MICRO4 BIOGAS

2021 - 2025

The Anaerobic Microverse

An In-Depth Roadmap on AD Plant Operations, Microbiome Innovation, and Economic Impact

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MICRO4 BIOGAS

MICRO4BIOGAS is a new European research project that aims to develop a deeper understanding of anaerobic microbiomes in particular for biogas production. Here, new ways of manipulating anaerobic microbiomes are to be explored, especially with regard to bioaugmentation. The project includes a comprehensive analysis of the European biogas landscape followed by a policy analysis, which is to be published as an e-Book in the framework of MICRO4BIOGAS.

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MICRO4BIOGAS - ABSTRACT

Since the 30.06.2021, the European Commission is funding the MICRO4BIOGAS research project. In the frame of the H2020 research programme, 14 partners from 6 European Countries will receive €5.7M for a duration of 4 years. The main goal of the project is to assess the manipulability of anaerobic microbiomes based on bioaugmentation. Before entering the anaerobic microverse, the present roadmap gives a detailed overview on the basic functionalities of anaerobic digester (AD) plants and as well the economic situation. This includes the technical functionality, different types of digester plants, but also inputs and outputs, such as substrates, biogas, emissions and digestate.

To reflect the European situation in the biogas sector, MICRO4BIOGAS is focussing on the 6 countries, which are represented within the project: The situation of the respective countries is very different. Among the countries considered, Greece had the smallest number of biogas plants in 2020 (60 units). Outgoing from about 20 plants in 2011, more and more plants for the utilisation of agricultural residues have been built. Most of the Greek AD plants are now using substrates from agriculture. Finland has a similar situation to Greece. Spain and the Netherlands each had between 200 and 300 biogas plants in 2020. However, these are mostly landfills and digesters for sewage sludge. Belgium had a similar amount as Spain and the Netherlands in 2020 (approx. 200 plants), but about 50% received their substrates from agriculture. Germany plays a pioneering role in the European biogas landscape with currently 11,000 plants, which provide now more than 70,000 GWhs. More than 90% of these plants use agricultural residues. The rapid increase in the amount of AD installations was possible mainly due to legislative support. In Germany, the Renewable Energy Sources Act (EEG) established a feed-in tariff and grandfathering for biogas plants, which ensured a stable economic situation in the long term. It must be emphasised at this point that Germany started early with the construction of biogas plants. There were already more than 8,000 biogas plants in 2011. The case in Germany shows in a positive way what potential biogenic residues have. Supported by current political events (tensions with Russia and the war in Ukraine), this leads to continuously increasing prices per kWh, which in turn increases the competitiveness of biogas plants. This shows even more that biogas is a good alternative to fossil fuels. It also reduces the dependency of international fossil fuel trading. However, biogas plants are still dependent on economic support (e.g., through the feed-in tariff). Further developments are necessary to ensure that biogas plants have a long-term future. Here, new marketing strategies could take effect. Potential improvements could be achieved in relation to emissions trading, new kinds of substrates, or new ways to better integrate biogas plants into the bioeconomy (e.g., through hydrogen production or extraction of organic acids for fine chemistry). Another solution, which is the focus of MICRO4BIOGAS, is the optimisation of biogas plants based on bioaugmentation and microbiome manipulation.

Bioaugmentation refers to the introduction of microorganisms into certain systems, such as contaminated soil, polluted water or even biogas plants. The challenge here, however, is to find microorganisms that could not only optimise the biogas process. These respective organisms must also be able to assert themself against the large number of existing, indigenous microorganisms.

To find microorganisms that are both efficient and robust, the MICRO4BIOGAS project is specifically looking for microorganisms that are already native to biogas plants. Currently, the project partners are working on one of the most comprehensive metagenome studies in relation to biogas. About 80 different samples were collected from many biogas plants. Process chemical parameters were collected for the corresponding plants. Further analyses are now being carried out using 16S-rRNA gene amplicon high-throughput sequencing

or metagenome sequencing. Numerous microorganisms are to be isolated from the most promising samples and tested for suitability for bioaugmentation. In line with the goal of optimising anaerobic microbiomes, the underlying roadmap also describes the structure of anaerobic microbiomes and addresses their manipulability. The word microbiome stands for the totality of all microorganisms found in a defined habitat. The microbiome in biogas plants comprises about 300 key microbial groups and is thus a highly diverse habitat. Besides bacteria, archaea, fungi, protista and bacteriophages are found. While the work in MICRO-4BIOGAS focuses on bacteria and archaea, the other microbial groups are also of interest for future projects. Both anaerobic fungi and bacteriophages have important functions in the anaerobic microbiome. Anaerobic fungi have outstanding degradation strategies and bacteriophages have considerable influence on the taxonomic composition. Nevertheless, the number of publications on these organisms in relation to biogas is extremely low. Amongst bacteria, the so-called Candida Phyla Radiation (CRP) could also play an importance in future research. One also speaks here of nanobacteria, and an interesting representative is Gracilibacteria. This genus is not viable on its own. They can physically dock to other microorganisms and are thus kept alive. They seem to have important functions in the degradation of substances that are difficult to break down, e.g. in petroleum-contaminated waters, and they have also been observed be present in taxonomic profiles, which comprise methanogenic archaea. The anaerobic treasure chest of the biogas sector certainly holds further surprises and MICRO4BIOGAS would like to help realise this potential.

Independent of optimisation of anaerobic microbiomes, MICRO4BIOGAS would also like to give an update on the current situation of the European biogas landscape. In this context, an intensive cooperation with the European Biogas Association (EBA) is planned and an extensive survey is already in preparation. At least 200 people are to be interviewed, whereby stakeholders but also people without a connection to biogas are eligible. Of interest is the general level of knowledge about the biogas industry, the future potential of biogas plants for the bioeconomy, technical and legal approaches to improving biogas plants, and the need for new funding instruments. The keyword "microbiome" also plays an important role here.

Finally, the cooperation with the town of Aras de los Olmos should be mentioned. The town is in the northernmost part of the Community of Valencia. Since the "Covenant of Mayors for Climate and Energy" in 2014, the town has committed itself to decarbonisation, capacity building, sustainable and affordable energy. The goal is a complete switch to renewable energy, and biogas will play a role here. Even though the goals of Aras de los Olmos are pursued independently of the MICRO4BIOGAS project, the project consortium accompanies Aras de los Olmos on its way and presents their project as a case study in this roadmap. Aras de los Olmos is currently building a biogas plant. Should this be completed within the project framework, synergies with the MICRO4BIOGAS project might be possible. The plant biogas plant might be available for test trials within the framework of MICRO4BIOGAS.

Specific tasks within work package 1 - MICRO4BIOGAS

The MICRO4BIOGAS project is divided into 8 work packages. The MICRO4BIOGAS roadmap is part of work package 1, which is divided into 5 tasks. The following paragraphs are giving an overview of the specific tasks of work package 1. The MICRO4BIOGAS roadmap is the focus of task 1.5. Although task 1.1 – 1.4 are separate from the roadmap, they are partly reflected in the present roadmap as well.

Task 1.1: State of the art analysis

Biogas production is an industrial area with various structural solutions. There are different types of biogas reactors, but several reactors of different designs can also be combined with one another. In addition, there are additional process differences with regard to the selected process chemical environmental parameters. In particular, the number of possible substrates is highly variable. Finally, many industrial areas are known that are related to the biogas industry. The first chapter on the project roadmap is intended to show the current status in the biogas industry and to reflect its versatility. We would like to present the structural standards of different types of biogas plants against the background of biological processes, operational safety, environmental friendliness, and ecological considerations. We would also like to point out weaknesses and opportunities for improvement.

Task 1.2: Policy Analysis

A comprehensive overview of the political situation, political objectives, target markets and future development opportunities for the biogas industry is planned. The role of biogas plants as a networking element of the bioeconomy and industry should be emphasized. In connection with MICRO4BIOGAS, three fundamental questions arise:

1. Can bioaugmentation improve the efficiency of biogas plants and thus the market situation for biogas plants?
2. Can multi-stage biogas plants help to substitute petroleum-based raw materials at least partially?
3. Could bioaugmentation help to better interconnect the biogas industry with other branches of industry in the biorefinery or with the provision of biological resources?

All three questions will be answered based on an extensive literature research. A survey with key players in the European biogas landscape is also to be carried out.

Biogas production, as commented above, is linked to agriculture and cattle industry among other sectors, having thus a great influence over the development of several EU rural areas. MICRO4BIOGAS will study which existing EU policies can foster biogas production in rural areas and how to improve them, as well as the existing rural networks able to push towards Biogas production. In addition, and linked to WP6, we will analyse the public funding options and alternatives in such a low margin market.

Task 1.3. New technologies and innovations

The rise of next-generation sequencing technologies in the last few years has stimulated the research of anaerobic digester microbiomes. This tremendous sequencing effort will now be gathered and exploited by the MICRO4BIOGAS consortium. With the help of curated literature and text mining strategies, all publicly available sequencing data and their associated scientific papers will be identified, integrated, and analysed, with a special focus on the manipulability of anaerobic microbiomes and on the accessibility and standardisation of the sequencing data, metadata, methods and protocols from each study. Once all possible data is integrated, several aspects and their effects on the anaerobic digestion process will be deeply analysed, for example, increase of hydrogen forming and synthrophic bacteria, DIET and its links with phototrophy, variation of typical process parameters (pH, Temperature, viscosity, etc.), increase of cellulase-producing bacteria, and alterations of signalling/quorum sensing. The final goals are to gain insight on potential bioaugmentation strategies, and to apply machine learning techniques to predict the behaviour of a specific microbiome and its implication in the biogas production process.

Task 1.4. AD-Microbiomes vs. Europe

The key contribution of this task will be the addition of industrial microbiome approach to the European biogas landscape (EBL). To do so, the EBL will be deeply characterized from several points of view:

1. Innovations, technological advances and in silico analysis associated to this field.
2. Public policies and public networks involving local governments.
3. Biogas stakeholders' map.

We will reinforce the biogas innovation scenario, particularly those aspects related to the industrial microbiomes of anaerobic digesters, including microbiome manipulation, big-data and bioaugmentation. The EBA (European Biogas Association), one of the most important bodies linked to the European Biogas Network, is supporting MICRO4BIOGAS in this task.

T1.5. e-Book

This task will see the compilation of an e-Book showing the roadmap of the project through the integration of data collected from all relevant contributions from partners. Summarized reports of each task will be aggregated and complemented with graphical abstracts towards turning the useful information into a user-friendly e-Book that will be publicly available through the project website. Furthermore, this e-Book with the project roadmap will be used for widespread dissemination among potential end-users, policy makers, and biogas plant operators, among others.

Involved partners: The MICRO4BIOGAS project is coordinated by Universitat de València (Spain) and comprises the following partners:

- **Gasterra BV** (Netherlands)
- **ABS International** (Belgium)
- **AEV Energy GMBH** (Germany)
- **Ayuntamiento de Aras de los Olmos** (Spain)
- **Bioenergie Verbund EV** (Germany)
- **Technische Universität Dresden** (Germany)
- **Draxis Environmental SA** (Greece)
- **Bioclear Earth BV** (Netherlands)
- **Universitat Politècnica de València** (Spain)
- **Universiteit Gent** (Belgium)
- **Finrenes OY** (Finland)
- **Darwin Bioprospecting Excellence SL** (Spain)
- **Scienseed SL** (Spain)

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You can also follow us on social media: @micro4biogas

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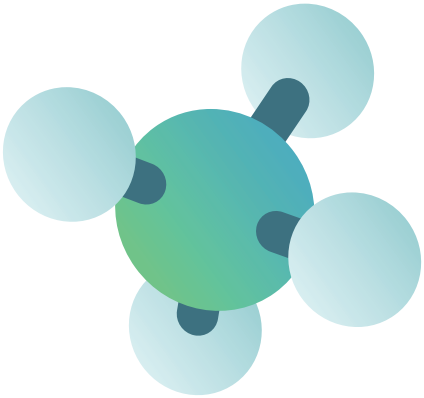
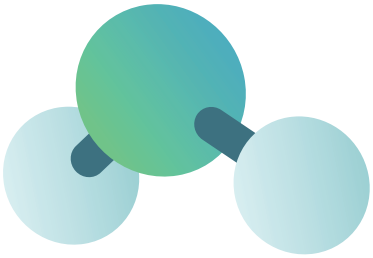




State of the art

Authors from the AEV Energy GmbH: Benjamin Rocktäschel, Astrid Palen, Phillipp Kyas.

Abstract:

The present chapter gives an overview on anaerobic digestion in general. This includes the purpose of anaerobic digestion and its basic principles. Different types of anaerobic digesters and their functionality are explained, as well as different scenarios of biogas usage. The chapter also contains environmental consideration and as well potential for improvement. Anaerobic digestion is especially interesting for stakeholders related to agriculture, livestock, waste disposal, green waste, and water treatment. Anaerobic digestion has further importance for technological innovators, as anaerobic digestion holds still potential for many bioproducts, which remain unexploited.

Agriculture: For biogas production, substrates from agriculture such as corn silage, grass silage, whole plant grain maize, and grain meal are preferred. According to the state of the art, these can be digested easily and deliver high biogas yields. They are also referred to as energy crops. It makes economic sense to produce renewable raw materials on one's own agricultural land. Unlike the production of food, the whole biomass produced can usually be used here. The resulting digestate can be returned to the fields as fertilizer. This creates a closed nutrient cycle. Through subsidies on the sale of electricity and better utilisation of the cultivated biomass, a biogas plant can contribute to the economic viability of an agricultural company.

Livestock: Manure and dung from cattle and pigs is also a popular substrate for biogas production. The process stability is even greater with manure and dung from cattle and pigs, compared to the use of plant biomass. The demands on the process technology are lower. Excreta from poultry such as chickens or turkeys contain a lot of nitrogen. These can only be fermented with conventional methods in combination with other substrates. All animal excreta have in common that the expected gas production is lower than when using plants such as corn, grain, or grass. Often, residual materials such as liquid manure or dung can be acquired at very low-cost if long transport distances are not necessary. It is even cheaper for livestock farms to use their own residues. Thus, the construction of a small biogas plants often makes economic sense even for smaller livestock farms. The direct use of slurry and manure as fertiliser results in considerable methane emissions, especially in the case of cattle slurry. These can be effectively prevented by digestion in a biogas plant.

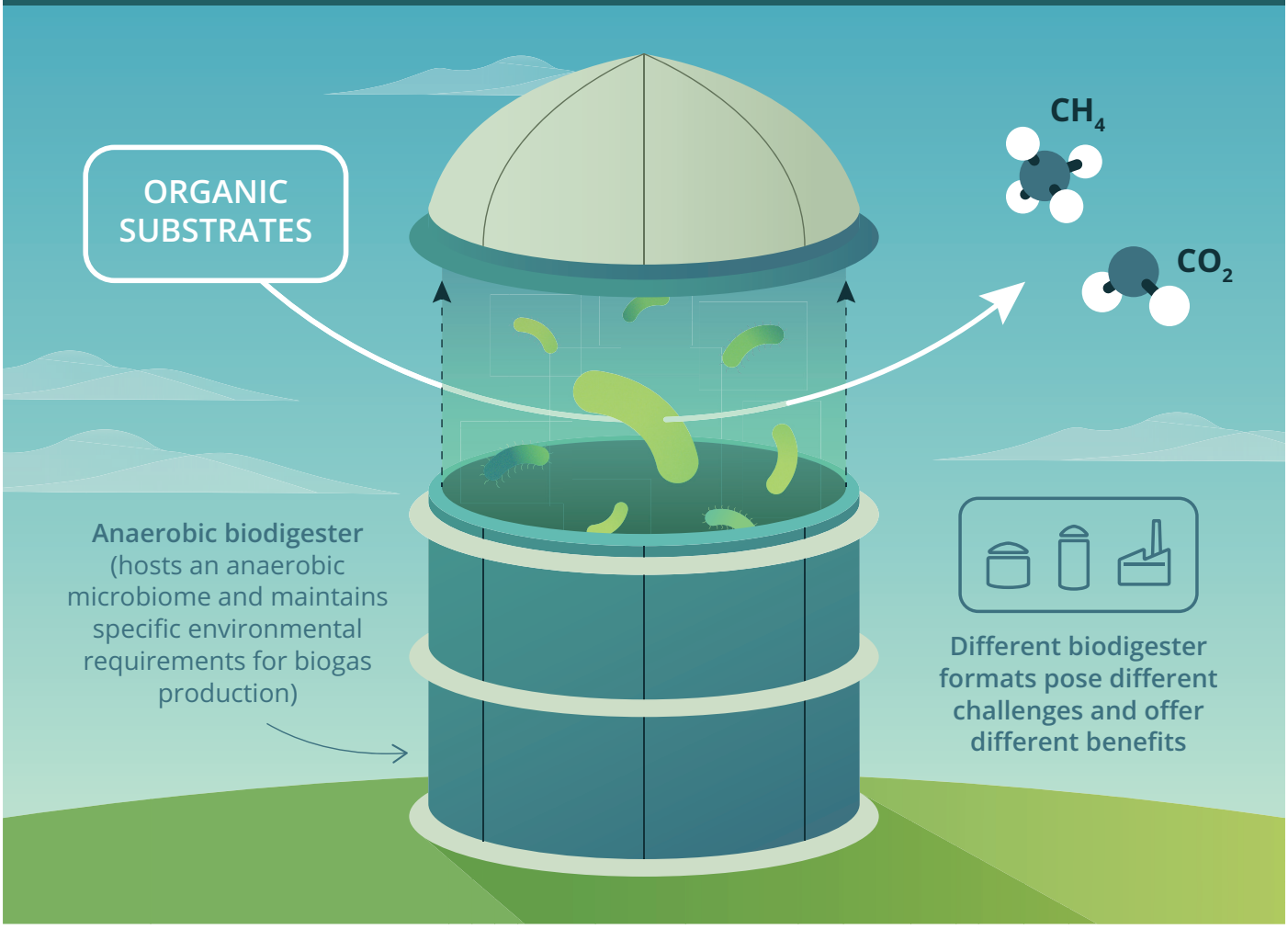
Waste disposal: The use of organic waste fractions for biogas production is attractive because waste does usually not have to be paid for. Under certain circumstances, it may even be possible to make a profit from the purchase of waste. Whether the entire process is economical depends on the effort that must be made to process the available waste and the expectable methane yield. In most cases, pre-treatments such as separation by fraction or shredding are necessary. There are special types of digesters for the treatment of stackable substrates. Batch systems are particularly suitable for waste. Using this technology, pretreatment can be omitted, and the waste can be freed from organic fractions in this way. The potential for producing biogas from waste varies greatly from region to region. Especially in regions where waste recycling is not widespread, there are often large amounts of waste that can be digested. In these regions, the positive ecological effect is also greatest.

Green Waste: Green waste can be interesting as a substrate for biogas plants if the proportion of lignified plant parts is low. Normally, these substrates are used seasonally and together with other substrates such as energy crops. As long as the dry matter content of the material is not greater than about 40%, the technical requirement is similar to the fermentation of energy crops. Whether it makes economic sense to use depends on whether a sufficiently large quantity of substrates with a constant quality is available. With a dry matter content of over 40% and larger proportions of lignified plant parts, fermenters for stackable substrates can also be used.

Wastewater treatment: Sewage treatment produces sewage sludge, which can be treated anaerobically to produce biogas. However, the technical requirements (tanks, agitators, separators, etc.) for this process are fundamentally different from the biogas production described below in the context of agricultural plants. The legal framework conditions are also completely different in most cases. In the case of anaerobic wastewater treatment, the focus is more on the decomposition of organic substances and economic advantages when combined with aerobic processes. Biogas production from wastewater is therefore not considered in the following.

New bioproducts: The primary components of biogas (CO_2 and CH_4) can in principle be separated and further utilised. In the case of methane, this is done by biogas upgrading. The biomethane produced can be fed into the natural gas grid and is mostly used to produce electricity and heat. In the European Union, the production of biomethane has been steadily increasing since 2010. In 2020, 32 TWh of energy was produced in this way. CO_2 is also a potential feedstock for syntheses of carbon-containing compounds. However, the cost of carbon dioxide production is too high compared to other chemical processes to make it economically viable. There is also research into the production of other chemical substances during fermentation. However, these are not part of the state of the art, can be omitted, and the waste can be freed from organic fractions in this way. The potential for producing biogas from waste varies greatly from region to region.

Biogas plants are available in many different designs, with different solutions for every feedstock and every industry. In this capter, we provide a clear summary that compares the various process engineering solutions and shows the advantages and disadvantages.



1.1. Introduction

1.1.1. What is biogas?

Biogas is a combustible gas mixture and consists mainly of methane and carbon dioxide. Biological processes form the basis to produce biogas. A combustible gas mixture is produced from organic biomass under anaerobic conditions. The formation of biogas can be observed in natural processes. These take place in bogs, in sediments at the bottom of water bodies and in the faeces and stomachs of cattle **(Fachagentur Nachwachsende Rohstoffe, 2013)**. Biomass is decomposed by various microorganisms in several steps, mainly to carbon dioxide, methane and water. The gaseous components form the so-called biogas. This gas mixture consists of a methane content of 50 to 75% by volume and a carbon dioxide content of 25 to 50% by volume **(Fachagen-**

tur Nachwachsen stoffe, 2013). In addition, it contains other components such as nitrogen, water (as water vapour), ammonia, hydrogen sulphide and hydrogen in small quantities. In the following, only the technical production of biogas is considered. The raw materials of the biomass are referred to as feedstocks. Plants for the targeted and priority production of biogas are called biogas plants. In biogas plants (also referred to as anaerobic digesters), biomass is used as the starting material for microbial degradation. Feedstocks include excrements from farm animals, biowaste, residues from food production as well as energy crops (Streicher *et al.*, 2016). The resulting biogas can be used in combined heat and power plants to produce electricity and heat, or it can be purified and fed into the natural gas grid. Biogas plants make it possible, especially for companies that have unused biomass available, to save costs for disposal, prevent emissions and generate income through the sale of electricity and heat or gas.

Composition of biogas: The composition of biogas is determined by various influencing factors, which also influence each other. The most important factors are the microorganisms involved, the process conditions and the chemical composition of the feedstock used. The following table (**table 1**) gives an overview of common gas compositions (Rasi, S., 2009).

Table 1: Composition of biogas (Fachagentur Nachwachsende Rohstoffe, 2013).

Gas	Concentration
Methane (CH_4)	50 - 75 Vol.-%
Carbon dioxide (CO_2)	25 - 45 Vol.-%
Water (H_2O)	2 - 7 Vol.-% (20-40 °C)
Hydrogen sulphide (H_2S)	20 - 20,000 ppm
Nitrogen (N_2)	< 2 Vol.-%
Oxygen (O_2)	< 2 Vol.-%
Hydrogen (H_2)	< 1 Vol.-%

Methane – CH_4: Methane is the main combustible component of biogas and therefore the target product in technical production. The higher the methane content in biogas, the higher its calorific value. The more methane is produced, the more electricity and heat can be generated, and thus the more revenue. For utilisation in CHPs, the methane content must normally not be below 40% - 45%. Under normal process conditions, this methane content is achieved without any problems. Biochemically, methane is formed in various metabolic pathways (Rasi, 1984).

Carbon dioxide – CO_2: Carbon dioxide is the second main component of biogas after methane. Since it is not combustible, it is a by-product from a technical point of view. Carbon dioxide behaves like an inert gas during combustion in the CHP unit and does not contribute anything to the calorific value. If the biogas is to be processed and fed into the natural gas grid, the CO_2 must be separated. Carbon dioxide is formed at many points during microbial degradation. It is formed during hydrolysis/acidification and during methane formation. It is the end point of the oxidative degradation of carbon compounds (Abdeen *et al.*, 2016).

Water – H_2O: Water is found in biogas, mainly as water vapour. After its formation, biogas usually has a relative humidity of almost 100%. In (inactive) combustion engines, this moisture can condense and, in combination with hydrogen sulphide, lead to corrosion. Biogas must therefore often be dried and H_2S should be removed.

Nitrogen - N_2: Nitrogen is not formed during the decomposition of biomass under anaerobic conditions, but it reaches the biogas plants in small quantities when air is added. Biochemically, nitrogen behaves inertly and is therefore negligible for the technical process.

Oxygen – O_2: In the presence of oxygen, the energy-rich hydrocarbons in the biomass are completely degraded to carbon dioxide and water without methane being

formed. Biogas production therefore takes place in the absence of air. The addition of very small amounts of oxygen with the substrates can hardly be prevented technically, but oxygen is often added to the biogas in a controlled manner to remove gaseous sulphur compounds. Under anaerobic conditions, small amounts of oxygen are consumed very quickly, which is why it is usually only found in very small quantities in biogas. It generally has an inhibitory effect on methane-forming microorganisms. In larger concentrations, oxygen forms an explosive mixture with methane, which is why the oxygen content is kept as low as possible in technical biogas production.

Hydrogen sulphide – H_2S: H_2S is formed as a gaseous end-product during the decomposition of sulphur-containing compounds of biomasses. It is technically undesirable due to its corrosive properties. The content of H_2S in biogas depends strongly on the sulphur content of the outgassing products. Sulphur compounds can have an inhibitory effect on microorganisms. However, such concentrations are usually not reached in agricultural biogas plants. Hydrogen sulphide is toxic to humans, and thus provides an increased hazard potential when found in biogas **(Guidotti, 1996)**.

1.1.2 Biochemical conversion to biogas

In the following, the biochemical basics of biogas formation will be briefly presented. Further information and an introduction to the microorganisms involved can be found in *chapter 5*. Biogas formation is also known as anaerobic digestion (AD). The anaerobic digestion can be divided mentally into four sub-steps. The process is shown schematically in **figure 1**. In technical language, all sub-steps are usually summarised as methane formation, since all biochemical processes often take place together in one container. **(Weiland, 2010)**

Hydrolysis: The first phase of biochemical conversion to biogas is hydrolysis. In the chemically broader sense, hydrolysis means the splitting of molecules under the reaction with water **(Planet Biogas, online accessed**

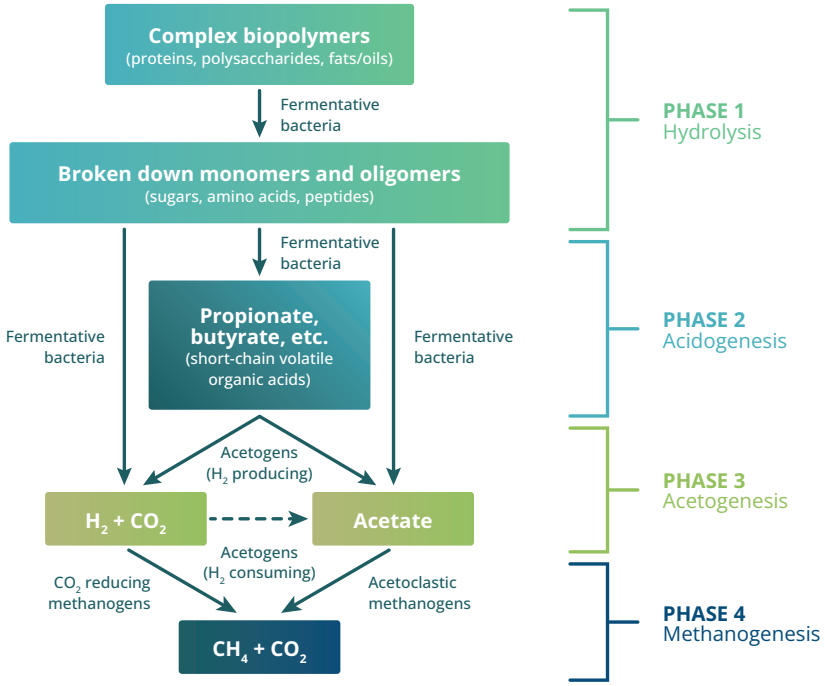


Figure 1: Schematic representation of anaerobic fermentation (Adapted from Dutton, online accessed 2021).

2021). Hydrolysis can take place under anaerobic conditions. Polymers present in the biomass such as cellulose, proteins and fat (complex macromolecules) are split in this phase to oligo-, di- and monomers (shorter split products) (Nwokolo *et al.*, 2020). Bacteria involved in this process release enzymes that biochemically decompose the substrates (Bauer *et al.*, 2009).

Acidogenesis: In the second phase, also called the acidification phase, the hydrolysis products (mainly from the sugars, fat, and proteins) are converted into hydrogen, carbon dioxide, alcohols, and fatty acids by acid-forming bacteria. The fatty acids are short organic fatty acids such as acetic, propionic, and butyric acid (Schnürer, 2016). For the process, it should be noted that ammonia is formed from nitrogen compounds. In excessive quantities, this is toxic for the microorganisms and thus has a process-inhibiting effect (Aberle, 2016).

Acetogenesis: The third phase of anaerobic digestion is acetogenesis (acetic acid formation). The initial products of acidogenesis are converted into even smaller molecules by acetogenic bacteria. Acetic acid, hydrogen and carbon dioxide are formed. A too high hydrogen content inhibits the conversion of the intermediate products of acidogenesis (Drake, 1994).

Methanogenesis: In the final phase of biogas formation, the acetic acid, hydrogen and carbon dioxide are converted into methane by means of strictly anaerobic methanogenic archaea. The starting materials of methanogenesis are methane, carbon dioxide and water. The production of methane can be divided into two groups. Hydrogenotrophic and acetoclastic methanogenesis. In hydrogenotrophic methanogenesis, methane is produced from hydrogen and carbon dioxide; in acetoclastic methanogenesis, methane is produced by acetic acid cleavage (Schnürer, 2016). The microorganisms involved in methanogenesis are called methanogenic archaea. These are light and temperature sensitive (Wu *et al.*, 2002; Olson *et al.*, 1991). In anaerobic digester plants, mainly acetoclastic and hydrogenotrophic methanogenesis is relevant for methane production. The respective methane formation follows the following reactions (Zengler *et al.*, 1999):

Acetic acid-splitting (acetoclastic methanogenesis):

- $CH_3COOH \longrightarrow CO_2 + CH_4$

Hydrogen-utilising (hydrogenotrophic methanogenesis):

- $CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$

Different microorganisms are involved in the individual degradation stages, and these have different optima in respect to process chemical conditions (e.g. temperature, pH value). From a process-technical point of view, a compromise must therefore be found which takes into account above all the methanogenic microorganisms. These have a low growth rate and react fragilely to disturbances. Therefore, the conditions of the biocenosis must be adapted to their requirements. The biocenosis must, therefore, allow fermenting bacteria and methanogenic archaea to coexist. The milieu conditions and operating parameters required for this are discussed below. Depending on the type of feedstock and the design of the biogas plant, different environmental conditions can arise in the individual fermenter stages. The environmental conditions have an impact on the microbial biocenosis and thus influence the metabolic products (Boe *et al.*, 2010).

1.1.3 Milieu conditions

Oxygen: Methanogenic archaea formed about three to four billion years ago. Therefore, they are amongst the oldest living organisms of our planet. Since the atmosphere had a completely different composition at that time, archaea depend on an oxygen-free environment. Even small amounts of oxygen can already have a toxic effect on archaea. An input of oxygen into the fermenters cannot be completely avoided, but this does not lead necessarily to inhibition of the involved methanogenic archaea. These archaea live

in symbiosis with oxygen-consuming bacteria from the previous degradation steps. Therefore, oxygen is consumed before causing toxic effects on the archaea involved (Pedone *et al.*, 2004).

Temperature: Although the reaction rate of chemical reactions increases with temperature, the temperature optimum must not be exceeded. There are different temperature optima for the metabolic products of microorganisms (**table 2**). If these are exceeded or not reached, the underlying processes can be inhibited, and the microorganisms can be irreversibly damaged. The temperature optima for biogas plants can be divided into three groups (Dobre *et al.*, 2014).

Table 2: Temperature optima of participating microorganisms (according to Dobre *et al.*, 2014).

Thermal stage	Temperature range
Psychrophilic	< 25 °C
Mesophilic	37 – 42°C
Thermophilic	50 – 60°C

Due to the low temperatures in the case of psychrophilic microorganisms, it is not necessary to heat up the fermenter. Nevertheless, the gas yield per unit of time is significantly lower under psychrophilic conditions due to the lower reaction rates. Mesophilic and thermophilic microorganisms have a significantly faster gas production (Zinder *et. al.*, 1984).

Compared to mesophilic and psychrophilic temperature, the thermophilic temperatures allow a faster biogas formation, but it has a lower process stability. Under thermophilic conditions, cultures exist in a lower species range than in the mesophilic group. This means that the fermentation process is more sensitive to the introduction of new substrates, as well as to the operation and certain disturbances. The fermenter must also be brought to a high temperature level, which leads to an increase in maintenance costs. The most widely used biogas plants are those that operate in the mesophilic temperature range. They offer a good compromise between fast biogas production and good process stability (Briones and Raskin, 2003).

For the cultures involved, the temperature should be kept as constant as possible. The microorganisms can react and adapt to slow temperature changes, but this is not possible with too high fluctuations in short intervals (Chae *et al.*, 2008). When microorganisms break down carbohydrates, self-heating occurs, which influences the temperature in the fermenter. The temperature can rise to a level of 43 - 48°C if the fermenter is operated in a mesophilic mode (Lindorfer *et al.*, 2006).

pH value: Characteristic for the biodegradation process are the decrease of dry matter and an increase of the pH value. Different microorganisms require different pH values for optimal growth. The pH optimum of the individual bacteria and archaea is listed in **table 3**.

Table 3: pH values optima of microorganisms involved (Fachagentur Nachwachsende Rohstoffe, 2013).

Microorganims	pH-value
Hydrolysing and acidifying bacteria	5.2 - 6.3
Acetic acid-forming bacteria and methanogenic archaea	6.5 - 8

The pH value within a fermenter is self-adjusted due to the underlying biochemical processes. The alkaline and acidic metabolic products formed during anaerobic degradation are decisive for this. The pH value can drop if the acidic metabolic products of acidogenesis accumulate. This is possible, for example, if the substrate supply is too high or if methane formation is inhibited. If the pH value drops, the inhibiting effect of hydrogen sulphide and certain organic acids may increase, and the fermenter may tip over.

1.1.4 Inhibitors

Inhibitors can interfere with processes of the individual degradation stages. If an inhibitor reaches a too high concentration, the degradation process can even be completely stopped. Inhibitors can either enter the fermenter via the substrate or they arise as intermediate products of the individual degradation stages. Excessive addition of substrates can also be classified as inhibitory. Microorganisms can adapt to inhibitors, but their adaptability is limited. Examples of inhibitors that are introduced via the substrate include antibiotics, disinfectants or solvents, herbicides, salts, or heavy metals, which inhibit the process even at low concentrations **(Theuerl *et al.*, 2019)**. Some of the inhibitors are described below as examples.

Nitrogen / Ammonia is a prerequisite for the growth and activity of microorganisms. If the added substrate is rich in nitrogen compounds (especially proteins), this leads to an increase in the ammonia concentration in the fermenter. Ammonium (NH_4^+) and ammonia (NH_3) are produced during hydrolysis and are in equilibrium with each other **(Purwono *et al.*, 2017)**.

- $NH_4^+ + OH^- \rightleftarrows NH_3 + H_2O$

An increase in pH increases the NH_3 content. Also the temperature influence the ammonia concentration, as with rising temperature there is a shift from ammonium to ammonia **(Fuchs *et al.*, 2018)**.

Sulphur / Hydrogen sulphide is an essential building block for the microorganisms involved and thus essential for the production of biogas. Dissolved hydrogen sulphide (H_2S) can be toxic and have an inhibitory effect at high concentrations. Elevated H_2S concentrations cause corrosion and can lead to damage in the CHP. H_2S can be converted with the help of sulphur bacteria, which biologically convert the H_2S into harmless sulphur **(Aberle, 2016)**.

- Sulphur hydrogen oxidation: $H_2S + O_2 \longrightarrow S_2 + 2\,H_2O$

1.1.5 The technical production of biogas

According to **Weiland (2010)**, an agricultural biogas plant can basically be divided into four process steps:

- Feedstock management (delivery, storage, processing)
- Biogas production
- Digestate management
- Biogas utilization (incl. storage and treatment).

The individual process steps are shown in detail in **figure 2**. The individual process steps are not decoupled from each other but are closely connected. For example, process step four provides the required process heat for the second step **(Fachagentur Nachwachsende Rohstoffe, 2013)**.

Components of a biogas plant: The components used in a biogas plant vary depending on the design of the plant. In the following, only the core components of a biogas plant will be discussed. Further information on the individual plant components can be found in chapters 1.2 - 1.5. **Figure 3** shows the schematic structure of a biogas plant.

Feedstocks/Substrates: Feedstock are plant and animal substances that can be fermented in biogas plants **(Person *et al.*, 2019)**. They originate from agriculture as well as industry, commerce, and municipalities. Feedstocks differ in their degradability and biogas yield due to different material properties. In addition to residual materials, renewable raw materials are also used, which are produced specifically for the use in biogas plants **(Person *et al.*, 2019)**. The different substrates are discussed in further detail in *section 1.2*.

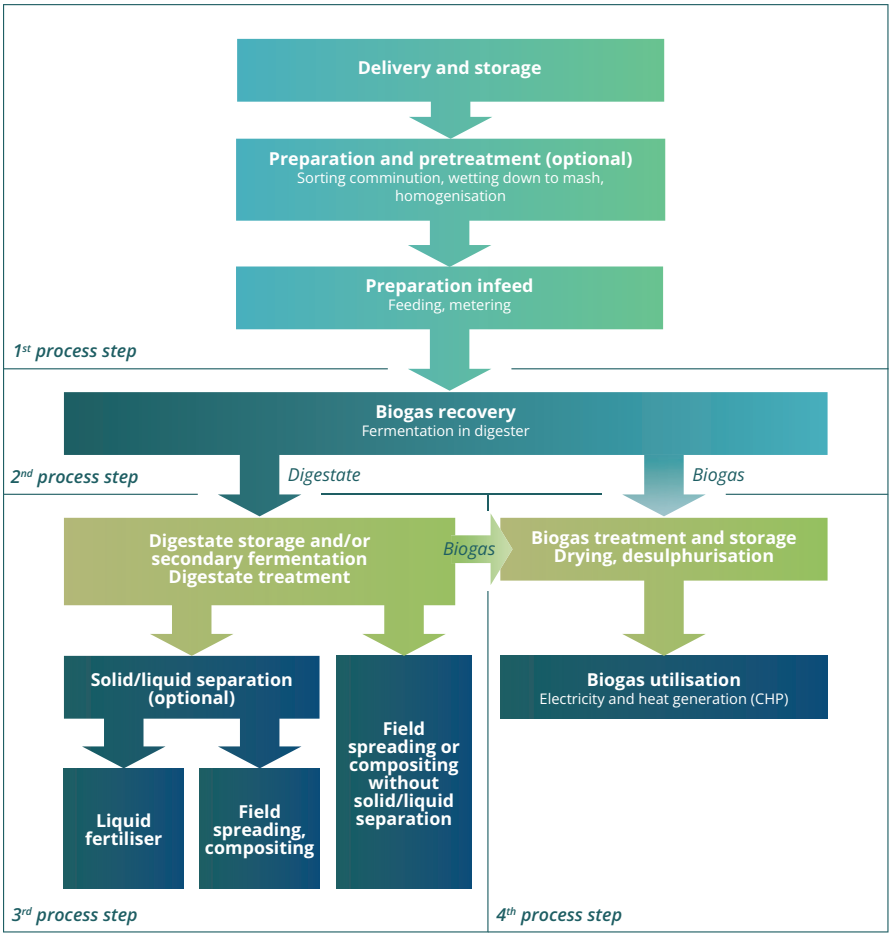


Figure 2: General process of biogas recovery (Adapted from Fachagentur Nachwachsender Rohstoffe, 2013).

Preliminary pit, silo and receiving feeder: The liquid substrates are temporarily stored in the preliminary pit. This is usually a round container or tank, which is embedded in the ground **(Person *et al.*, 2019)**. The building material is usually ferro concrete, which is often used in the form of prefabricated parts or as cast-in-place concrete. The preliminary pit is located before the digester and has the task of storing several days' rations of liquid substrate. The respective tanks can be open or closed. In addition to liquid, pumpable and agitable substrates, it is also possible to introduce solid substrates to a limited extent. However, it is more common to store solid substrates in silos. Silos are usually piles of solid substrates that are covered with a membrane to protect them from rain. Energy crops (or parts thereof) are stored in silos after harvesting and then continuously removed over the course of the year. During storage in the silo, lactic acid fermentation occurs. Due to the lowered pH from lactic acid fermentation, siloed substrate becomes durable and more usable for anaerobic digestion. Solid substrates are conveyed from the silo to the receiving dosing unit using a wheel loader or a similar machine. A receiving feeder consists of a receiving container, a dosing device and often also crushing and weighing technology. The substrates are are further conveyed via either a chute or a solid's feeder such as a screw conveyor **(Person *et al.*, 2019)**. The comminution of the substrates serves to protect the system from blockages and also significantly increases the usable surface area for the microorganisms **(Sreekrishnan *et al.*, 2004)**.

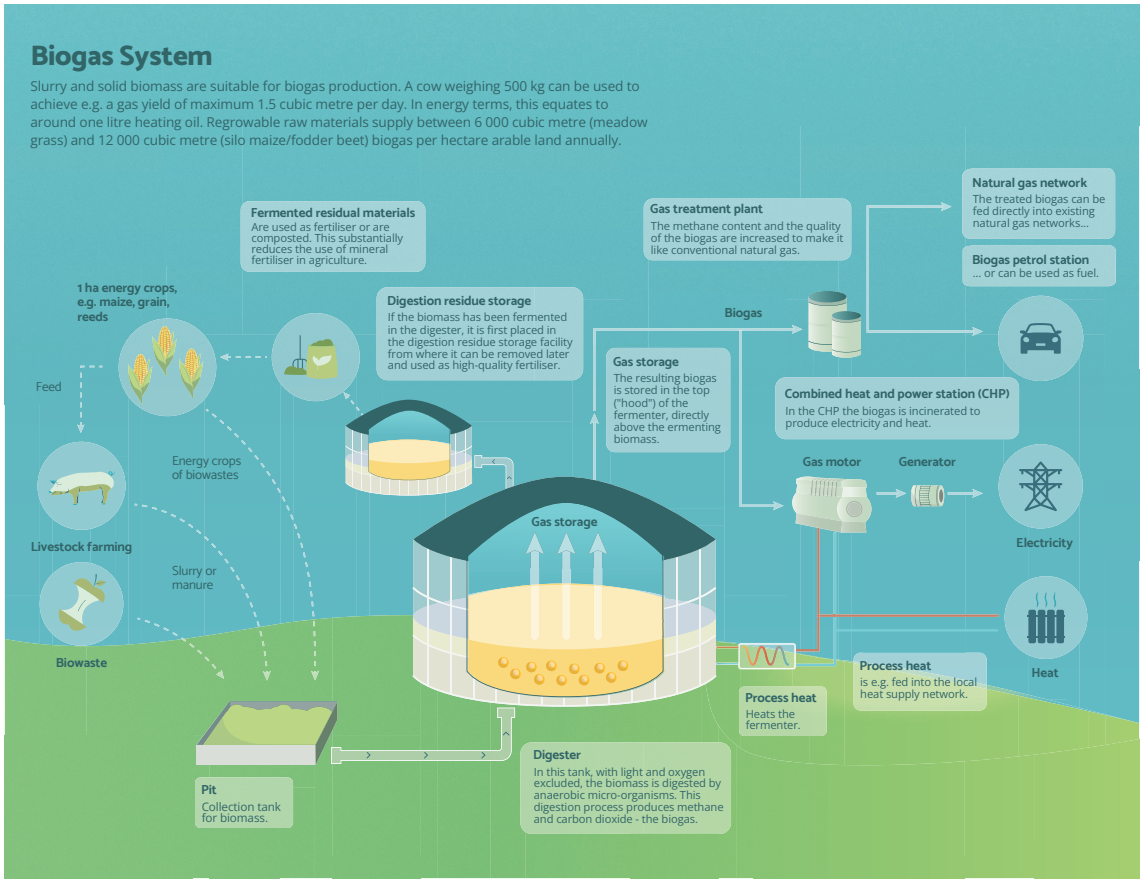


Figure 3: Components of a biogas plant (Planet Biogas, accessed 07.10.2021).

Fermentation tank/digester: The biochemical degradation process takes place in the fermentation tank. Different microorganisms gradually decompose the introduced substrates under exclusion of oxygen. In the process, biogas is produced in the fermentation tanks. The decomposition takes place under exclusion of air and light. Fermentation tanks are often made of ferro concrete, but constructions made of steel enamel or stainless steel are also common. The airtight cover of the tanks is provided by a flat solid or foil roof with integrated gas storage. To facilitate the heating of fermentation tanks, they are usually insulated. Heating is usually provided by the waste heat from a CHP unit. Systems for mixing, usually agitators, are installed in the tanks; these prevent differences in concentration and temperature within the fermentation substrate. In doing so, they mix the bacteria and archaea in the tank with the fresh biomass. Agitators also prevent sedimentation and flotation of the fermentation substrate and, depending on the viscosity, are also important for gas discharge.

Secondary digesters: In two-stage processes, a secondary fermenter is connected down-stream of the fermentation tank/digester. This is where substrates that are more difficult to degrade are converted. A secondary fermenter is often necessary to meet the requirements for a minimum retention time in the gas-tight system and thus avoid emissions (Schmidt-Eriksen, 2011).

Separator: The digestate from the fermenter is separated into a solid and a liquid phase in the separator. The liquid phase is collected and discharged as liquid fertiliser after intermediate storage. If required, it can also be used to mash the solid substrates in the fermenter. The solid phase has a reduced volume and can be used as a highly concentrated fertiliser.

Digestate storage tank: The digested fermentation residues are collected in the digestate store. These are then used as fertilizer in agriculture. Digestate tanks are

located downstream of the fermenter and the separator. Depending on the intended use, the digestates are stored in different ways (Effenberger *et al.*, 2008). The digestate storage must be large enough to store the digestate for the next thinning in spring or autumn. The liquid phase of the digestate is stored in round basins or lagoons, the solid phase on paved areas.

Gas storage: Gas storage tanks are used to buffer fluctuations in gas production and to store biogas for flexible operation. This enables flexible operation of the CHP units, which then do not have to be operated permanently, but can also be shut down for several hours. The plant can thus be operated according to demand and feed in electricity when it is needed and best remunerated. In the event of technical faults at the CHP unit or during maintenance work, gas storage facilities prevent the loss of biogas for a limited time. Gas storage tanks, as well as all gas-tight covered fermentation tanks, are equipped with an over-vacuum safety device. In the event of overpressure, they release biogas into the atmosphere and in the event of under pressure they let in air. This is to prevent damage. Safety shutdowns, operating methods and the gas flare must ensure that a release of biogas through the overpressure safety devices occurs as rarely as possible.

Heat reservoir: Heat reservoirs are water tanks with an insulating layer. They store generated heat even during times when no CHP is in operation. The stored heat can be used, for example, to heat the fermenter or to dry substrates. Heat reservoirs are necessary if the CHP units are not applied full-time, i. e. if they are operated flexibly.

Gas flare: A gas flare is almost always connected to the gas pipe system of a biogas plant. This burns biogas in an emergency to prevent uncontrolled release in the event of overpressure. This is to prevent emissions and explosive atmospheres on the plant.

Combined heat and power plant (CHP)/ gas utilisation: The utilisation of biogas usually takes place in so-called combined heat and power plants. There, electricity, and heat are generated from the biogas. Biogas utilisation is mostly carried out by means of gas Otto engines with flange-mounted generators. Thermal and electrical energy produced is reused in the biogas plant or fed into the public grid. The thermal energy produced is used in the fermenter, temporarily stored in buffer tanks, fed into a district heating network or used for drying plants. If heat utilisation is not possible (e.g. in summer when the heat demand is low), the CHP units have emergency coolers that dissipate the excess heat to the ambient air. The electrical energy is fed into the public grid via a transformer station.

1.2. Substrates

1.2.1 Feedstocks for biogas production

The composition of the substrate mixes and the nature of the individual substrates (dry matter content, structure, origin, etc.) determine the design of the process technology (DBFZ Deutsches Biomasseforschungszentrum, 2021). The biogas yield is not only substrate-specific, as one could assume based on **figure 4**, but it also depends on the boundary conditions such as HRT, temperature and the plant operation. Therefore, different biogas yields can occur with the same substrate input (Weiland, 2010).

When using renewable raw materials, the operator should make use of different feedstocks to be able to react to crop failures and price fluctuations. In Germany, biogas plants are often operated primarily based on animal by-products such as liquid or solid manure and renewable raw materials (Nwokolo *et al.*, 2020). Substrates can be differentiated according to their material properties, degradability, and gas yield. The methane yield of the substrate depends on its composition and the content of protein, fat, and carbohydrates (see **table 4**).

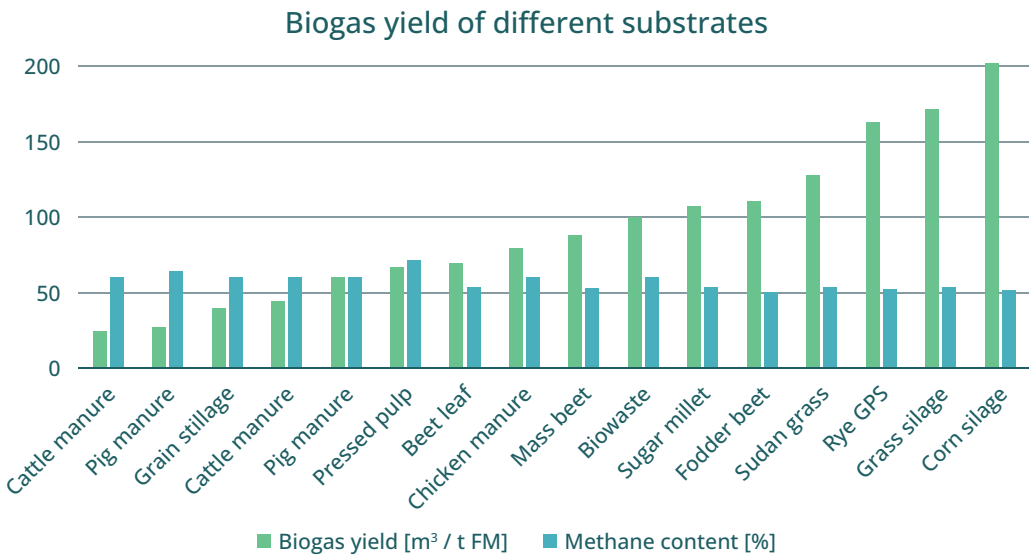


Figure 4: Components of a biogas plant (Planet Biogas, accessed 07.10.2021).

The lifetime of a biogas plant also depends on the substrate used. Material with a high protein content increases the concentration of H_2S, which is harmful to humans and machines as well as (above a certain concentration) the biogas-producing microorganisms. Monitoring the gas composition with different substrates is therefore necessary **(Effenberger *et al.*, 2008)**.

Species and origins of different substrates: The origin of substrates according to **(Persson *et al.*, 2019)**:

From agriculture:

- Animal farming (farm manure/animal excrement)
- Crop production - cultivation (renewable raw materials such as corn, cereals, Sudan grass, sweet sorghum)
- Crop production – residues (grain plaster, straw)

From industry, commerce, municipality:

- Food processing industry (production waste, incorrect batches, returns, wastewater)
- Food trade (market waste, superimposed food)
- Food processing industry (gastronomy, catering)
- Municipal waste management (bio bin, green waste, agricultural care, residual waste)
- Special industrial sectors (pharmaceutical industry, food supplements)

Table 4: Estimation of the maximum theoretical methane yield and biogas percentage composition (Nwokolo *et al.*, 2020).

Nutrient	Methane Yield (m^3/kg VS)	CH_4 [%]	CO_2 [%]
Carbohydrate	0.42	50	50
Protein	0.50	70	30
Lipid	1.01	70	30

Animal by-products are divided into three categories. Category one material may not be used in biogas plants. This includes animals suffering from mammalian diseases, pets, zoo and circus animals, as well as kitchen and food waste from airports. Category two and three materials may be used into biogas plant. Category two includes manure, dung, stomach and intestinal contents, colostrum and inhibitor milk. Manure fermentation makes a major contribution to reducing greenhouse gas emissions, not only due to the production of biogas, but especially due to the reduction of CH_4 emissions from open storage **(Weinrich *et al.*, 2020)**. The use of plant by-products such as straw and crop residues in biogas plant can be economical and leads to process stability when used together with ABP. This reduces the ammonia content and ammonia inhibition **(Fuchs *et al.*, 2018; Verein Deutscher Ingenieure, 2006)**. Plant by-products include brewer's grains, waste bread, starch, molasses, peels, fruit and vegetables and spoiled fodder silage.

For waste, water content and degradability are important factors in the selection of suitable substrates. Fermentable materials that are too moist for composting can be used for digestion. Residual waste comes from private households or public places. The organic fraction in residual waste can be separated using sieves, air sifters and metal separators. Following this, the organic fraction can be transported to the biogas plant for further processing **(Nwokolo *et al.*, 2020)**. Organic bins are also used to separately collect organic material. Garden and park waste is collected in green waste containers at decentralised collection points. Municipal sewage sludge is obtained from the various purification steps of wastewater treatment. It should be noted that sewage sludge may contain antibiotics, which have an inhibitory effect on methanogenesis. Restaurants, kitchens and canteens collect food waste in bio bins or containers. Liquid waste from beverage production and sludge from industrial processes is collected in tanks. This type of waste requires a high level of processing, for example removal of packaging, but provides a very high biogas yield **(Steffen *et al.*, 1998)**.

1.2.2 Delivery and storage of substrates

Delivery: Substrate preparation is the first process step in biogas production. In biogas plants that are integrated into agricultural or food-producing operations for the utilisation of residues, the transport is carried out internally. If substrates are produced off-site, they are delivered in substrate-specific containers. The quantity and quality of the substrates are recorded in the delivery area **(Persson *et al.*, 2019)**.

Non-farm liquid substrates are delivered in tankers, liquid manure drums and silo vehicles. Weighing is one way of recording quantities. Alternatively, the incoming material flow can be measured by means of a volumetric flow meter. The closed construction of the transport vehicles reduces odour emissions. Filling the substrate also produces few odour emissions, as it is pumped into closed receiving containers via a hose connection. In the case of open unloading processes, e. g. intake via an open bottom flap, the closed system (transport and storage) is interrupted, and odours are formed **(Nwokolo *et al.*, 2020)**.

The delivery of solid substrates depends on the type of substrates. Bulk agricultural substrates are transported using standard agricultural transport technology. Other bulk substrates, e. g. biowaste, are transported in container vehicles, foodstuffs can be delivered on pallets or in cartons. The simplest way to record incoming substrates from outside the farm is to count the delivery vehicles at defined transport volumes, which allows a rough estimate of the amount of material. Precise measurement of incoming substrates is ensured by weighing equipment. These can be external or installed directly in the biogas plant. Silage can be recorded after storage by measuring the silo body. Agricultural substrates are mostly transported in mobile silos; the resulting odour emissions and dust developments can be considered marginal. Agricultural substrates that have to be stored dry are stored in high silos **(Koch *et al.*, 2017)**.

Storage: Substrate storage allows a steady substrate supply. The substrates should be stored with the lowest possible energy losses and without any harmful impact on the environment. There are great differences in local rules and regulations, especially with regard to proper storage in terms of emissions, which must be taken into account during planning.

The stored biomass contains large amounts of chemical energy. For the full utilisation of a plant with a electrical output of 500 kW, about 9,500 tonnes per year of corn silage must be stored **(Amon *et al.*, 2015)**. For substrate storage in biogas plants, there are solid manure storage facilities, silage camps or flat bunkers, tower silos (dry bulk silos), tower silos for moist preservation, foil tube silos, bale silos, discharge bunkers and reception halls **(Persson *et al.*, 2019)**. In the following, the receiving halls, the silage camps and the tower silos will be dealt with in more detail. As an alternative to storage on the farm premises, temporary silo facilities can be built directly on the field. However, these field silos are not permissible in many areas, as degradation products are released unhindered into the environment **(Thaysen, 2010)**.

Receiving halls: Halls for receiving substrates are technically complex and costintensive; they are only used in very special cases, for example, when substrates release special emissions or may not be stored openly, for example for the storage of waste. The storage area is determined by the expected substance quantities and storage periods. If problematic substances are delivered as co-substrates from industrial origin, they must be stored in a receiving station separate from the agricultural operation. Before hygienization of the corresponding substrate, it must not be mixed with harmless substrates. To minimise emissions, the storage should be closed. The exhaust air should be able to be discharged in a targeted manner and, if necessary, fed into an exhaust air purification system. To prevent odours from escaping, such halls are equipped with a negative pressure system which, in addition to airwash, prevents odours from escaping. Halls offer the advantage that the technology used is protected from the weather, and the same applies to the performance of maintenance work. The enclosure in halls makes it possible to comply with noise protection legal requirements and regulations. The employees in the halls must be protected from hazardous gases. This applies in particular to hydrogen sulphide (H_2S). Protection can be achieved through the targeted discharge of gases from storage containers before staff access and through technical equipment such as warning alarms **(Fachagentur Nachwachsende Rohstoffe, 2013)**.

Silage camps: Silage camps are surface structures that are open to the atmosphere and used in biogas plants. Silage camps are used as storage for stackable biomass and bulk material. With regard to the planning requirements, the quality assurance of the silage must be ensured, and the negative environmental influences must also be minimised as far as possible. The silage camps system should be designed in such a way that the stored biomass can be sufficiently compacted. It must be possible to seal the biomass airtight by means of a cover. Fermentation juice/leachate (produced by hydrolysis and lactic acid fermentation) and rainwater must be able to be discharged separately in some cases. Biomass losses due to leachate formation should be minimised. No biomass should be discharged uncontrolled into water bodies. The silo walls cannot be built to any height, as the amount of fermentation juice increases significantly from a stack height of five to six metres. For high silage quality, professional placement of the biomass is indispensable, as is ensuring sufficient compaction and covering with a suitable foil/cover. It is recommended to divide the silos into chambers, which facilitates the professional and low-emission management of the silos. To allow good access, it is recommended that the chambers measure at least four times the width of the vehicle in the transverse direction. The gating surfaces should be designed to minimise solar radiation. The silo plate must be liquid-tight and resistant, no surface water from the surroundings may flow into the plate. Fermentation juice, leachate and contaminated rainwater must be collected within the floor slab

and must not flow next to it. The walls must be impermeable to liquids. Fermentation juice, leachate and contaminated precipitation water are collected in a container and pumped from it into the fermentation tank or into the digestate storage tank **(Postel *et al.*, 2009)**.

Tower silo: In addition to agricultural use, tower silos are also used in the biogas plant sector. They are usually placed outdoors, but smaller containers can be placed in halls or buildings. For biogas plants, they serve as storage for grain, maize grains or corn-cop mix (CCM). They serve as a template for the addition of energy-rich substrate. They are less common than mobile silo systems. A distinction is made between dry silos and high silos for moist preservation. In ventilated dry silos, relatively dry substrates such as cereals and grain maize are stored, while in high silos for moist preservation, substrates such as crushed grains are mixed with water and preserved as a pumpable slurry under exclusion of air. High silos can be considered emission-free. The equipment often consists of cylindrical containers with bolted steel segments with a roof, extraction hopper and the conveying equipment **(Persson *et al.*, 2019)**. Alternatively, the container materials consist of plastic reinforced with glass fibre, fabric or concrete **(Persson *et al.*, 2019)**. For the construction of high silos, it is essential to build a load-bearing foundation. Blowers, screw conveyors, elevators and pumps act as filling and removal equipment. In addition, elevated silos have crushing technology and thus have a high degree of automation with regard to the storage of the substrate. The conveyor technology transports the substrates directly into the fermenter or into a feed tank/mixing tank. High silos can optionally be attached to weighing cells; this is helpful for precise metering and level measurement. The advantage of this plant component is the low space requirement and the highest possible degree of automation, which results in low labour costs when using high silos. The disadvantage of dry silos is that the storage time depends on the moisture content of the grain and the high purchase costs **(Thaysen, 2010)**.

Figure 5: Silage camps (Alberle, 2016).

1.2.3 Substrate feeding

Distinction is made between pumpable substrates such as liquid manure and stackable substrates such as corn silage. Substrates with a low dry matter content (DM content) of approx. 4% - 15% are particularly suitable. At less than 4%, economic utilisation by means of mono-digestion is difficult, as much non-utilisable water is contained. Above 15%, the substrate usually has too high viscosity, so that the stirring and pumping is no longer possible. Feeding can be

Figure 6: Tower silo (Photo by AEV Energy GmbH, Biogasplant Ketzin, Germany)

done continuously, quasi-continuously and discontinuously. Substrate feed can, for example, be carried out with the help of feed systems such as a solid's feeder with elevators, feed screws, feed shafts, preliminary pits and pipelines **(Piekutin *et al.*, 2021)**.

Liquid feedstocks: The following criteria must be observed when dimensioning pumps **(Kissel *et al.*, 2014)**:

- Medium (composition, dry matter content, pH value and temperature).
- Geodetic transport height.
- Pipeline (diameter, length and number of fittings).
- Volume flow.
- Tank (diameter and height)

The substrate feeding is usually carried out with a pump for liquid substances. The following pumps are usually used (**figure 7**):

Only small amounts of solids may be conveyed when using pumps. The substances are added via a preliminary pit. A rod mixer or a submersible motor propeller unit is usually installed in this. Ideally, the digester should be fed continuously; this guarantees a stable fermentation process. In practice, the fermenter is fed quasi-continuously, in short time intervals at regular intervals, with a feed quantity that is as constant as possible. The pumping process is usually fully automated. Slurry contains litter as well as feed residues, so the pumps for substrate delivery must have special characteristics **(Biomin, accessed 18.10.2021)**. In agricultural biogas plants, mainly centrifugal pumps and positive displacement pumps are used to convey the liquid input material. To protect the pumps, they are often preceded by cutting and crushing devices and foreign body separators. The pumps must have a high capacity to avoid clogging. **Table 5** shows the design of the pumps and their characteristics in comparison.

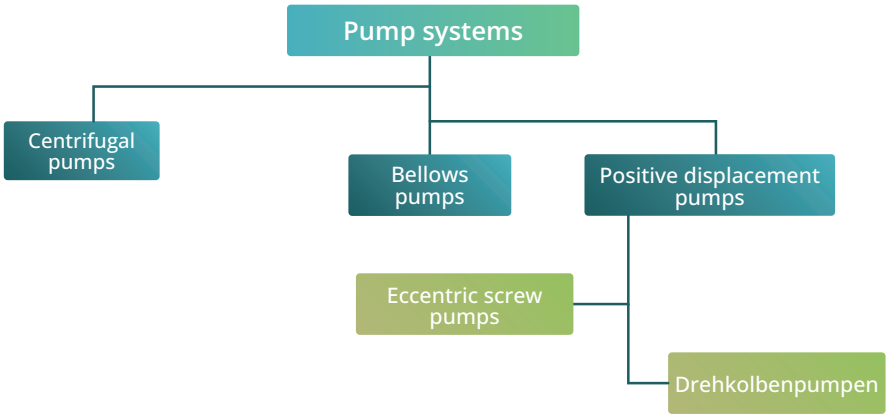


Figure 7: Pumps for liquid feeding.

Centrifugal pumps have a relatively simple technology, they are robustly built and are used for substrates with a dry matter content of 0% - 12%. The delivery rate of this type of pump depends on the delivery head. The flow rates vary between 2 and 6 m^3/min, with a power consumption between 3 and 30 kW **(Effenberger and Lebuhn, 2011)**.

Positive displacement pumps are used when viscous materials need to be pumped. The quantity pumped can be determined by the number of rotations. This theoretically increases the process stability and the control of the system. Positive displacement pumps are more susceptible to faults than centrifugal pumps; it is advisable to connect a macerator in front of the pump to protect it by crushing to coarse organic matter **(Roskatski, 2019)**.

Bellows pumps are particularly suitable for viscous pumpable substrates with high levels of impurities. They are more robust than positive displacement pumps and insensitive to dry running, but only pump very small quantities **(Roskatski, 2019)**.

Eccentric screw pumps (see **figure 8**) can also be used to pump viscous substrates; in contrast to bellows pumps, only a small proportion of impurities may be present. Furthermore, eccentric screw pumps are sensitive to dry running. However, they can self-primate from a depth of up to 8.5 m and reach a discharge pressure of 24 bar; the delivery rate is generally lower than that of centrifugal pumps **(Kissel *et al.*, 2014)**.

Figure 8: Eccentric screw pump of the company Armatec FDM GmbH & Co. KG (accessed 13.10.202).

Rotary lobe pumps can also be used; they are less sensitive to impurities than the eccentric screw pump, have a high delivery rate and are characterised by a simple design **(Biomin, accessed 18.10.2021)**.

Solid feedstock: The solid substrates can also be introduced via a feed or conveying screw. In this case, the solid material is pressed below the liquid level in the fermenter by tamping screws. This prevents gases from escaping (**figure 9**).

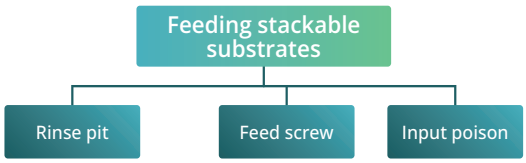


Figure 9: Feeding of stackable substrates.

Table 5: Pump comparison (BioBG, accessed 25.10.2021).

Type	Centrifugal pumps	Positive dispalcement pumps
Construction methods	Submersible centrifugal pump	Eccentric screw pump
	Centrifugal immersion pump	Rotary lobe pump
Properties	High flow rate, low pressure, not basically self-priming	Meterable, self-priming, constant pressure, also for viscous materials

Another possibility is to introduce the solids by means of a piston (**figure 9**). A hydraulically operated feed cylinder presses the substrates into a pressure channel that is below the liquid level of the fermenter (Hopfner-Sixt, *et al.*, 2007). After the substrates have been introduced, the channel is closed again by a hydraulic slide valve. The introduction of the solids by means of feed pistons enables automatic dosing at any time intervals. With this method, there is a risk that the substrate introduced will form a sink layer and clump together, which means that it is no longer optimally degradable for the microorganisms (Kamarád *et al.*, 2013).

Containers with and without crushing tools are used to feed the feed screws and feed pistons (Kissel *et al.*, 2014). Size reduction increases the substrate surface for microbiological degradation, which has a positive effect on methane production. The higher the comminution, the faster the degradation takes place, but the gas yield does not necessarily increase (Naegele *et al.*, 2014).

1.3. Anaerobic Digesters

The digester is the main component of a biogas plant. In this reactor, microorganisms convert organic biomass into biogas. The digester must provide optimal growth conditions for the microorganisms to ensure the highest possible biogas production. Essentially, a digester consists of a fermentation tank, which is thermally insulated, a heating system, mixing units and, if necessary, discharge systems for sediments and the fermentation substrate (Fachagentur Nachwachsende Rohstoffe, 2013). Biogas plants can be divided according to the type of fermentation process into continuous wet fermentation, continuous dry fermentation and discontinuous dry fermentation (Effenberger and Lebuhn, M., 2011). In 2016, an operator survey (reference year 2015) was conducted from 310 samples (Nwokolo *et al.*, 2020). The result of the survey can be seen in **figure 10**. The most commonly used reactor types were stirred tank fermenters, followed by multistage systems in which the stirred tank fermenter is followed by another reactor type (usually as a secondary fermenter). With a three percent share, the plug flow reactor was named second most frequently. Newer reactor types such as ring-in-ring solutions or those operated according to the double-chamber process (Pfefferkorn principle) were only rarely mentioned. The small number of special processes can be attributed to the low rates of expansion of biogas plants after their market saturation (Fachagentur Nachwachsende Rohstoffe, 2013).

1.3.1 Types and designs of containers

General construction types of containers: Fermenter designs can be differentiated according to their geometry (round, angular, horizontal, or vertical), their mixing (stirred tank, plug flow), their process requirements (parallel, serial), their biological arrangement (single-stage, multi-stage) and their constructional design.

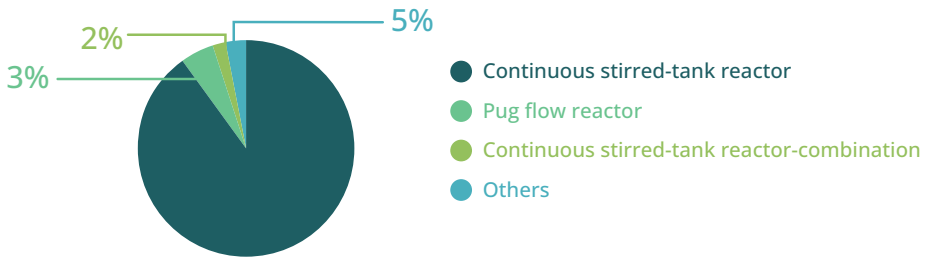


Figure 10: Percentage distribution of the fermenter systems used (operator survey 2016, reference year 2015) (Adapted from Planet Biogas, accessed 07.10.2021).

In digesters with complete mixing, the substrate fed in is distributed evenly over the entire contents of the digester. When fresh substrate is added, the corresponding amount of fermentation substrate is removed from the reactor.

With plug flow, the substrate is fed to the beginning of the reactor. A defined amount of substrate fed in will theoretically not mix with another defined amount until the end of the reactor. In practice, however, this does not apply due to recirculation flows.

In batch digesters, neither mixing of the biomass nor continuous feeding of further substrate is necessary **(Samer, 2012)**. **Table 6** shows a comparison of the properties of different fermenter technologies.

Biogas reactor with complete mixing (storage-flow process): As shown in **figure 10**, stirred tank fermenters are the most common type, with a share of 90%. They are continuous stirred tank reactors (CSTRs) and are mainly used in the field of agricultural biogas production. They are fully mixed reactors in cylindrical upright design. A scheme of a typical CSTR is shown in **figure 11**.

The CSTR reactor is characterised by its simple, robust and reliable technology. **(Weiland, 2010)** CSTRs are designed to maximise the contact between the biomass and the substrate, which increases the fermentation performance. Feeding is automated to the greatest possible extent. The digester is designed as a tank with a concrete floor and walls made of steel or ferro concrete. It can be fully or partially sunk into the ground or built completely above ground **(Mutungwazi *et al.*, 2018)**. Foil roofs or concrete ceilings are built on top of the tanks for gas-tight covering. Mixing is achieved with one or more agitators located in or on the reactor. The agitators are arranged either centrally or laterally. Hydraulic fermenters without agitators are also possible. The mixing devices must be very efficient. Agitator systems are discussed in more detail in *chapter 1.3.3*. A size up to 6,000 m^3 is possible. As the size increases, mixing and process control become increasingly demanding. In principle, all substrate types can be introduced, ideally pumpable substrates with a low to medium dry matter content. This type of fermenter is basically suitable for continuous, quasi-continuous and discontinuous feeding. Advantages are the low-cost construction, the variable operation as flow-through or flow-through-storage system and low maintenance efforts, which can be carried out without emptying the fermenter. Disadvantages are high costs due to energy-intensive agitators, short-circuit flows (parts of the fresh substrate can get into the outlet) as well as floating blankets and sink layers **(Fachagentur Nachwachsende Rohstoffe, 2013)**.

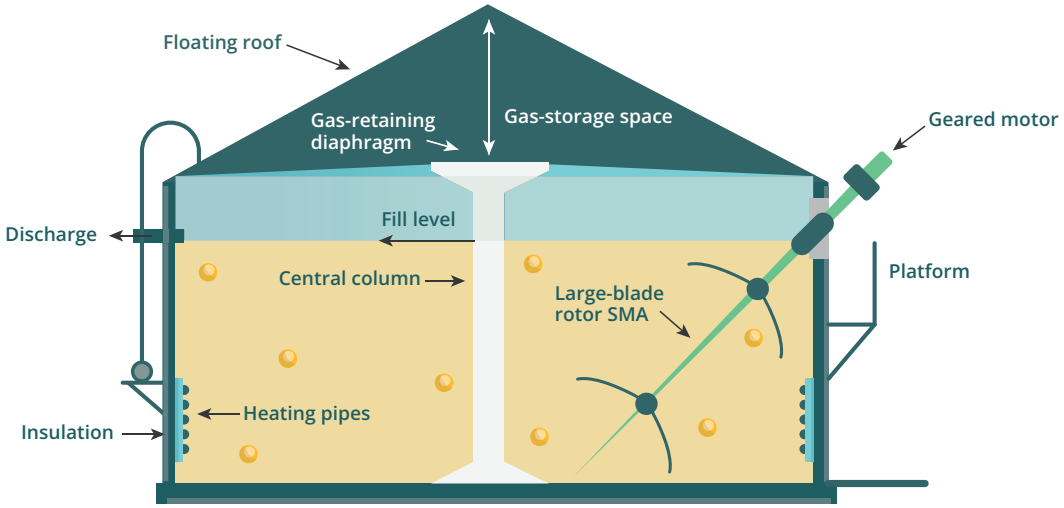


Figure 11: Stirred tank reactor with long shaft agitator (Adapted from Energypedia, accessed 15.10.2021).

Plug flow biogas reactor: Plug flow fermenters are designed in the form of horizontal pipes with a round or rectangular cross-section. They use the displacement effect of the substrate to achieve a plug flow through the fermenter. The substrate is continuously fed into the reactor via the substrate feeder (usually with a screw pump). The feed device creates a pressure that guides the substrate through the reactor chamber. The plug flow leads to a separation of the fermentation stages within the tank. The schematic construction is shown in **figure 12**. Agitators are needed for mixing. Special agitators are needed due to high content of solids, mostly slow-running paddle shafts, which cause mixing across the direction of flow (direction of movement of the substrate), but not in the longitudinal direction. There are horizontal and vertical plug-flow fermenters; horizontal reactors are mostly used in agriculture. Standing reactors are rarely used; they also exist in designs without agitators **(Veluchamy *et al.*, 2019)**. Standing reactors use gravity and pumps to mix the biomass.

Plug flow fermenters are mostly made of steel or stainless steel as well as ferro concrete. The size of horizontal digesters is about 800 m^3 for horizontal digesters and up to 2500 m^3 for vertical digesters. For economic reasons, horizontal reactors made of steel or stainless steel are usually only manufactured up to a volume of 300 m^3; in these size regions, the advantages of the compact and cost-effective design outweigh the disadvantages. The fermenters are built in the factory and transported to their place of use, which limits the size of the tanks **(Fachagentur Nachwachsende Rohstoffe, 2013)**.

The tanks are thermally insulated, often offer several devices for gas extraction, substrate connections as well as digestate discharge, agitator, and heating pipes. Heating coils can be integrated into the paddle agitators.

Biogas reactors with plug flow are suitable for wet fermentation, mostly pumpable substrate with high dry matter content is used. Quasi-continuous or continuous feeding of the tanks is envisaged **(Veluchamy *et al.*, 2019)**. The compact design is an advantage, which is why these digesters are preferably used in small plants. Due to the construction, floating ceilings, sinking layers or sedimentation of solids are usually avoided. The retention times are usually adhered to and can be determined relatively accurately, as short-circuit flows cannot occur as with other reactor types. Due to the small size, there is less heat loss, and the reactor can be heated effectively. The small size of the fermenters is a disadvantage, and the fermenter must be completely emptied for maintenance work. The process stability is lower than with stirred tank fermenters, and the operating and construction costs are usually higher **(Edelmann and Engeli., 2015)**.

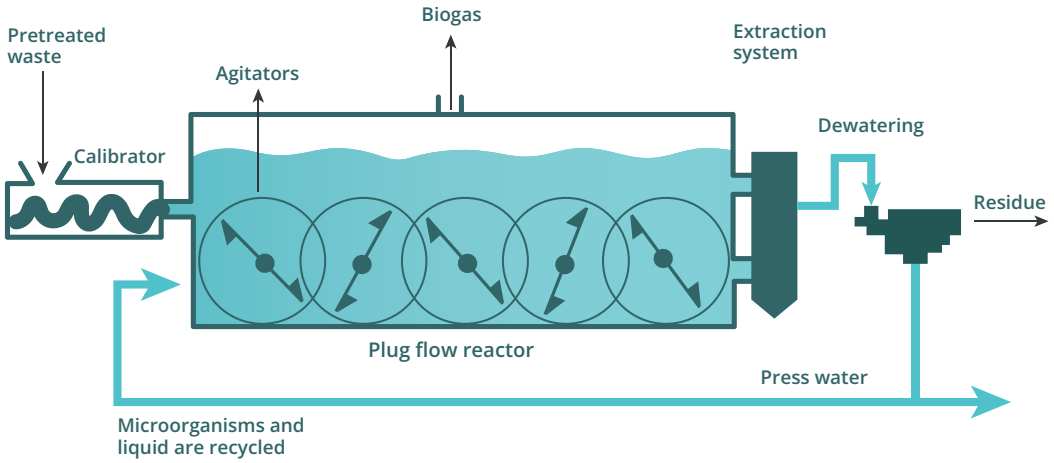


Figure 12: Plug flow reactor (Adapted from Persson *et al.,* 2019).

Leachbed processes: For discontinuous operation, so-called boxes or garages are used. These can usually be opened via a gate and emptied and filled directly. The box fermenters are made of ferro concrete or steel. In practice, several containers are connected in parallel and operated at different times to be able to operate the downstream plant continuously. Two to eight units, usually four units, are used in parallel. This enables quasi-continuous gas production. Box digesters are particularly suitable for dry fermentation, often using pourable substrates such as corn and grass silage. This type of reactor is particularly advantageous for substrates that cannot otherwise be handled, such as organic waste with impurities (Qian *et al.*, 2016). **Figure 13** shows the process of a box fermenter from BEKON GmbH.

In leachbed processes, the fermenters are filled with substrate, sealed airtight and only opened after outgassing. Both the filling process and the emptying are usually carried out with a wheel loader (Clarke, 2018). The ratio of fresh material to inoculation material is 40% to 60%. This ensures that the substrate is supplied with sufficient anaerobic bacteria. For humidification, the substrate is sprayed with percolate (leachate of the substrate, which is recirculated) with nozzles attached to the ceiling throughout the entire residence time. The percolate migrates through the substrate, is collected, and pumped into a storage tank. The temperature of the biomass is controlled by means of attached wall and floor heating. Gas collection pipes are attached to the fermenter, in which the biogas is collected and discharged (Fu *et al.*, 2018; Weiland, 2010).

Leachbed digesters are usually single-stage processes in batch operation and the various degradation reactions (hydrolysis, acid and methane formation) take place in one process step.

Double chamber process (Pfefferkorn principle): Another approach to wet fermentation is the use of double-chamber processes. A biogas plant based on the Pfefferkorn principle (after the Austrian inventor Herbert Pfefferkorn) is a plant that works without an agitator in the fermenter. The reactor contains a main fermentation tank with an enclosing secondary fermentation tank with hydraulic mixing. This process eliminates the need for agitators, which saves electrical energy. In addition, the maintenance effort is reduced, as no agitator units must be changed or serviced, which also saves costs. The substrate used consists

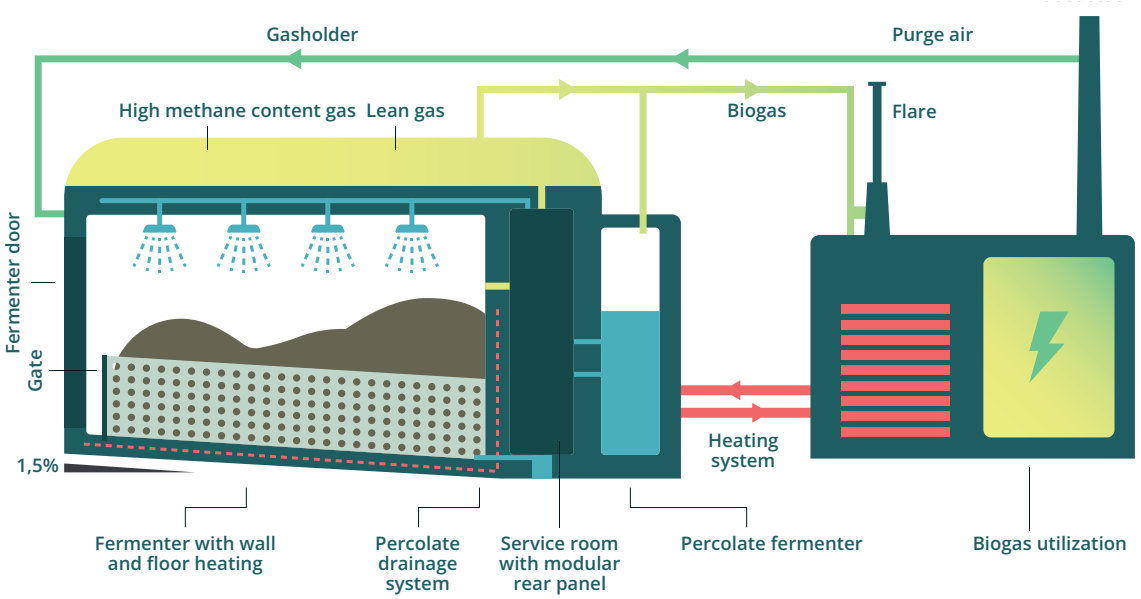


Figure 13: THE BEKON PROCESS, Process of a box fermenter (Adapted from Bekon gmbH, accessed 18.10.2021).

mainly of liquid manure and small amounts of corn silage and grain meal. The construction effort is much higher than with stirred tank fermenters. The fermenter volume is between 400 and 6,000 m^3 **(Fachagentur Nachwachsende Rohstoffe, 2013)**. Mixing takes place through pressure surges. The pressure surges are generated when the gas at the end is not extracted but displaces the liquid phase and thus leads to different filling heights of the two tanks. Due to a sudden pressure equalisation, the filling levels then balance out abruptly. A major disadvantage of the system, apart from the high construction costs, is the enormous technical effort required to deal with any blockages that may occur.

Table 6: Pump comparison (BioBG, accessed 25.10.2021).

Reactor	Feeding	Temperature	Stirring	Substrate	Reliability
Stirred tank reactor	Continuosly	Mesophilic or thermophilic	Flow-through fermenter with agitators, hydraulic fermenter without Agitators	Easy to pump, Use for various substrates	Foreign substances can cause technical problems
Plug flow reactor	Continuosly	Usually thermophilic, also mesophilic possible	Longitudinal or trans-verse to the flow, vertical systems with-out agitators	Pumpable, mainly used for municipal biowaste	High toler-ance to Foreign substances
Box fermenter	Dis-continuosly	Mostly mesophilic, also thermophilic possible	No agitators, Percolation system	Stackable, mainly used for municipal organic waste	Robust fermenter without moving parts

1.3.2 Construction types of tank heaters

To ensure a stable fermentation process, there should be no temperature fluctuations in the fermenter. Temperature fluctuations must, therefore, be kept low in terms of temporal temperature fluctuations and as well in respect to temperature gradients within the fermenter. Excessive fluctuations as well as exceeding or falling below certain temperature limits can inhibit the process or even cause it to come to a standstill **(Han *et al.*, 2020)**. According to the German agency for renewable resources **(Fachagentur Nachwachsende Rohstoffe, 2013)**, temperature fluctuations can lead to:

- The supply of fresh (cold) feedstock
- Temperature stratification or temperature zone formation due to insufficient thermal insulation, ineffective or incorrectly dimensioned heating, insufficient mixing
- Location of the heaters
- Outside temperatures
- Failures

External or internal heat exchangers or heaters provide the required process temperature. They compensate for heat losses and heat the substrate. Accordingly, heating technologies can be divided into system-integrated and external systems.

Integrated heating: For the integrated heating solutions, a distinction can be made between wall heating, floor heating and heated stirring unit. Wall heating is carried out by installing heating pipes inside the tank in the wall as well as on the outer wall **(Persson *et al.*, 2019)**. Stainless steel pipes, PVC or PE are used as material for the pipes. Stainless steel pipes have a better heat transfer than plastic pipes, turns can be saved, but they are also more expensive. In the tank, the use of stainless-steel pipes means that less heating surface is required, which in turn reduces the flow resistance. Plastic pipes are installed at the inner tank wall or inside the wall (**figure 14**). **Table 7** shows an overview of integrated heaters. **Figure 14** shows different types of integrated heating systems.

External heat exchanger: When using external heat exchangers, the substrate is heated outside the fermenter. This can be done in wet fermentation plants by heating in circulation or in the substrate feed **(Persson *et al.*, 2019)**.Heated percolate can temper the fermentation substrates in solid-state fermentation plants. **Table 8** shows an overview of external heat exchangers. External heat exchangers can be used to avoid temperature fluctuations during substrate introduction.

Figure 14: Stainless steel heating pipes installed in the digester (inside) (left); installation of heating hoses in the digester wall (centre, right) (Fachagentur Nachwachsende Rohstoffe, 2013).

As a rule, stainless steel is used as material for heat exchangers, and they are usually designed as spiral or double-tube heat exchangers. External heat exchangers are basically suitable for all types of fermenters; they are often used in plug-flow fermenters. Heat exchangers are well suited for thermophilic operation. External heat exchangers ensure good heat transfer, the entire biomass volume is reached by the heating. They can be easily cleaned and maintained and offer good temperature controllability **(Fachagentur Nachwachsende Rohstoffe, 2013)**.

1.3.3 Stirring systems

Functions of the stirring systems: According to **Kissel *et al,* (2014)**, agitators must fulfil the following tasks in biogas plants:

- Homogenization of the ingredients, this enables a uniform conversion of substances for the formation of biogas.
- Suspension of heavy inorganic substances that are introduced via the substrate.
- Reverse suspension: light, floating substances must be fed back into the bulk phase at varying levels. This serves to avoid the formation of swimming layers.
- Heat exchange in the container, uniform temperature distribution throughout the fermenter room for uniform material conversion.
- Complete circulation flow without demolition and formation of stagnant zones, for the discharge of biogas and even distribution of freshly introduced substrates.

Table 7: Tank heating integrated (Postel *et al.,* 2008).

Type	Description	Range of application	Advantages	Disadvantages
Wall heater	Inside of tank	All types of fermenters, rather standing	Pipes accessible for work, e.g. for lime deposits, very good heat transfer	Flow resistance, deposits, risk of tearing
	In the tank wall, heating hoses	In-situ concrete, not segment construc-tion	Pipes protected	Temperature gradient in wall (stress cracks), reduced heat transfer
	Container exterior	Steel tank only	Pipes easily accessible	Reduced heat transmission
Floor heating	Common floor heating cation lines	All standing fermenter	Longitudinal or transverse to the flow, vertical systems without agitators	Low heat transfer, especially with sink layer formation
Heating in stirring unit	Double wall pipe system	All digester types, rather horizontal	No agitators, Percolation system	Damage to welds difficult to detect, less heat transfer during standstill

Table 8: External tank heating (Postel *et al.,* 2008).

Type	Description	Range of application	Advantages	Disadvantages
Heating in circulation	Circulation of the fermenting liquid, e.g. via double-pipe or spiral heat exchanger	Wet fermentation	Tanks and walls are free of fix-tures	Increased electrical energy consumption due to pumping; deposits possible
Heating in the feed	Design of the inlet as a double-tube or spiral heat exchanger	Wet fermentation	Flexible handling, e.g. combination with hygienization	More elaborate tank insulation required; additional heating may be necessary; storage possible
Heated percolate	From temperature-controlled percolate storage for trickling over digestate in the solid's fermenter	Solid's fermentation	No or low-power heating devices required at the solid's fermenter	Carbonates in percolate liquid clog pumps, pipes and nozzles

- Uniform ground flow.
- Avoidance of blockages on the agitator.

The design of the agitators is individual for each plant; there are no uniform concepts, as the ingredients and the process control vary depending on the plant. The technical requirements for the agitator can be subdivided according to the process. Biogas from wastewater plants can be classified as technically rather simple, since the solids content ranges between 3% - 6% **(Gomez, 2016)**. The solids content in biogas plants is considerably higher, up to 22% **(Krieg *et al.*, 2016)**. The technical effort to realise an effective agitation system is higher. In the following, some frequently used agitators are discussed in more detail.

Submersible mixer (propeller): This type of agitator is very common and has been used in biogas plants for a long time. Submersible propeller agitators are used in fully mixed fermenters as well as in digestate stores. In the fermenter, this type of agitator is often combined with other types of agitators **(Mohammadrezaei *et al.*, 2018)**. It is also possible that several agitators are operated in one tank.

The propeller and the electric motor form a unit that is completely immersed in the medium. Therefore, the entire agitator unit must be watertight and corrosion-resistant. Attached to a vertically arranged guide rod, the height of the agitator can be adjusted. The direction of action of the propeller can be changed by means of a crank, and the agitator unit can also sometimes be swivelled up and down by 30°. This makes them easily adjustable in position and is good against floating covers and sinking layers **(Brehmer *et al.*, 2016)**. The fermentation substrate cools the motor in the submersible mixer. In thermophilic fermenters, one has to verify that the cooling capacity is sufficient **(Bauer *et al.*, 2019)**. Submersible mixers are counted among the fast-rotating agitators, they are equipped with small agitator blades (Ø 400 - 1000 mm) and have propeller speeds of up to 600 rpm. Due to the high speed, submersible mixers generate a large directional thrust, which is suitable for mixing low-viscosity fermentation substrates. The agitators have an output of between 0.25 and 35 kW. For fermenters between 24 and 28 m in diameter, common sizes are between 12 and 18 kW **(Persson *et al.*, 2019)**. With viscous media, such agitators only function to a limited extent; an effective mixing effect is only achieved with long agitation times. Viscous media damage or wear the agitator blades very quickly, which is why they should not be used in this application. For higher viscosity, modified submersible mixers are available, which have larger agitator blades (Ø >1000 mm) and reduced rotational speed. There are also special designs of submersible mixers that are driven hydraulically and not electrically, and these are operated by hydraulic units, which are installed outside the fermenter **(Bauer *et al.*, 2019)**.

Submersible mixers are well suited for fermentation tanks with variable filling heights. However, the agitator blades must be completely submerged during operation to prevent imbalances. An advantage is that submersible mixers can also work at very low fill levels. Fast-running submersible mixers are better suited for stirring floating layers than slow-running submersible mixers, as they have a higher suction and pushing effect. The disadvantage is the relatively high energy demand, and that moisture can penetrate the motor.

Rod mixer (Figure 16 & 17): Like submersible mixers, rod mixers have long been used in biogas plants. They are used in fully mixed fermenters with a constant liquid level and are often combined with other agitators **(Mohammadrezaei *et al.*, 2018)**.

For low-viscosity media, the combination of submersible mixers and high-speed rod mixers has proven successful. For viscous fermentation substrate, paddle or long axis agitators are combined with slow-running large blade rod mixers. Propellers are attached to a drive shaft of the agitator, the number of which can vary. In the field of biogas production, rod mixers are driven by electric motors located outside the fermentation tank. Compared to submersible mix-

Figure 15: High-speed submersible mixer with cable guide on the suspension cable (Kissel *et al.,* 2014).

ers, they require less maintenance and have a longer lifetime, which reduces costs. Like submersible mixers, rod mixers are fast agitators. The diameter of the agitator blades is relatively small (<700 mm) and the speed of the propellers is high (up to 600 rpm). There are also other types of hand blenders with slower speeds (<100 rpm) and larger blades (Ø > 1200 mm). These slow-running stick mixers are used in the fermentation of substrates like corn or silage, which leads to an increase in dry matter content and viscosity **(Kissel *et al.*, 2014)**.

By changing the direction of rotation, the mode of action of the agitator can be changed from pushing to pulling. The shape of the agitator blade, the orientation of the agitator and the direction of rotation must be adjusted.

The rod mixers are mounted in the upper area of the fermenters, either guided through the fermenter wall or, in the case of a concrete ceiling, through the fermenter ceiling. To be able to counteract the formation of floating layers, the rod mixers are designed to swivel hydraulically, and their inclination can be varied **(Bauer *et al.*, 2019)**. There are multiple scenarios on how rod mixers are applied. For example, there is scenario, were sinking layer are avoided by mounting the mixer close to the floor. For

Figure 16: High speed rod mixer with smaller propeller (Kissel *et al.,* 2014).

this specific case, short agitator shafts are used, which are not swivelling. Compared to the submersible mixers, however, swivelling stick mixers are limited in their adjustment possibilities and cannot, for example, work tangentially to the container circumference.

Long-axis agitators (figure 18): This type of agitator was specially developed for the use in biogas plants. It is well suited for fermentation substrates with a high viscosity and for mixing viscous media. Due to their low speeds, they are not suitable for stir-

Figure 17: Wall- and ceiling-mounted rod mixer with hydraulic (left) and mechanical (right) (Kissel *et al.,* 2014).

ring floating blankets at variable filling levels and for thin-bodied fermentation mixtures. Long-axis agitators are combined with slow-running large vane mixers or large vane submersible motors to achieve a good agitating result **(Krieg *et al.*, 2001)**.

Due to their large blade diameters (>1.5 m), long axis agitators manage with low speeds (<40 rpm) for effective mixing. Long-axis agitators are mounted on the tank bottom as well as on the digester wall/ceiling. On tanks with a concrete ceiling, they are guided through the ceiling. In the case of fermenters with an attached gas storage and a weather protection foil, they are guided through the fermenter wall **(Bauer *et al.*, 2019)**. The motor unit of the agitator is mounted outside the fermenter/digester, which makes it easy to maintain. The speed of the agitators is adapted to the respective requirements with frequency converters. As soon as the medium is in motion, the agitator can switch to partial load operation, which means lower energy consumption **(Bauer *et al.*, 2019)**.

Paddle agitators (figure 19): Like the long-axle agitators, this type of agitator does not originate from slurry technology but was developed for the fermentation of renewable raw materials and high dry matter content in the fermenter. Paddle agitators are available in vertical and horizontal versions.

Figure 18: Wall- and ceiling-mounted rod mixer with hydraulic (left) and mechanical (right) (Kissel *et al.,* 2014).

Horizontal paddle agitators: This type of agitator is mainly used in fully mixed fermentation tanks with a liquid level that is as constant as possible. They are rarely used in fermentation residue storage. In highly viscous media, they are combined with slow-running large vane rod mixers or large vane submersible motor agitators. Highly viscous media are present in fermenters that have a low proportion of liquid and a high proportion of solids. If the agitator shaft is at the same level as the substrate level, the direct effective radius is a paddle length.

Paddle agitators are usually very slow-running agitators, they run at a low speed of 1 - 20 rpm. The agitator shaft is equipped with large-dimension paddles, it is guided horizontally through the wall and mounted on a rod mounted in the middle of the fermenter. The drive motor is flanged to the agitator shaft outside the fermenter and is equipped with a torque stop and a frequency converter. This type of agitator is also easy to maintain in case of malfunctions. Horizontal paddle agitators are susceptible to corrosion processes, as creeping currents can occur in the agitator. To counteract this, care should be taken to ensure sufficient earthing of the agitator shaft.

Vertical paddle agitators: This type of agitator is also called an axial agitator and can be arranged centrically or eccentrically. Standing paddle agitators require a concrete ceiling to be technically attached. They are mounted on the ceiling and on the tank bottom. In most cases, the paddles are rigidly mounted on the agitator shaft and can be arranged in pairs or individually. They are suitable for use in fully mixed fermenters and for homogenising digestates in digestate stores. Vertical paddle agitators are often combined with other standing paddle agitators or other slow-running agitators.

Special forms: There are multiple types of agitators, which occur less frequently than the types listed above. This includes reel agitators, central agitators, hydraulic stirrers, and pneumatic mixers. They have been designed for special applications, which is specified further below.

Reel agitators (figure 20): This type of agitator has a long horizontal agitator shaft to which large agitator arms are attached. Compared to paddle agitators, the agitator shaft extends over the entire fermenter. With longer fermenters, an intermediate bearing is necessary to prevent sagging, which could otherwise lead to damage. Reel agitators have a very low speed of 1 - 5 rpm, they are operated without pause intervals. The drive units are located outside the fermenter **(Weiland, 2010)**.

Figure 19: Suspended paddle agitator with drive unit (Kissel *et al.,* 2014).

The reel agitator was developed for use in horizontal fermenters (plug flow) with high dry matter contents (>14%) in the fermentation mixture. The aim of the agitation process is not to mix the fermentation substrate completely, but to prevent mixing in the horizontal (axial) direction. This causes a spatial differentiation of the decomposition phases. The agitator is not responsible for substrate transport. Instead, the material is transported into horizontal direction due to the material feed (plug flow principle). The advantage of the system is that the fermenter can be loaded more heavily and that short-circuit currents do not occur **(Bauer *et al.*, 2019)**.

A heater can be integrated into the agitator shaft. Heating water flows through the agitator shaft, which heats up the fermentation substrate. The failure of an agitator of this type should be avoided in any case, because the swelling of the fermentation substrate can damage the fermenter or the pipes. In the event of segregation of the fermentation substrate, the power of the motor is usually insufficient to restart the agitator. Warning alarms and an emergency power supply should therefore be installed to prevent any damage **(Bauer *et al.*, 2019)**. Usually, reel agitators are available on the market for round pit fermenters. In these fermenters, they should intensively mix the entire fermenter contents. Difficult substrates can be fermented here, which have a strong tendency to segregate and push other agitators to their limits. Due to the high costs, they are only very rarely used in pit fermenters **(Bauer *et al.*, 2019)**.

Central agitators (figure 21): Like the reel agitator, it is used rather rarely. Central agitators are used for mixing highly viscous fermentation suspensions. To be able to mix effectively, the fermenter geometry must have a certain ratio of fermenter diameter to fermenter height (0.8 to 1) **(Alberini *et al.* 2022)**.

Central agitators are guided centrally through the concrete or stainless-steel ceiling of a high-level fermenter and mounted there. Two to three specially shaped agitator paddles are mounted on the agitator shaft in several levels. The agitator paddles are arranged in such a way that a downward flow is generated near the agitator and an upward flow at the edge of the fermenter. This allows viscous materials to be mixed effectively, and flow breakers are also used in this system to create further turbulence.

Central agitators are operated continuously to prevent the formation of floating layers. The drive unit is located outside the fermenter; for maintenance tasks on the agitator, it can simply be pulled out of the tank by means of a crane. The fermenter therefore does not need to be emptied.

Figure 20: Reel agitator in a horizontal fermenter with drive unit (Kissel *et al.,* 2014).

Hydraulic stirring (figure 22): With hydraulic mixing, liquid is circulated in the fermenter, which should not be confused with the hydraulic drive of the mechanical agitators. Pumpable substrates are particularly suitable for hydraulic mixing. Hydraulic mixing has its origins in wastewater treatment, where mostly low-viscosity sludges need to be heated. The disadvantage of this technology is that floating and sinking layers can hardly be mixed. This technology is mostly used in small standing digesters (<1500 m^3). In compact biogas plants, there is an increase in the hydraulic mixing system. These digesters have a working volume of about 120 m^3 **(Bauer *et al.*, 2019; Persson *et al.*, 2019)**. During hydraulic stirring, liquid with low viscosity from the lower part of the fermenter is drawn off by circulation pumps and pumped to the top of the plant. If pumping is sufficiently long or continuous, this can achieve complete mixing of the fermentation substrate. In direct comparison, mechanical agitators have a considerably higher installation cost. The circulation pump is located outside the tank and is easy to maintain. There are no moving parts in the fermenter that are susceptible to failure **(Weiland, 2010)**.

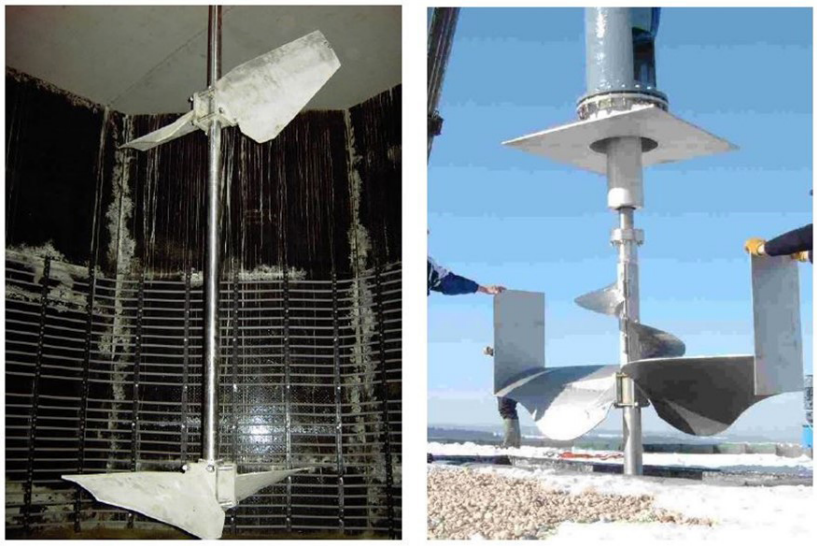


Figure 21: Central agitator in fermentation tank (Kissel *et al.*, 2014).

Pneumatic mixing: With this agitator technology, the fermentation suspension is circulated by injecting biogas into the fermenter from below. Mixing occurs due to the displacement effect of the gas bubbles rising in the fermentation substrate. To achieve this, the hydrostatic pressure of the fermentation substrate must be overcome. Sufficiently powerful compressors and large gas pipes must be available. This technology is very rarely found in agricultural biogas plants. The process originated in wastewater treatment and is used in waste management plants with flowable substrates. The process is rather unsuitable for classical biogas plants, as the dry matter content in the fermentation mixture is too high. Pneumatic mixing is suitable for the exclusive fermentation of pure liquid manure, as the solids content is low enough. A resulting floating layer can hardly be stirred up, especially if substrates containing fibres with a tendency to form floating layers. The disadvantages of the process can be compensated for if they are combined with mechanical agitators. To increase the stirring performance, the gas is introduced into the fermenter via several nozzles. By recirculating the biogas, part of the introduced CO_2 is converted into methane, which increases the methane yield. There are no moving parts in the digester, the compressor is located outside the digester and is therefore easy to maintain **(Bauer *et al.*, 2019; Persson *et al.*, 2019)**.

Figure 22: Centrifugal pump with cutter as circulation pump in the bypass line of a digester (Kissel *et al.,* 2014).

1.3.4 Conveying technology for liquid digestate

For substrate transport within a plant, sufficient mixing and the degree of comminution are the decisive factors. For this purpose, pipelines, fittings and pumps that transport liquids are considered. Gas pipes are dealt with in *chapter 1.4.2* Conveying technology.

Fittings: Manually operated flat gate valves **(Persson *et al.*, 2019)** are often used as fittings for shutting off containers. In smaller biogas plants, manually operated control levers are used to control the material flow. Larger systems are equipped with automatically actuated valves for control. These automatic control valves can be operated pneumatically or electrically. Pneumatic systems are preferred because they are significantly less expensive than electric valves. If the device is located far away from the compressor/compressed air system, electric fittings are used **(Persson *et al.*, 2019)**.

Pipes: Pipes made of steel or plastic are used to transport liquids. For pipes laid underground or in enclosed spaces, PE-HD pipes are mostly used, while stainless steel is used outdoors. Pipes laid underground must usually have a second casing to detect leaks. Plastic pipes are rarely used above ground because they tend to become brittle under UV radiation and require more elaborate support due to their lower mechanical strength. Steel pipes, on the other hand, tend to corrode when laid underground. Steel pipes are considerably more expensive than plastic pipes, which is particularly noticeable in the case of long stretches. Depending on the section of the system, the pipe may need to be thermally insulated, heated or frost-proofed. The pipelines are usually laid in such a way that the occurring operating cases can be controlled via easily accessible gate valves.

Pumps: In biogas plants, pumpable substrates are usually conveyed by pumps driven by electric motors (**figure 24**). Together with the corresponding fittings, they can be controlled via process computers, which automate the process completely or partially. The pumps are often located centrally in one or more pump or control houses. Usually, one pump is used for as many pumping paths as possible. Substrate distributors are used to control what is pumped by which pump.

Even with good substrate preparation, the pumps can become blocked. They should therefore be easily accessible and there must be sufficient working space around them. The moving parts of a pump are wearing parts that need to be serviced or

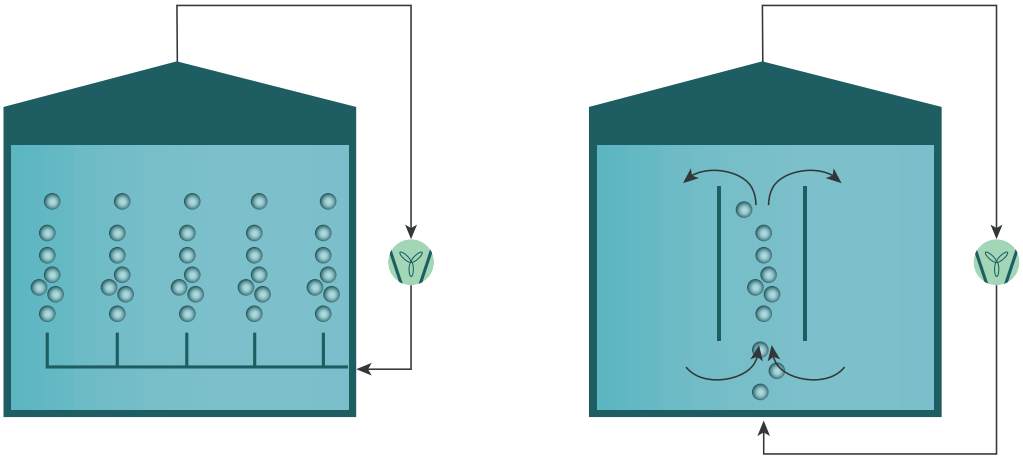


Figure 23: Basic principles for pneumatic mixing; gas spraying (left), gas lift (right) (Adapted from Postel *et al.,* 2008).

replaced regularly without the biogas plant having to go out of operation. It must therefore be possible to disconnect the pumps from the mains by means of a gate valve before carrying out maintenance work. As described in *chapter 1.2.3*, centrifugal or positive displacement pumps are mostly used, which are also used in slurry technology **(DBFZ Deutsches Biomasseforschungszentrum., 2021)**.

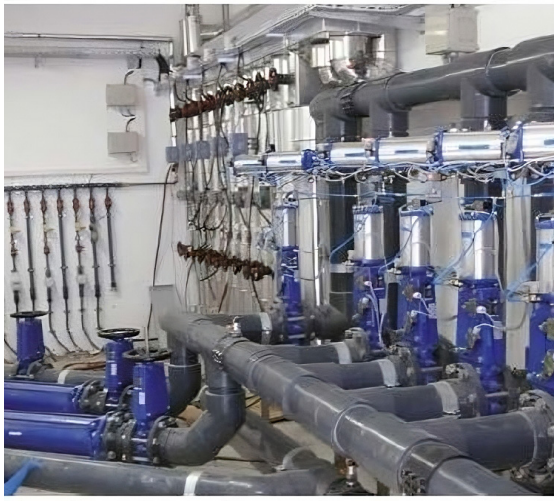


Figure 24: Pumps in a biogas plant [WELTEC BIOPOWER GmbH] (Fachagentur Nachwachsende Rohstoffe, 2013).

Centrifugal pumps: Centrifugal pumps originate, as already mentioned, from slurry technology. Inside the pump, an impeller rotates in a stationary housing. The medium is accelerated by means of the impeller and the resulting increase in speed in the discharge connection of the centrifugal pump is converted into delivery head or delivery pressure. **(Bachmann, 2013)** These pumps are also available in designs whose impeller is equipped with hardened cutting edges for substrate comminution. Common centrifugal pumps deliver a delivery pressure of up to 6 bar, which results in a delivery rate of from 2 m^3/min up to 6 m^3/min. The power consumption is strongly substrate-dependent, but good orientation values are 3 kW for 2 m^3/min and 15 kW for 6 m^3/min. The pumps are generally suitable for a dry matter content of <8%, making them suitable for low-viscosity substrates with a low straw content. Their robust design is simple and compact, they have a high flow rate and can be used flexibly. The disadvantage of this type of pump is that it is not self-priming, which means that the pumps have to be positioned below the level of the substrate to be sucked in. For this purpose, they can be placed in a shaft. Due to the design, there is a high dependency between the delivery rate and the delivery pressure or delivery head. In terms of their design, centrifugal pumps are available as submersible pumps or as dry-installed pumps **(Fachagentur Nachwachsende Rohstoffe, 2013)**.

Eccentric screw pumps: Eccentric screw pumps are self-priming, valveless, eccentri-

cally rotating positive displacement pumps. The pumping principle is based on a corkscrew-shaped rotor that runs oscillating in a stator made of elastic material. The two components are matched to each other, forming a delivery chamber through which the medium is transported. The design of the rotor and stator means that there is hardly any pulsation or shear forces. The medium can therefore be moved continuously. The flow rate can be regulated via the speed, and in combination with a frequency converter, the flow rate can be precisely controlled. The pumps reach a delivery pressure of up to 48 bar and enable a delivery rate from 0.055 m^3/min up to 8 m^3/min. They draw a power of 7.5 kW at 0.5 m^3/min and 55 kW at 4 m^3/min. The power consumption is strongly dependent on the substrate being pumped. The advantage of these pumps is that they are self-priming, have a simple and robust design, are highly meterable and the direction of rotation can be reversed. They have a stable delivery at fluctuating pressures. Dry-running protection can be integrated. The disadvantages are that they have a lower delivery rate than centrifugal pumps, they are sensitive to dry running and they also react sensitively to impurities such as stones or metal particles. The pumps are very durable, easy to maintain and require only a short maintenance period **(Fachagentur Nachwachsende Rohstoffe, 2013)**.

Rotary lobe pumps: Rotary lobe pumps (see **figure 23**) are self-priming, valveless positive displacement pumps. They have two counter-rotating two- to six-bladed rotary pistons. The rotation of the pair of pistons creates a vacuum on the suction side. The pistons do not touch each other. The vacuum draws the liquid into the pump chamber and, by further rotating the pistons, transports it into the pressure area. Rotary lobe pumps can generate a delivery pressure of up to 12 bar. The flow rate is between 0.1 m^3/min and 16 m^3/min. The power consumption is between 2 and 55 kW. They are suitable for viscous, pumpable substrates. They have a simple and robust design, are self-priming up to 10 m water column and suitable for substrate dosing. They convey larger foreign and fibrous materials than eccentric screw pumps, are insensitive to dry running, require little space and are easy to maintain **(Fachagentur Nachwachsende Rohstoffe, 2013)**.

1.3.5 Power2Gas

In the power-to-gas process, electrical energy is temporarily stored in the form of gaseous energy carriers **(Zapf, 2017)**. Excess electricity from renewable energies is used for this purpose and is available when production is greater than demand. The gaseous energy carrier can be ammonia, hydrogen or methane. In the context of biogas technology, the production of hydrogen and methane from hydrogen is interesting. Hydrogen is obtained from water by electrolysis using electrical energy.

- $2\,H_2O \rightarrow 2\,H_2 + O_2$

Hydrogen formation: There are various technical options for realizing this. The best

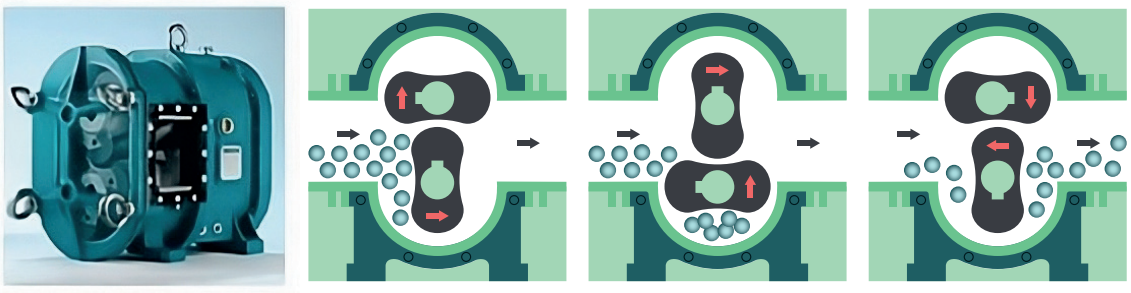


Figure 25: Rotary lobe pump (left), rotary lobe pump principle (right) [Börger GmbH (left), Hugo Vogelsang Maschi-nenbau GmbH] (Adapted from Fachagentur Nachwachsende Rohstoffe, 2013).

researched are alkaline electrolysis (AEL) (Brauns and Turek, 2020), electrolysis on polymer electrolyte membranes (PEM) (Carmo *et al.*, 2013), and solid oxide electrolysis (SOEC) (Wang *et al.*, 2019).

- **Alkaline electrolysis:** Alkaline electrolysis is the most technically mature variant of hydrogen production by electrolysis. It is carried out at high pH values with KOH or NaOH as electrolyte. Commercial plants producing hydrogen by alkaline electrolysis have been available for many years (Zoulias *et al.*, 2004). These plants can reach outputs of up to several MW (Götz *et al.*, 2016).
- **Polymer electrolyte membranes electrolysis:** This variant of electrolysis is characterised by the use of a solid membrane that only allows protons to pass. On an industrial scale, the method is less widespread than alkaline electrolysis, but there are also industrial applications with outputs above one MW (Ayers *et al.*, 2010).
- **Solid oxide electrolysis (SOEC):** This process is already well researched, but not yet established on an industrial scale. The process is characterised using a solid body as an electrolyte. At high temperatures around 900°C, this becomes conductive for oxygen ions. The high temperature also causes a drop in the necessary voltage and thus in the electrical energy requirement (Wang *et al.*, 2019).

Hydrogen conversion: Hydrogen can be used directly in many ways, however, the aim of power-to-gas processes is to store electrical energy in chemical form. The storage of hydrogen is problematic because of the high diffusion rate in most materials and the high corrosiveness towards steel (Li *et al.*, 2020). More practical is the conversion and storage of methane. Methanation can take place chemically or biologically and essentially follows the following reaction equation (Götz *et al.*, 2014).

- $CO_2 + 4\,H_2 \rightarrow CH_4 + 2\,H_2O$

Chemical H_2-methanation: In chemical methanation, carbon monoxide is first formed as an intermediate, which then reacts with hydrogen to form methane and water in a modification of the Fischer-Tropsch synthesis (Wei *et al.*, 2011). The reaction is carried out at high temperatures and pressure on metal catalysts. Chemical methanation has been established as a large-scale process for fuel production for decades. The required carbon dioxide can be obtained from exhaust gases of combustion engines, from biogas (only after adsorption of H_2S) or from off-gas from biomethane production plants.

Biological H_2-methanation: Hydrogen can also be converted to methane by microorganisms (biological methanation). The process is known as hydrogenotrophic methanogenesis from biogas formation and therefore also takes place in biogas plants. A big difference is that in biogas plants the hydrogen is formed in situ in the aqueous phase and immediately consumed again. In biological methanation, the hydrogen is present as a gas and must first be dissolved in the aqueous phase. Due to the poor solubility of hydrogen at normal pressure in water, this is a technical obstacle (Jensen *et al.*, 2021). Since hydrogenotrophic methanogenesis is part of the formation process of biogas, the idea of feeding hydrogen from electrolysis into a biogas plant is obvious. It allows the usage of the existing infrastructure and reduces investment costs. Alternatively, the process can also take place in a stand-alone reactor and detached from a biogas plant (Thema *et al.*, 2019). In a stand-alone methanation reactor, both CO_2 and biogas (approx. 50% CO_2) can be used as a carbon source. The process is not yet technically mature, but a general advantage is that a stand-alone reactor can be tailored specifically to the needs of hydrogenotrophic methanogenesis and the corresponding archaea. The transport of hydrogen into the aqueous phase can be specifically improved. Various reactor types are being developed for this purpose (Shiga *et al.*, 1998).

As already mentioned, the introduction of hydrogen directly into a tank of the biogas plant is also possible. Here, the entry of hydrogen into the liquid phase is a critical point **(Jensen *et al.*, 2021)**. It would be conceivable to inject the gas into the liquid or to mix the gaseous and liquid phases. However, both methods lead to an increase in energy demand and thus to a reduction in energy efficiency. An interesting approach is also the direct implementation of an electrolysis cell in the aqueous phase of a biogas reactor. Here, hydrogen could be formed and consumed in situ, which would reduce the handling of gaseous hydrogen to a minimum and avoid the problem of mass transport. However, there is still a lot of research to be done on this **(Marchese *et al.*, 2020)**.

No matter which technique is used for biological methanation, the product is biogas with an increased methane and hydrogen content. Higher hydrogen contents (>5%) are a considerable problem for conventional biogas plants because the membranes of the gas storage tanks are too permeable for hydrogen. There is then a risk of explosive gas mixtures of hydrogen and air forming in the vicinity of the tanks. The utilisation of biogas containing hydrogen is also not easy, as it would cause malfunctions in combustion engines as well as in gas treatment at high concentrations. If the biogas is to be upgraded to biomethane and fed into the natural gas grid, the limits for hydrogen in natural gas must also be observed **(European Association for the Streamlining of Energy Exchange-gas, 2008)**. The hydrogen must therefore be dosed in such a way that it can be consumed continuously. In Germany, the gas storage tanks of a biogas plant must be able to temporarily store at least the gas from 6 hours of gas production. Then the additional methane (produced during the day from surplus solar electricity) could be consumed at night for electricity and/or heat production. Another technical hurdle is the changing methane content of the final gas. Since power-to-gas only really makes sense with surplus electricity, it must be assumed that the methane content in the biogas fluctuates greatly over the course of the day, depending on whether additional hydrogen is introduced or not. However, both the gas control system of the unit power plants, and the control system of the gas treatment plants are set to relatively fixed methane contents. Special units would have to be used here to record the methane content and automatically readjust it.

Projects implemented in Europe: In recent years, about 100 Power to X plants with an output (based on the electrical output of the electrolysis) of 5 - 600,000 kW have been planned and built in Europe **(Wulf *et al.*, 2018; Wulf *et al.*, 2020)**. More than 30 of these are power-to-gas plants, about half of which are biological and half chemical methanation plants. The largest plant for biological methanation was built in Germany in 2019 **(pfi Germany, 2017)**. This has an electrolysis capacity of 1800 kW. The methanation is carried out biologically in a column. The resulting biogas is desulphurised, dried and fed into the natural gas grid. The largest chemical methanation plant is planned in France with an electrolysis capacity of 600,000 kW **(Wulf *et al.*, 2018)**.

1.3.6 Two stage digestion

For decades, the operation of biogas plants was mainly linked to the operation of agriculture and animal farming. The focus was and is therefore often on investment costs and reliable and simple operation. A single-stage process in a tank that only has to be stirred, fed and heated is much easier for a farmer to handle than two process stages that have to be operated in coordination with each other. Therefore, it is not surprising that single-stage biogas plants are much more common than two-stage plants. However, two-stage plants offer advantages that could contribute to an improved utilization of the energy potential of the feedstock in the future **(Theuerl *et al.* 2019)**.

In two-stage biogas plants, acidification and methane formation take place in different reactors (hydrolysis stage and methane stage). By spatial separation, the digestion conditions can be optimized for the respective microorganisms **(Köllmeier, 2014)**. The interaction of the two processes can also

be improved by selective dosing of the substrate flow from the hydrolysis stage to the methane stage, which improves the performance of the overall process. Gas yield can be increased, residues can be reduced, less substrate needs to be fed, and plants can be made smaller due to better space-time yield (Hahn *et al.*, 2012).

Hydrolysis of substrates is usually faster compared to methanogenesis (depending on the complexity and degradability of the substrate). Hydrolysis tanks can therefore usually be built smaller than a digester for the same input quantity, since the necessary residence time is shorter. An advantage of separate hydrolysis is the possibility to convert substrates that are more difficult to degrade, for example those containing cellulose or even lignocellulose (Čater *et al.*, 2014). But also solid substrates like potato peels which need more time for microbial digestion due to their particle size (Parawira *et al.*, 2005). Separate hydrolysis is also interesting for particularly easily degradable substrates such as sugar-containing residues from the food industry. In single-stage processes, the use of such impurities quickly leads to an accumulation of organic acids once methane formation is inhibited (Ziemiński and Kowalska-Wentel, 2017). This then often causes a lowering of the pH value, which then permanently inhibits methane formation. This danger can be reduced by using the already hydrolyzed material as an input. In contrast, separate hydrolysis does not make sense when already acidified substrates are used, such as silage from grasses or corn. Also, for plants using cattle slurry (partly also for manure and slurry from other animal species) as substrate, a two-stage operation is not reasonable. Cattle manure contains a lot of water and relatively few carbohydrates, the cells have already been broken down by the stomachs of the cattle, methanogenic archaea from the stomachs of the cattle are already present in the substrate and, in addition, manure often has a high buffer capacity for organic acids (Torrellas *et al.* 2018).

A two-stage design is also interesting if the substrates contain impurities that are not to enter the entire plant but only a specially equipped area. In this case, percolation of the substrate with a liquid phase is often used. The solid components or impurities are retained and only the degradable compounds are to be dissolved from the substrate by the percolate. Such plants are usually realized in the "garage design" in which individual chambers are fed discontinuously and emptied again (Qian *et al.*, 2016).

Hydrolysis stage: The designs for hydrolysis vessels are selected according to the substrates. Although round stirred tank reactors are also common here, special designs are also often encountered. The spatial separation required for separate hydrolysis is usually achieved by two separate reactors. Often, special crushing equipment or other mechanical pretreatment or collection systems are integrated. Since hydrolysis, just like methanogenesis, only takes place in the absence of oxygen, a hydrolysis vessel must also be gas-tight. In addition, gas is also produced during this process, which must be collected and recycled. The underlying biochemical process produces mainly carbon dioxide and hydrogen. Methane is produced only in very small quantities. The resulting gas can be used in various ways. The simplest is to discharge it into the methane stage (Weiland, 2010). There, the mixture of hydrogen and carbon dioxide can be further metabolized to methane. The bacteria used in hydrolysis are more robust compared to the metanogenic archaea (Köllmeier *et al.*, 2012), which allows for example the use of higher loading rates, which in turn improves the turnover rate of substrates (Hansen *et al.*, 2021). In general, a lower pH is observed in hydrolysis stages than in methane stages (Dobre *et al.*, 2014). This places special demands on the materials used. For example, concrete must be protected from corrosion by special protective coatings. In any case, it makes sense to monitor the pH value of a hydrolysis tank (Hahn *et al.*, 2012).

Methane stage: In two-stage plants, the methane stages are fed with the output of the hydrolysis stage. Since the formation of organic acids has already been completed, the required amount of substrate can now

be precisely metered. In essence, their operation is similar to that of the classic fermenters. Differences can arise, however, due to the fact that solids have already been broken down in a separate hydrolysis stage, or sometimes these solids are specifically retained (cf. percolation). In these cases, the stirring technology can be run with less power. If the gas from the hydrolysis is to be further metabolized in the methane stage, it must be brought into contact with the liquid phase by a suitable technology.

1.4 Utilisation of biogas

1.4.1 Intermediate storage in the gas storage facility

Flexibilization: Usually, biogas is produced constantly and with continuous feeding. Biogas production should be kept at a constant level in the long term. To compensate for small differences between gas production and gas utilisation during continuous electricity generation, biogas plants have gas storage facilities. In the case of demand-oriented feed-in (flexible operation), biogas storage has an additional significance. Discontinuous utilisation of the biogas is made possible by regularly emptying and filling of the gas storage tanks. In a flexible mode of operation, the storage system holds the biogas to be able to run at increased output during attractive hours **(Fachagentur Nachwachsende Rohstoffe, 2018; www.biogas-netzeinspeisung.at, accessed 07.10.2021)**.

The dimensioning of the gas storage depends on the operating strategy of the biogas plant. With constant gas output, the necessary size of the gas storage capacity increases with the degree of flexibilization. With increasing plant output, the necessary storage capacity grows. On one hand, the storage size determines the maximum idle time and, on the other hand, the maximum running time of the CHP unit. The capacity of the biogas storage should generally be more than 12 hours, because the peak price periods that occur in the typical daily rhythm have approximately this time interval (daily flexibility) **(Fachagentur Nachwachsende Rohstoffe, 2018)**. With a larger storage volume, the costs of interval operation for the CHP can be reduced because it has to be started less frequently **(www.biogas-netzeinspeisung.at, accessed 07.10.2021)**. In principle, the construction costs for flexible plants are higher than for non-flexible ones. Whether this makes economic sense depends mainly on whether demand-responsive feed-in is a prerequisite for government subsidies for electricity remuneration.

Gas storage: Gas storage tanks must be pressure-resistant, gas-tight, resistant to media, UV, temperature, and weather **(Weiland, 2010)**. Before commissioning, they must be checked for tightness. Gas storage tanks are equipped with mechanical or hydraulic over pressure and under pressure safety devices to ensure that the internal pressure remains within the prescribed limits.

Container-bound storage systems: This refers to a round container that is covered with a gas-tight dome-shaped roof. A general advantage of container-based storage systems is the space saving compared to external gas storage systems, as the containers are already available. If the fermenter or the secondary fermenter itself is used as a gas storage, foil roofs are usually used. In the case of a concrete cover and a constant gas volume, the gas space cover merely provides a gas-tight seal of the fermenter against the environment. With variable gas volumes, the gas storage serves to compensate for fluctuating gas production. For the subsequent section, the gas storage tank provides a constant gas volume flow and gas pressure.

The tank covers can be designed as a concrete cover, made of stainless steel, from a single-membrane storage tank or double-membrane storage tank. Only the last two tank covers mentioned are suitable for flexibilization. A comparison of the different covers can be found in **table 9**. When using

foils, these are attached gas-tight to the upper edge of the container. A support frame is built onto the tank on which the foil can rest when the gas storage tank is completely empty. Roofs consisting of only one foil must be expandable and must be able to expand depending on the filling level of the gas storage tank. Supporting air roofs (see **figure 26**) or double membrane storage tanks consist of two, non-expandable foils.

The upper film is a weather protection film that is applied over the actual storage film. The weather protection foil serves to protect against environmental influences (especially wind gusts) and to absorb acting loads. Air is blown into the space between the two foils. The supporting air blown into the gap ensures the stability of the outer film and causes a relatively constant pressure on the inner membrane. The upper weather protection foil is always in a taut, stretched state, while the storage foil adjusts variably to the amount of biogas to be stored. With this system, the gas pressure is kept as stable as possible **(Weiland, 2010)**.

In principle, the container cover is independent of the type of material used in the fermenter. Container-bound gas storage systems are always pressure less or low-pressure storage systems; the pressure range is between 0 and 5 mbar for simple foil storage systems and 0 - 50 mbar for double membrane storage systems. The sealing of the foil roofs is achieved by means of a so-called Seeger seal or with clamping rails **(Persson *et al.*, 2019)**.

Gas storage membranes have a certain diffusion rate of 1‰ - 5‰, concrete and steel covers are emission-free. Storage foils are mostly made of PE or fabric-reinforced PVC. Some odour emission is released by diffusion, which does not require any further measures during normal operation. The odour emission is reduced in double-membrane storage tanks **(Schmidt-Eriksen, 2011)**.

External storage systems: The gas storage tank is spatially decoupled from the fermenter/digestate storage. They are available as low-pressure, medium-pressure and

Figure 26: Biogas plants with load-bearing roofs (AEV Energy GmbH)

high-pressure storage tanks. The advantage is that less gas is lost when the fermenter is opened. The disadvantage is the increased space requirement **(Khan *et al.*, 2017)**.

External low-pressure storage tanks can be designed in the form of foil cushions or supporting air storage tanks on concrete foundations. To protect them from the weather, they can be covered with an additional foil or housed in a building. The advantage of this system is the low cost compared to other systems, and the usable volume of foil cushions is relatively large. The disadvantage is the short life of the foil if it is exposed to the weather. The basic principle is the same

as for internal double-membrane storage. An outer foil is supported by supporting air, which protects the movable storage membrane inside (Persson *et al.*, 2019). An overview of the types of storage tanks in the low-pressure range is given in **table 10**; an example of an external double-diaphragm storage tank is shown in **figure 27**.

1.4.2 Conveying technology for biogas

In normal operation, only biogas is transported in the gas-carrying system. This must comply with special safety regulations, as the escape of gas or the entry of air can form explosive mixtures. In case of malfunctions, the liquid level in the fermenter may rise above the opening edge of the gas outlet, thus digestate or foam may enter the gas line. Gas pipes must be inspected and maintained regularly; corrosion increases the risk of gas leaks and explosions. Raw biogas also contains water vapour, hydrogen sulphide, hydrogen and ammonia, which are reactive gases that can attack metallic pipelines. Water collected in the condensate separator can corrode the pipes. The pipelines must therefore be resistant to media and corrosion; they are made of stainless steel, polyethylene (PE-HD) or PVC-U. In general, stainless-steel pipelines are often used above ground; plastic pipelines must be protected from mechanical and thermal damage and

Table 9: Overview of container covers and container-bound gas storage tanks (Postel *et al.,* 2008).

Type	Description	Application area	Advantages	Disadvantages
Concrete ceiling	Attached steel concrete plate	Only for concrete tanks	Stable, can be walked on, partly driven over, suitable for agitators	Constant gas volume not suitable as "lung" function
Enamelled steel or stainless steel	Steel construction segments, foil as inner membrane	Concrete tanks, steel tanks	Lighter than concrete, better to install	Gas volume somewhat larger, but still inflexible
Single-membrane storage tank (single shell)	» Foil cover made of a single membrane » Mast supported	All standing tanks widespread in older and small plants	» Larger storage volume » Low cost » Possibility of optical process control	» Constant gas volume, susceptible to weather, wind and snow » Foils can tear under negative pressure
Double-membrane storage tank (double-shell)	» Inner foil as gas-tight membrane, outer foil as protective cover » Mast supported » Supporting air roof	For all standing containers, is standard	» Larger storage volume, more weather-resistant than single-skin » Possibility of optical process control » Permanently low pre-pressure supplied	» More expensive due to increased film requirements » Foils can tear under negative pressure » Energy requirement for supporting air

need more supports than stainless steel pipes when laid above ground. The connections are flanged, welded, glued or screwed. All fittings and pipelines must be protected against frost. The pipes are always laid with a gradient, this enables targeted condensate collection. It must be possible to drain condensate from all gas pipes, all fittings must be easily accessible and easy to maintain. When laying pipes in the ground, ensure good compaction, the laying must be stress-free, if necessary, compensators or U-bends must be planned.

1.4.3 Gas treatment

In addition to methane (CH_4) and carbon dioxide (CO_2), general raw biogas also contains considerable amounts of hydrogen sulphide (H_2S) and ammonia. The combination of hydrogen sulphide and the water vapour contained in the biogas results in acid formation **(Fachagentur Nachwachsende Rohstoffe, 2013)**, which leads to corrosion. Most modern CHP units are equipped with oxidation catalysts; sulphur atoms can occupy the active centres of the catalysts even in moderate concentrations and thus render the catalysts ineffective. Water vapour in larger quantities is adsorbed by the activated carbon and inhibits the absorption of sulphur. In the case of biogas plants, desulphurisation and drying of the biogas is usually carried out. The manufacturers of CHP units set minimum requirements for the quality of the fuel gases used; these should be complied with in order to be able to operate the CHP units effectively and to protect them from damage **(Fachagentur Nachwachsende Rohstoffe, 2018)**.

Desulphurisation: Possible processes (for comparison see **table 11**) can be divided into biological, chemical and physical separation processes. In addition to the gas composition, the flow rate is a decisive parameter for the desulphurisation equipment. The flow rate can fluctuate; it is increased when fresh substrate is fed in and during operation of the agitators. Power peaks of the flow rate can therefore occur by more than 50% above the average value. Therefore, the plants

Table 10: Overview of external gas accumulators in the pressure less and low-pressure range (Postel et al., 2008).

Type	Description	Application area	Advantages	Disadvantages
Foil pillow feeder	» In fixed old buildings (silo, barn) » In new building, e.g., lightweight hall » On concrete ceiling of a digester » Exposed or under roofing	» Farms that have buildings available » Special cases, e.g., if digester is under-ground » Tanks with concrete ceiling » Smaller storage tanks	» Inexpensive with existing building » Easily accessi-ble » Space-saving » Easily accessible	» Volume (shape/ quantity) limited » If necessary additional space required » Relatively small volume » Exposed to weather and sun
Complete system	Double membrane accumulator	Accumulator can be positioned on terrain	Flexible and modular installation	Additional space requirement

Figure 27: Example of freestanding double diaphragm accumulator (AEV Energy GmbH).

Figure 28: Two tanks with piping and pressure-relief device (AEV Energy GmbH).

Biological desulphurisation in the fermenter: Biological desulphurisation is often carried out directly in the fermenter. By means of the bacterium Sulfobacter oxydans, hydrogen sulphide is converted with oxygen into elemental sulphur **(Ramos *et al.*, 2013)**.

$$2\,H_2S + O_2 \longrightarrow 2\,S + 2\,H_2O$$

The sulphur accumulates on the surface of the tanks and eventually falls back into the substrate. The elemental sulphur can therefore be discharged from the reactor via the fermentation residue. The bacteria are already present and do not have to be added separately. The oxygen is introduced into the fermenter by injecting defined amounts of air (max. 5% of the biogas formed in the same period). This process is very cost-effective, no additional chemicals are required. The technology is low-maintenance and low-failure. Sulphur can then be discharged as fertiliser. The introduction of oxygen may impair the process and methane oxidation is possible. It is difficult to react to different gas production rates. Another disadvantage of this process is the possible formation of sulphuric acid. Bacteria can develop (in the presence of atmospheric oxygen) that oxidise the resulting hydrogen sulphide to sulphuric acid. This leads to corrosion damage on surfaces susceptible to this (concrete and metal materials). The use of biological desulphurisation has proven itself in technology; its effectiveness depends on the sulphur content. It is hardly possible to reduce the hydrogen sulphide concentration to the level required for combustion in a CHP with oxidation catalyst, but the process improves the gas quality in a simple way to such an extent that downstream methods can work more economically. It is therefore mostly used in combination with other processes **(Okoro and Sun, 2019)**.

Biological desulphurisation in external reactors - trickling filter process

Biological desulphurisation is also possible outside the plant. Separate containers with biological desulphurisation columns can be used for this. In the droplet process, hydrogen sulphide is absorbed with the help of a scrubbing medium. Degradation rates of 99% can be achieved, which can lead to a residual gas concentration of less than 50 ppm sulphur. The technology is available for all biogas plant dimensions and is basically suitable for all biogas plants. This process is rather unsuitable for feeding into the natural gas grid due to the high air input. The advantage is that the air input takes place outside the fermenter, so the process is not impaired by the oxygen input. Targeted automatic regulation of hydrogen sulphide decomposition (through nutrient, air supply and temperature management) is possible. No use of chemicals is necessary. The disadvantage is that an additional, costly unit is

needed and the high air input in the biogas. Another disadvantage is that maintenance is very expensive **(Okoro and Sun, 2019)**.

Sulphide precipitation: Sulphide precipitation is a chemical process which, like biological processes, is used for coarse desulphurisation. With sulphide precipitation, hydrogen sulphide values between 100 and 150 ppm are achieved. By adding ferric chloride or ferric hydroxide, the sulphur is chemically bound in the substrate, thus preventing its release as hydrogen sulphide.

- $Fe^{2+} + S^{2-} \longrightarrow FeS$
- $2\ Fe^{3+} + 3\ S^{2-} \longrightarrow 2\ FeS + S$

The iron salts are dosed via the preliminary pit or the solids dosing unit or directly into the fermenter. The hydrogen sulphide formed is still bound in the liquid phase of the fermenter as sparingly soluble iron sulphide. The iron sulphide remains as a solid in the digestate and can be discharged from the system with the solids discharge. The exact dosing quantity depends on the respective system; it is usually between 100 - 220 giron/tsubstrate. The dosing quantity is strongly dependent on the substrate used. The binding of H_2S directly in the fermenter can have a positive effect on the microbiological processes taking place **(Fachagentur Nachwachsende Rohstoffe, 2013)**. Sulphide precipitation is also often used in combination with biological desulphurisation **(Okoro and Sun, 2019)**.

Figure 29: Left figure: Gas control for air injection (Fachagentur Nachwachsende Rohstoffe, 2013). Right figure: Sulphur in the fermenter (Biomin, accessed 18.10.2021).

Adsorption on activated carbon: For the upgrading of biogas to biomethane and its injection into the natural gas grid and for the operation of CHPs with oxidation catalysts, very low H_2S concentrations are necessary in the biogas. To achieve this, fine desulphurisation is necessary. Activated carbon filters are very often used for this purpose. This process uses adsorption on activated carbon based on the catalytic oxidation of the hydrogen sulphide on the activated carbon surface. The hydrogen sulphide is adsorptively bound in the pore system and then catalytically oxidised. Impregnation or doping of the activated carbon to improve the reaction rates and the loading capacities is possible. Potassium iodide and potassium carbonate are used as impregnating agents. During operation, the activated carbon filters are loaded once with carbon. This is used until the loading limit is reached. When the activated carbon loses its effect can be determined by measuring the hydrogen sulphide content after desulphurisation. The loaded activated carbon is then either regenerated or disposed of in a waste incineration plant, for example. In order to increase the service life of activated carbon filters, a combination with coarse desulphurisation is recommended, which makes the process more economical **(Okoro and Sun, 2019)**.

Figure 30: External biological desulphurisation, on the left bio-trickling bed reactor, on the right bio-moisture reactor [S&H GmbH & Co. Umweltengineering KG] (Fachagentur Nachwachsende Rohstoffe, 2013).

Drying: To optimise combustion in CHP units and meet the requirements of subsequent purification stages, water vapour must be removed from the raw gas. The amount of water vapour in the biogas depends on the gas temperature. In the fermenter, the relative humidity is close to 100% and the biogas is saturated with water vapour. The processes used to dry the biogas are condensation drying, adsorption drying and absorption drying **(Okoro and Sun, 2019)**.

Condensation drying: To separate the condensate, cooling takes place within the gas pipe. The operating principle of this process is based on cooling the biogas below the dew point. In small systems, the cooling of the gas as it passes through buried pipes can already remove sufficient water. For this purpose, the gas pipes are laid at an incline, and at the lowest point of the pipe the condensate is collected at a condensate separator. For the gas to cool down, it must have a certain residence time in the pipeline. In addition to the water vapour, other undesirable constituents such as water-soluble gases and aerosols are removed from the biogas. The separator must be emptied regularly, so it must be easily accessible for maintenance work. In larger plants, the flow velocity is often too high for all the water to be separated in this way. In this case, additional gas cooling systems are used to reduce the dew point. Dew points of 3 - 5°C are thus possible. The efficiency can be further increased by prior compression **(Fachagentur Nachwachsende Rohstoffe, 2013)**. After gas cooling, the absolute humidity is lower; the relative humidity is adjusted by reheating the gas. This value is important for the operation of activated carbon filters and combustion engines. Condensation drying is usually sufficient for the needs of a biogas plant.

Figure 31: Activated carbon filter biogas with base and bypasses (BioBG, accessed 25.10.2021).

Adsorption drying: The process is based on zeolites, silica gels or aluminium oxide and allows dew points of up to -90°C. The adsorbers are mounted in a fixed bed and are operated alternately at an ambient pressure of 6 to 10 bar. The process is suitable for small to medium volume flows but is rather untypical for biogas plants **(Gaj, 2020)**.

Table 11: Process overview desulphurisation process (Fachagentur Nachwachsende Rohstoffe, 2013).

Procedure	Energy demand		Operating supplies		Air input	Purity in ppm	Problems
	el.	them.	Consumption	Disposal			
Biological desulphurisation in the Fermenter	++	0	++	++	yes	50-2,000	Inaccurate process control
External biological desulphurisation	-	00	+	+	yes	50-100	Inaccurate process control
Bioscrubber	-	0	-	+	yes	50-100	High procedural effort
Sulphide precipitation	0	0	--	0	no	50-500	Inertial process
Activated carbon	0	0	--	-	yes	<5	High disposal volumes

1.4.4 Combined heat and power plant

With a combined heat and power unit (CHP), electricity and heat can be generated simultaneously. The heat is produced as a co-product and is used to provide the process energy and for other external purposes **(ASUE, 2014)**. Combined heat and power plants with combustion engines coupled to a generator are used almost exclusively. The engines run at a constant speed, which allows the generator to provide electrical energy whose frequency is compatible with the grid frequency. Usually, ignition jet and gas Otto engines are used, but alternatively micro gas turbines, Stirling engines or fuel cells can be used **(Thomas, 2008)**.

A CHP module (see **figure 32**) consists of the following components: an internal combustion engine, a generator, a heat exchanger system, cooling water and lubricating oil circuits, hydraulic equipment for heat distribution and electrical switching and control equipment **(Weiland, 2010)**.

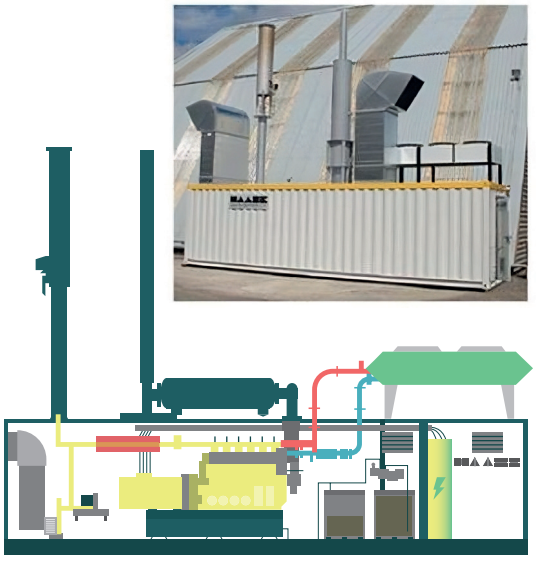


Figure 32: Biogas CHP, complete module in compact design with emergency flare (Adapted from Fachagentur Nachwach-sende Rohstoffe, 2013).

Gas-Otto engines: These are engines specially developed for gas operation, which work according to the Otto principle. To minimise nitrogen oxide emissions, gas Otto engines in biogas plants are operated as lean-burn engines. This leads to a reduction in performance and less fuel is convert-

ed in the engine. Gas Otto engines rely on a minimum methane content of 45%; at lower concentrations, the engines shut down. Gas Otto engines are mainly used in the higher power range. They achieve mechanical efficiencies of 40% - 42% at full load (at a rated power of >300 kW). The overall efficiency is high at up to 90%, and the operating time is about 80,000 - 96,000 operating hours **(Persson et al., 2019)**. The electrical output is up to >1 MW, rarely less than 100 kW. Engines of this type are basically suitable for all biogas plants, but economically they are more suitable for larger plants. They are specially designed for gas utilisation; emission limits are largely complied with. They require little maintenance and the overall efficiency is higher than that of ignition jet engines. The disadvantages are the higher investment costs compared to ignition jet engines and a lower electrical efficiency than ignition jet engines in the lower power range **(ASUE, 2014)**.

Dual Fuel Engines: Engines of this type work according to the diesel principle. The biogas is added to the combustion air via a gas mixer. Ignition oil is fed into the combustion chamber via an injection system and ignited together with the gas mixture. The proportion of ignition oil in the fuel supplied is approx. 2% to 5%. Ignition jet engines are operated with a high air surplus, they are mainly used in the small power range up to 350 kW. The operating time is about 35,000 operating hours, they achieve a mechanical efficiency of 30% to 44%. The efficiency increases with increasing plant size.

In principle, these engines can be used for all biogas plants, but for economic reasons they tend to be used in smaller plants. They are less expensive than gas Otto engines and have a higher electrical efficiency in the lower power range compared to the gas Otto engines. Due to the relatively small amounts of ignition oil used, cooling is rather low and there is a risk of coking of the injection nozzles. This leads to increased exhaust gas loads and thus to shorter maintenance intervals. The overall efficiency is lower than with gas Otto engines, and an additional fuel (ignition oil) must be used. In the event of a biogas supply failure, it is possible to switch to a substitute fuel (diesel), for example to ensure heating of the fermenter **(Weiland, 2010)**.

Generators: The generator is the interface between the mechanical movement and the flow of electricity. The combustion engine generates movement, and this is converted into electricity by the generator. Synchronous generators are mostly used in CHP units, but asynchronous generators are also used in some cases. The generators are usually coupled directly to the shaft of the combustion engine. Digital voltage regulators are a component of the generators, which enables them to ride through voltage dips in the grid and support the dynamic grid support.

Heat extraction: Heat is generated during electricity production in a CHP unit, and heat exchangers can be used to utilise this heat. The largest amount of heat is produced in the cooling water circuit of the combustion engine. Due to its temperature level, this can be used to provide heating or process energy. Plate heat exchangers are usually used to extract the heat from the cooling water. The heat thus extracted is fed to the individual heating circuits via a distributor. The exhaust gases of combustion engines contain a lot of energy; their temperature level is approx. 460 to 550°C. The heat is usually extracted from the cooling water by means of plate heat exchangers. Exhaust gas heat exchangers made of stainless steel are mostly used for heat recovery; steam at various pressure levels, hot water and thermal oil are used as heat transfer media **(Fachagentur Nachwachsende Rohstoffe, 2013)**.

The heat produced can be used internally in the plant. The heat demand depends on the season; in winter it is high, in summer excess heat has to be dissipated by emergency coolers. The heat required for fermenter heating is about 20% to 40% of the total heat produced. In addition, plant rooms and living quarters can be heated. In addition to internal heat sinks, the heat can be consumed externally. Particularly due to the expensive purchase of renewable natural resources, an economical use of the heat produced is recommended and is often also remunerated with bonuses. Heat consumers can be

nearby commercial or communal facilities, for example horticulture farms, fish farms, wood drying, swimming pools and residential buildings. For heat transport, sufficient thermal insulation is recommended to potentially save heat (Effenberger *et al.*, 2008).

1.4.5 Biomethane injection

Biogas can be upgraded to biomethane (equivalent to natural gas) and fed into the natural gas grid. To do this, the non-combustible gases must first be removed from the biogas, and then the calorific value and pressure must be adjusted to the tolerances of the natural gas network. The entirety of these processes is called biomethane upgrading. Depending on the process and the plant constellation, this is either downstream of conventional gas treatment or does not require it. Biomethane feed-in is often operated in parallel with combined heat and power generation. An existing natural gas network in the vicinity of the biogas plant is advantageous for economical use.

Carbon dioxide separation: The processing step of carbon dioxide separation is required for the subsequent feeding of the product gas into the grid. By increasing the methane content, an adaptation to the required combustion properties of the product gas is possible.

Pressure Swing Adsorption (figure 33): Pressure swing adsorption is an adsorptive biogas upgrading process. Adsorption is the accumulation of gas particles (here CO_2) on the surface of solids (adsorbents) (Fachagentur Nachwachsende Rohstoffe, 2018). Activated carbon, zeolites or carbon molecular sieves are used as adsorbents. In addition to CO_2, other gas components such as water or hydrogen sulphide, nitrogen and oxygen are also retained in this process. With the process of physical adsorption and desorption alternating by pressure change, CH_4 yields of 97% are achieved (Daniel-Gromke *et al.*, 2018).

First, a pressure increase of approx. 4 to 7 bar takes place in this process. This is followed by water separation and desulphurisation. The gas is then fed into an adsorption column containing a molecular sieve. The carbon dioxide is retained on the molecular sieve, and the methane passes through the column almost completely. A small amount of methane is also retained and discharged with the CO_2 (off gas). The adsorbed gas components are desorbed by lowering the pressure. A first partial flow of the desorbed gas, which still contains CH_4, is fed into another column that is still unloaded. The methane flows through the column as completely as possible, whereby other components are retained again. A vacuum is applied to the first column, resulting in complete desorption of the column (Alonso-Vicario*et al.*, 2010). The methane yield can be further increased by recirculation and additional purging cycles. The disadvantages of this process are the relatively high-power consumption, the low controllability of the system and the high methane loss in the exhaust air stream (approx. 1% to 5%). Before using the PSA process, desulphurisation and drying of the raw gas is necessary. The heat requirement can be classified as low and no additional process chemicals are used (Alonso-Vicario*et al.*, 2010).

Pressure washing: This process is an absorptive biogas upgrading process. Absorption is the dissolving of gases in liquids (absorbents). In pressurised water scrubbing, water is used as the absorbent. The operating principle of this process is based on the different solubility of methane and carbon dioxide and other gases (Xiao *et al.*, 2014).

First, the raw gas is brought to a pressure level of approx. 7 to 10 bars by means of compression. It then enters an absorption column from below and flows through it. Water flows through the column from top to bottom in counterflow. Water, hydrogen sulphide, carbon dioxide, ammonia and any dust and microorganisms contained in the raw gas dissolve in the column (Fachagentur Nachwachsende Rohstoffe, 2013). The raw gas is then saturated with water, leaves

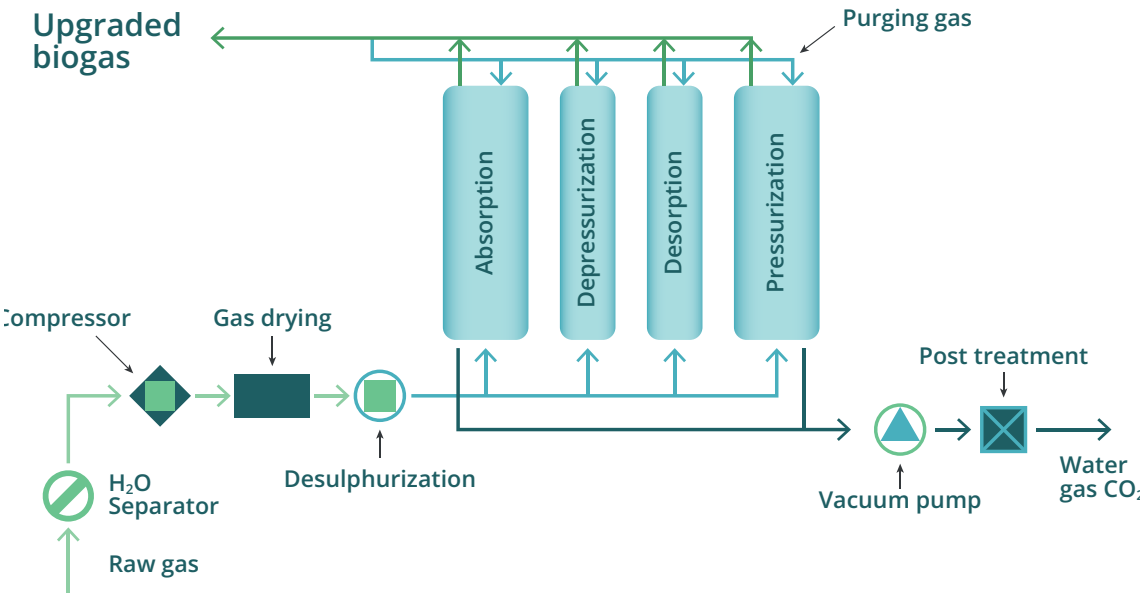


Figure 33: Pressure Swing Adsorption Technology for CO_2 Removal (Adapted from Ogunlude, 2019).

the column at the top and still must be dried. Methane is still bound in the absorbent; to remove this, it is partially depressurised in a flash column. The desorbed gas leaves the flash column at the upper end and is fed into the raw gas stream. In a desorption column, the water is expanded and brought to ambient pressure. The water thus regenerated can now be used again as a scrubbing agent for absorption. The dissolved exhaust gas leaves the desorption column at the upper end and usually must be fed into an exhaust gas after treatment system **(Xiao *et al.*, 2014)**.

A methane content of up to 98% can be achieved. It is not necessary to carry out desulphurisation or drying before the pressurised water scrubbing. Another advantage of this process is the flexible adjustment to the gas volume; depending on the CO_2 content of the raw gas, pressure, temperature, and throughput can be regulated. Automatic and continuous operation is possible, and maintenance can be classified as relatively easy. A disadvantage is the relatively high-power consumption and the high methane slip of approx. 1% **(Xiao *et al.*, 2014)**.

Amine scrubbing: Amine scrubbing is a chemical absorption process in which biogas is brought into contact with a scrubbing liquid. Monoethanolamine or diethanolamine (ethanolamine-water mixtures) are used as washing media for CO_2 separation. If H_2S is also to be removed, methyl diethanolamine or triethanolamine are used as washing media **(Capra *et al.*, 2018)**.

In contrast to physical washing processes, absorption can take place almost without pressure. There are also manufacturers who compress the gas to up to 4 bars before it enters the column. Co-absorption of H_2S takes place in the scrubber, so fine desulphurisation takes place simultaneously. Oxygen in the raw gas has a negative effect on the process; this can lead to undesired oxidation of the absorbent. Regeneration of the loaded absorbent takes place in the desorber with the addition of heat at 110 to 160°C **(Vo *et al.*, 2018)**.

The process is suitable for rather smaller volume flows. The regeneration stage by steam has a high thermal energy requirement, which speaks against this process. Another disadvantage is the high detergent requirement. An advantage is the very high product gas quality >99% (pre-requisite for this is low N_2 and O_2 concentrations in the raw gas) and the low methane slip of <0.1% **(Vo *et al.*, 2018)**.

Membrane process: A relatively new process, but one that is already in use. In terms of process technology, membrane processes effect the separation of gases through the different diffusion velocities of the gas molecules of different sizes (Chen *et al.*, 2015).

Because CO_2 molecules are smaller than methane molecules, they can pass through the micropores of the membrane more quickly. Methane accumulates on the high-pressure side of the membrane, while unwanted substances such as water vapour, ammonia, hydrogen sulphide and CO_2 pass through the molecular sieve. The process requires prior desulphurisation and drying and entails a very high-power requirement with low heat demand. The achievable methane content is about 96%. No additional chemicals are used, and the methane slip is relatively high (Chen *et al.*, 2015).

Cryogenic process: This process is based on gas liquefaction by rectification. Liquid CO_2 is produced, which is filtered out during a low-temperature separation. It is a very demanding process that requires prior desulphurisation and drying of the biogas (Bauer *et al.*, 2013).

The cryogenic process is only used in pilot plants, has a very high electricity demand, but no chemicals are used, and the methane slip is minimal (Tan *et al.*, 2017).

Processing to natural gas quality: The final processing stage of gas upgrading to natural gas quality can be divided into three process steps: odorization, calorific value adjustment and pressure adjustment (Bauer *et al.*, 2013).

Odorization: In this process, odorous substances are added to the biogas. Mercaptan or tetrahydrothiophene are mainly used. These strong-smelling substances serve to enable the human sense of smell to detect leaks. For technical and ecological reasons, sulphur-free odorants are increasingly being used (Fachagentur Nachwachsende Rohstoffe, 2013).

Calorific value adjustment: For injection, the biogas must have the same combustion properties as the adjacent natural gas. A measure of this is the calorific value, the relative density and the Wobbe index (Fachagentur Nachwachsende Rohstoffe, 2013). The actual values may lie within the permissible fluctuation range, the relative density may be temporarily exceeded, and the Wobbe index may be undershot. If the calorific value in the biogas is too high, the required parameters can be achieved by adding air. If the calorific value in the biogas is too low, regulation/adjustment is carried out by adding a propane-butane mixture (Fachagentur Nachwachsende Rohstoffe, 2013).

Pressure adjustment: To feed in different network levels, a slightly higher pressure is required than the existing network pressure. For low-pressure networks this is <0.1 bar, for medium-pressure networks between 0.1 - 1 bar, for high-pressure networks from 1 bar and for ultra-high-pressure networks from 16 bar. Screw and piston compressors are used to compress biogas (Fachagentur Nachwachsende Rohstoffe, 2013).

1.4.6 Thermal utilisation of biogas

Depending on regional regulations, alternative gas consumption facilities (as an alternative to the CHP) are usually prescribed for biogas plants. These serve to avoid gas emissions in the event of a CHP failure. As a rule, this is realised by means of emergency gas flares, which are sometimes already integrated into the CHP unit. Alternatively, gas burners can also be used if the heat is to be utilised.

Gas burner: In practice, the combustion of biogas for the exclusive generation of heat is usually only used in combination with cogeneration. This makes sense, for example, if the plant has a gas heating system anyway, which can then also use biogas as an alternative. Units with atmospheric burners and gas blower burners can be used for this

purpose. Atmospheric burners fire boilers, condensing boilers or instantaneous water heaters in the output range from 5 to 30 kW. The heat generators are usually integrated into a system with buffer storage to which the heat consumers are connected (Chandra *et al.*, 1991). Larger output ranges are covered by forced draught burners.

Direct biogas combustion is used almost exclusively in heating plants that belong to the industrial anaerobic wastewater treatment and sewage gas utilisation.

Figure 34: Emergency flare of a biogas plant [FNR/D. Riesel] (Fachagentur Nachwachsende Rohstoffe, 2013).

mergency flare (figure 34): Gas emergency flares are widespread safety devices on biogas plants; here, the focus is on safety; heat utilisation does not take place. A stationary emergency flare burns the biogas in the event of a malfunction or if the gas storage tanks can no longer hold any additional biogas. Other applications for the emergency flare include carrying out maintenance work or if the gas cannot be used further due to poor quality. Gas flares are also used when starting up a biogas plant. The gas flare prevents the uncontrolled release of biogas into the environment due to the overpressure protection of the gas storage tanks. Harmful substances are converted into less environmentally hazardous substances, and the methane contained in the biogas is burnt to form water and carbon dioxide. Depending on the type, they can burn biogas with a methane content of 10 to 15% by volume. Stationary plants are usually freestanding but can also be installed on the CHP as a system solution. The materials used are stainless steel or steel, and gas flares are designed for up to 3,000 m^3/h. The gas flares are installed directly upstream of the gas flare. A flame arrester must be installed directly in front of the gas flare to prevent open flames from flashing back into the gas-carrying system. They are basically suitable for all biogas plants (Trávníček *et al.*, 2019).

1.5 Digestate

1.5.1 Digestate storage

Digestate accumulate in the fermenter. Compared to the substrates used, the digestate has a low dry matter content due to microbial degradation, only a very low oDM content, a higher water content, less structural material and is more homogeneous. The macro- and micronutrient content is almost identical to that of the substrate. Before the digestates can be used on agricultural land in the fertilisation periods (spring and possibly autumn), they must be stored temporarily. The storage capacity to be aimed for depends on local regulations, and in Germany is up to 270 days. Usually, the digestate is separated before storage to reduce the amount to be stored. For practical reasons, digestate storage facilities for solid and liquid digestate are located directly on the premises of a biogas plant. The storage facilities are integrated into the operational processes of a biogas plant via pumps, pipelines, or other conveying equipment. In some cases, they are also built outside biogas plants. In newer plants, a gas-tight cover of the tanks is mandatory to prevent methane emissions. In practice, it sometimes makes sense to combine gas-tight storage tanks for liquid digestate with secondary fermenters. In this case, the digestate storage tanks are only partially emptied and thus used for gas production and digestate storage at the same time.

Solid digestate: These are produced during solid matter fermentation and as a separated

(solid) component of the fermentation product of wet fermentation. Solid digestates are stackable and pourable. Depending on the intended use, the digestates are stored in different ways. Storage can take place in the open air or in halls as well as in containers. It is important that the storage facilities meet the requirements of the fermentation product. Percolation liquid and press water must not penetrate the soil. Therefore, the floors of the storage facilities are made of concrete or asphalt. Escaping liquids are collected and returned to the fermenter or the digestate store, for example. Silage camps (see *chapter 1.2.2 Delivery and storage of substrates*) are used, for example **(Weiland, 2010)**.

An outdoor ground plate is constructed in concrete or mastic asphalt, it is liquid-tight towards the bottom. They are built with a slope for the drainage of liquids. In some cases, they are equipped with backfill walls and a roof. The advantage is that large storage volumes can be realised relatively cheaply. A disadvantage is the loss of nitrogen during long storage periods. If the floor slab is not covered, nutrients are washed out and very high liquid quantities may have to be collected. A storage hall with a concrete floor or mastic asphalt floor is sealed at the bottom. Like the floor slab, it must be able to capture seepage water. The advantage is that it is independent of the weather, the disadvantage is that ventilation is required, and the increased constructional and technical effort compared to outdoor storage. A container, usually made of steel, is another storage option. In contrast to the other storage applications, this one is transportable. If it is not roofed, nutrients may be washed out **(Persson *et al.*, 2019)**.

Liquid digestate: Liquid digestate is stored in earth basins (lagoons) and in cylindrical or rectangular containers. Earth basins are usually rectangular and are embedded in the ground. To prevent emissions, they are covered with a plastic film. Characteristic of this type of storage are the relatively low construction costs for a large volume. More frequently used are containers made of concrete or steel. These can be constructed above or below ground. As described in *chapter 1.3.3*, fermentation residue stores can be equipped with agitators. These serve to homogenise the digestates before removal **(Paolini *et al.*, 2018)**.

Earth basins are soil pits lined with plastic film, usually rectangular in shape. They are designed with two layers for leakage detection. Agitators can be used here. They are very inexpensive to realise and quick to construct. Construction with large volumes is possible, and maintenance costs are very low. NH_3 emissions can be reduced by using an emission protection foil. A disadvantage is the possible outgassing of CH_4, NH_3 and N_2O, a loss of nitrogen or fertiliser value and, in the case of open earth basins, odour emissions and precipitation can occur. Earth basins can now also be covered with gas storage tanks.

Figure 35: Open construction of digestate storage tank, surface of digestate is forming a dry crust (Vanek, 2011).

Storage tanks (figure 35) are usually cylindrical or rectangular, they are available as high or low tanks, and the digestates contained can be homogenised by means of an agitator. They are available in open or closed design, mostly unheated, but also with heating and insulation. The advantage is that they can be covered, so there is no precipitation, which also has a positive effect on any odour emissions. The quality of the fertiliser can be maintained. In a gas-tight design, the containers represent an additional gas reservoir. Open tank systems have the same disadvantages as open earth tanks. Fermentation residue storage usually results in the

formation of a floating layer (**figure 35**). This is desirable in most cases of open construction, as it reduces odour emissions. Due to the low dry matter content of the liquid digestates, there is no danger of sediments settling on a large scale. There is also no risk of disturbing the gas discharge, as the digestates usually only have a very small gas potential. The stirring technique can therefore be kept much simpler with digestates than with fermenters.

1.5.2 Solid/liquid - separation of digestates

Solids separation: The basic process of digestate treatment is solids separation. Solid's separation makes it possible to reduce the storage volume for liquid fermentation residues and to reduce the formation of sinking and floating layers. In addition, the solids separation enables a separation of the nutrients in the fermentation substrate, the soluble mineral nitrogen remains in the liquid phase, while organically bound nitrogen and phosphorus largely remain in the solid phase. The separated liquid phase can be spread or further processed, the separated solids can be composted or dried.

The properties of the digestate and the configuration of the separator determine the separation efficiency of the processes used. The higher the dry matter content in the digestate, the higher the possible volume reduction **(Lukehurst *et al.*, 2011)**.

Screw presses (figure 36): Screw presses are used as the first link in the mechanical processes for solids separation or on their own. In the presses, the digestate is pressed against a screen basket by means of a screw **(Lindner, 2020)**. Typical pressure levels of presses are around 30 to 50 bar. In addition to the technical condition of the press, a certain amount of structural material is necessary in the digestate substrate. This forms a plug, which considerably improves the pressing result. Due to the high-pressure levels and the mineral components present in the substrate, screw presses are subject to high levels of abrasion. The screw press is usually installed directly downstream of the fermentation process; the high temperatures and the pH value usually causes the release of ammonia. This can be compensated for by exhaust air collection and treatment.

Good separation efficiencies can be achieved with presses of this type; dry matter contents of up to 40% are possible **(Guilayn *et al.*, 2019)**.

Decanter / Belt filter presses (figure 37): Decanters as well as belt filter presses are used for the separation of digestates from wet fermentation plants, as well as for the further dewatering of the liquid digestate after the use of a press screw. When **decanter centrifuges** are used, it is important for a good separation result that the solids have a large density difference to the liquid phase. To improve the dewatering behaviour, flocculants can be added to accelerate the sedimentation or flotation of the suspending particles. Decanter centrifuges achieve a degree of separation of 15% solid phase and 85% liquid phase. Decanter cakes have a high density, in this state they are not compostable and must first be made compostable by mixing. **Belt filter presses** are continuous pressure filters with circulating screen cloths as filter media, in which the process sequence takes place in two stages **(Lindner, 2020)**. The first stage is a gravity filtration, the second stage is a squeezing out of the sludge between two screens, which are guided around several rollers. The degree of separation is up to 51%. A disturbing factor is the large amount of rinsing water required. Both the decanter centrifuges and the belt filter presses are connected downstream of a screw press during digestate preparation. The aim is to process the liquid phase for downstream processes (see *chapter 1.5.3 Processing and utilisation of digestates*) **(Lindner, 2020)**.

1.5.3 Processing and utilisation of digestates

Often, digestates are not processed further, but used directly as fertiliser. In plants that cultivate energy crops on their own land, it is essential to return the nutrients to their own land. In other cases, for example, when residues from other farms are used for biogas production, there is no internal use for them. Due to the high content of macro- and micronutrients and the large quantity, it is not possible to discharge the liquid phase into the sewage network. In this case, processing the digestate can help to increase economic efficiency. Under certain circumstances, the digestate can be sold as fertiliser; in any case, drying helps to save weight and thus transport costs. This is also a possibility to use waste heat from the CHP. The following drying processes correspond to the state of the art for the treatment of solid digestate. They differ greatly in their prevalence and functional reliability (see **table 12**). The processes for drying the solid phase are borrowed from other areas of application and have been tested; the degree of adaptation is to be regarded as low.

Figure 36: Screw presses for dewatering biowaste digestate (Raussen und Kern, 2016).

In other cases, nutrients must be removed from the liquid digestate in order not to overfertilize the soils. In these cases, separation of the nutrients and sale to other regions would be desirable. The processes for treating the liquid phase do not yet correspond to the state of the art, a high need for development is seen here. The membrane process is the most advanced, it is established on the market and is operated in several reference plants. There is still potential in this process, which can reduce energy consumption and wear. Evaporation and stripping processes are not yet far advanced in terms of large-scale continuous operation. In the future, stricter fertiliser regulations will greatly increase the problem locally. **Figure 38** shows an overview of different digestate treatment processes.

Evaporation of liquid digestates: This method is a multi-stage process in which the liquid is first heated and then the temperature is gradually increased to the boiling point under negative pressure. Evaporation under vacuum requires reduced electrical energy compared to evaporation without vacuum. Temperatures of 80°C are already sufficient in a vacuum **(Lindner, 2020)**. The pH value is lowered by adding acid, this avoids ammonia losses. The amount of fermentation residue is reduced by 70%. In this process, the fermentation residue is sanitised due to the high temperatures. Up to 4 times higher solids concentrations can be achieved, which significantly reduces storage and transport costs **(Lukehurst *et al.*, 2010)**. Evaporation requires upstream dewatering of the liquid digestate by means of a decanter or belt fil-

Figure 37: Decanter (l), belt filter press (r) - for further dewatering of press water from pressed biowaste digestate (Raussen und Kern, 2016).

ter press. Another disadvantage of this process is the high amount of thermal energy required. This process only makes sense if hygienization is necessary, e.g., when using slaughterhouse waste and a lot of excess heat is available **(Nag *et al.*, 2021)**.

Drying of solid digestates: For drying, established technology from other areas is used. For this process step, for example, drum, belt, or push-turn dryers can be used. In most processes, heat is transferred by hot air which flows over or through the material to be dried **(Lukehurst *et al.*, 2010)**.

A **belt dryer** (see **figure 39**) consists of a drying chamber and several conveyor belts. On these, the free-flowing material to be conveyed is usually transported on several conveyor belts running in opposite directions. The belts are permeable to air and are made of wire mesh or perforated steel plates. Hot air flows through the conveyor belts and the material is dried. The material is mixed and homogenised by transferring it to different belts. The temperature level of the belt dryers is between 80 - 120°C, for which dissipated heat from the CHP can be used. The exhaust air must be treated **(Lindner, 2020)**.

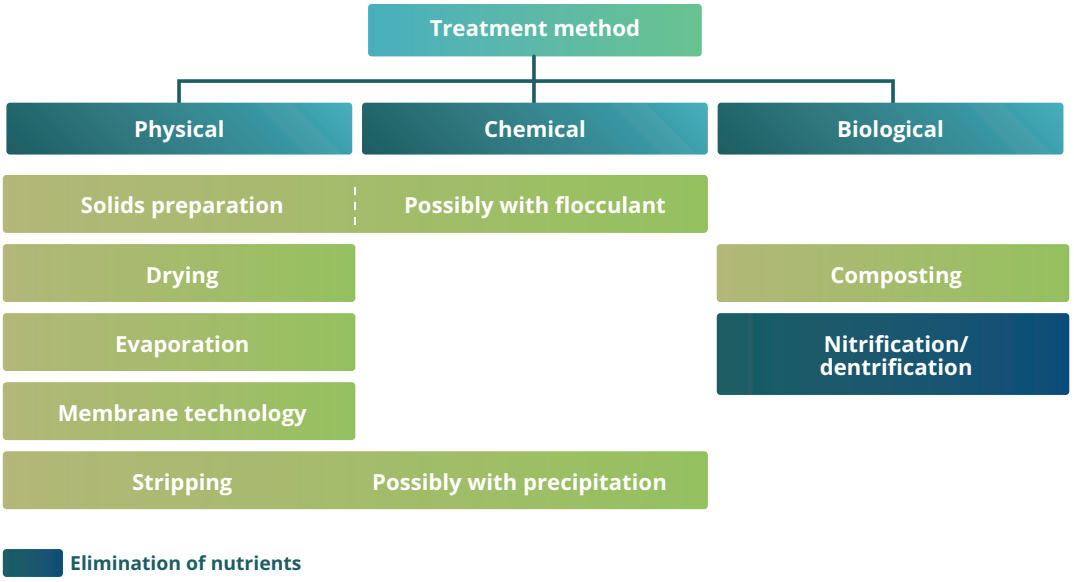


Figure 38: Classification of treatment processes by type (Fachagentur Nachwachsende Rohstoffe, 2010).

A **drum dryer** (see **figure 39**) consists of a slightly inclined rotary tube with an integrated hot air blower **(Lindner, 2020)**. Mechanically dewatered digestate is fed onto the raised end of the rotary pipe and passes through the rotating pipe on a long spiral track. For this purpose, multiple-pass drum dryers are used. Hot air at more than >200°C flows through the drum in co-current or counter-current. For economic reasons, it is advisable to use the waste gas heat of a CHP **(Lindner, 2020)**.

The ammonium contained in the solid phase passes into the drying air as ammonia during drying **(Fachagentur Nachwachsende Rohstoffe, 2013)**. Exhaust air treatment is necessary to prevent ammonia emissions and odour emissions.

Solid digestate composting: Composting is an aerobic process for treating organic waste. The aim of this process is to stabilise the organic components, kill pathogenic germs and weed seeds and reduce odour emissions. For composting, oxygen must be added to the digestate. The digestate is low-structure material, for successful composting it needs to have high-structure material such as bark mulch added to it and the material needs to be turned over. Only 55°C is reached during composting, not 75°C, which is necessary for hygienization. The reduced self-heating is caused by the anaerobic decomposition of carbon within the biogas plant; therefore, the treated material only reaches low temperatures during composting **(Nag *et al.*, 2021)**.

Membrane technology for liquid digestate: This process has its origins in wastewater treatment and is used there to treat water with a high organic load. In the field of biogas plants, it can be used to process the liquid digestate to such an extent that it can be discharged into the sewage network. The full treatment process has been adapted for biogas plants and is already in use. A unique feature is that no heat is required for this type of digestate treatment. It can thus be used in plants that do not have any surplus heat (for example, satellite CHPs). In this filtration process, decreasing pore size in combination with reverse osmosis produces a permeate from a liquid that can be discharged into the wastewater network and a concentrate enriched with nutrients. Phosphorus is retained in the ultrafiltration and is present in the retentate, while ammonium and potassium accumulate in the concentrate. The permeate is largely free of nutrients and can be discharged as wastewater. In this process, the dry matter content must be very low ≤3%, which requires solid-liquid separation by means of a decanter **(Fachagentur Nachwachsende Rohstoffe, 2013)**. Since the costs for this process are very high on a large scale, it only makes sense if another use of the digestates is ruled out.

Stripping of liquid digestate: Stripping is a process in which substances to be sepa-

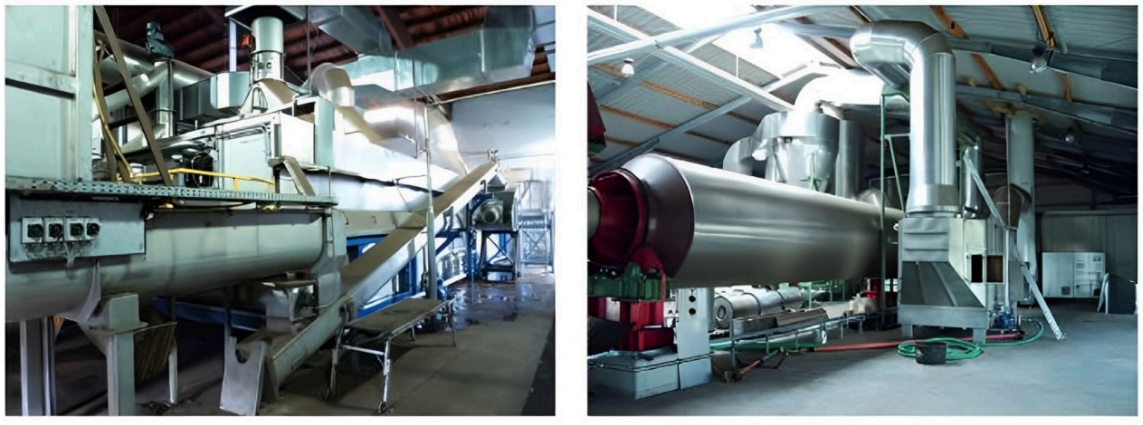


Figure 39: Belt dryer for fermentation residues of the biowaste fermentation Leonberg plant (left) Drum dryer for digestate (right) (Raussen and Kern, 2016).

rated are removed from liquids by passing gases through the liquid and the ingredients pass into the gas phase. The aim is usually to reduce the nitrogen content in the liquid digestate to such an extent that it can be used as fertiliser. To support the process, the temperature and the pH value can be increased. In the case of steam stripping, the temperature is increased, and the required gas volume flow decreases with increasing temperature. Desorption then takes place, the ammonia in the gas phase is converted into a disposable or recyclable product. The desorption of ammonia can be done by condensation, washing with acids or by reaction with an aqueous solution of gypsum. Ammonium sulphate is the end-product of desorption (Palakodeti *et al.*, 2021). **Table 12** gives an overview of possible processes for digestate treatment.

1.6 Environmental and plant protection

1.6.1 Avoidance of gaseous emissions and dusts

Gaseous emissions and aerosols: When operating CHP units, the combustion engines release gases such as nitrogen oxides, sulphur oxides and formaldehyde in addition to carbon dioxide. Depending on the type of engine used, its combustion settings and its condition, the quantity and composition of the gases released varies. The quality of the burnt biogas also has an influence on the assessment of engine emissions. The gases are released via the exhaust stack of the CHP unit (Persson *et al.*, 2019).

Operation-related emission sources can be represented by substrate and digestate storage, open pits or containers, and substrate preparation. Emitters are, for example, methane, ammonia, hydrogen sulphide and nitrous oxide. The amount of emitted gases depends on the construction and process-technological design of the biogas plant. To avoid emissions of gases and dusts, closed systems should be used instead of open systems. Gases may be released if components are leaking, if safety devices are activated or in the event of malfunctions (Persson *et al.*, 2019).

Aerosols are two-substance mixtures of suspended matter and a carrier gas. In biogas plants, there are various sources for the formation of aerosols. Examples include the dumping of dry substrates into storage containers, the improper handling of powdered operating materials, dust swirling up from vehicles or soot particles on the exhaust stack of a CHP unit (ASUE, 2014).

1.6.2 Avoidance of liquid emissions

Liquid emissions: The gases ammonia, carbon dioxide, carbon monoxide, hydrogen sulphide and nitrogen oxides can form aerosols and dissociate in water to form acids or alkalis. This brings the water hazard and possibly corrosive effects to the fore. Other liquid substances can escape due to incorrect storage, leaking components or incorrect handling. These substances can be silage leachate, liquid manure or digestate as well as operating fluids (Liebetrau *et al.*, 2013).

To detect the undesired escape of liquid phases, digesters must be equipped with leakage detection; pipes can be double-walled and equipped with leakage detection. All installations must be carried out professionally and checked for leaks before commissioning and regularly during operation. A wall can be built to collect escaping liquids. The concrete measures required can be derived from local laws and regulations and are determined during the approval process.

1.6.3 Emission reduction measures

For substrate delivery, the substrates should be delivered in closed containers; this ap-

Table 12: Comparative evaluation of digestate treatment processes (Fachagentur Nachwachsende Rohstoffe, 2013)

	Separation	Drying	Membrane technology	Evaporation	Stripping
Operational reliability	++	+/0	+	0	0
Dissemination status	++	+	+	0	0
Costs	+	+/0	0/-	0	+/0
Product usability					
Solid phase		+/0	0	0	0
Liquid (nutrient rich)	0	0	+	+	++
Liquid (nutrient poor)	0		+	0	0

++ = very good, + = good, o = average, - = poor

plies to odour-intensive and dusty substrates. The roads should be paved, and the biogas plant operator must ensure cleanliness on the premises. When storing the substrate, the silage must be covered; this reduces the seeping juices and dry matter losses. A film can be used for the cover. Dusty and odour-intensive substrates are to be covered and possibly stored in storage halls. Collection containers for the leachate must be installed and, if necessary, fed directly into the fermenter. Slurry systems are to be covered with an odour-mitigating film. The state of the art for substrate introduction is to cover the preliminary pit and the introduction opening. The filling level of all types of gas storage tanks must be monitored. Before the gas storage tank releases gas via the pressure relief valve due to overfilling, the gas utilisation system (CHP or gas emergency flare) must be activated. Safety-relevant components such as the supply of supporting air in the case of load-bearing roofs or the compressed air clamping in the case of single-skin foil roofs are designed redundantly and integrated into the emergency power supply. The emergency power supply must be designed in such a way that the supply is guaranteed after 20 minutes at the latest. This can be achieved by automatically starting generators or 24-hour standby service. In the case of load-bearing roofs, the supporting air must be checked regularly for methane content and the entire system should be checked annually for leaks using a methane-sensitive, optical gas camera. For additional gas consumption units, the following applies: before the overpressure safety devices are activated, another gas consumption unit (flare) must automatically go into operation. The maximum biogas flow must be assumed for the entire biogas plant. The over pressure and under pressure safety devices may only be activated after the biogas flare has been operated and in the event of a malfunction. All liquid-carrying pipes in gas-carrying containers must always be submerged to prevent biogas from flowing in. For this purpose, the filling levels must be monitored. This also applies to the condensate line from the gas system **(Liebetrau *et al.*, 2013)**.

In the field of biogas utilisation for CHP units, gas boilers, gas turbines, internal engine measures are taken to reduce emissions and exhaust gas purification devices such as ox-

idation catalysts are installed downstream. For biogas upgrading, post-treatment of the exhaust gas is necessary for all processes (except for amine scrubbing); this can be, for example, thermal post-combustion or catalytic post-combustion. For separation during digestate processing, these should be enclosed and have an exhaust air cleaning system. Ideally, the digestate should be processed immediately, and the solid fraction should be compacted and covered until it is spread. Drying should also be enclosed and have exhaust air purification. If necessary, the digestate storage facilities must be covered gas-tight (strongly dependent on the retention time and the substrate used) and have a connection to the gas utilisation system. A hydraulic retention time of 150 days in the gas-tight system should be observed for substrates that require a long retention time, such as straw. When transporting fermentation residues, the transport should take place in closed containers **(Beil *et al.*, 2021)**.

1.6.4 Noise protection

Noise emissions during the operation of biogas plants are mainly caused by vehicles and the operation of the CHP. One way to minimise noise emissions is to use an automated substrate feed system. This can minimise the frequency of use of wheel loaders or forklifts. The emissions of the CHP unit can be reduced by a soundproof cover and by installing it in closed rooms, halls, or containers. Silencers are installed at the supply and exhaust air openings and at the exhaust pipe. The use of low-noise air coolers as well as the avoidance of structure-borne noise transmission via the exhaust air stack, cooler or motor by means of sound-decoupled mountings are technical measures to reduce noise emissions. Often, sound predictions must be made before approval **(Liebetrau *et al.*, 2013)** and sound level measurements are carried out on the finished installation to confirm compliance with the limit values.

1.6.5 Explosion protection

Biogas can form an explosive gas mixture in combination with air. Depending on the state variables methane/carbon dioxide content, temperature, pressure, and humidity, they can shift the explosion limits. Above the limits, there is no longer a risk of explosion, but fires can be caused by open fire, switching functions of electrical equipment or lightning. It must therefore be assumed that there is an increased risk of explosion and fire in the vicinity of fermentation tanks and gas storage tanks. Different areas of the plant are therefore legally divided into different "potentially explosive atmospheres". Special labelling, precautionary and safety measures apply to these Ex-zones. The explosion zones of the individual components of biogas are shown in **table 13**. It is mandatory to draw up a risk assessment documenting where explosive atmospheres may form and describing appropriate countermeasures **(Cividino *et al.*, 2014)**.

Zone 0
In the hazardous area of zone 0, an explosive atmosphere occurs continuously over a long period of time or predominantly over time **(Fachagentur Nachwachsende Rohstoffe, 2013)**. These zones are generally not found in biogas plants **(Friedl and Keckstein, 2022)**.

Zone 1
In these areas, an explosive atmosphere may occasionally occur during normal operation of a biogas plant. These areas are usually in the immediate vicinity of entry points to the gas storage tank or on the gas-carrying side of the fermentation tanks and in the vicinity of blow-off devices, overpressure protection or gas flares **(Fachagentur Nachwachsende Rohstoffe, 2013)**. In the case of free ventilation, these areas must be provided with safety measures within a radius of 1 m. Only equipment approved for Zone 0 and Zone 1 and explosion-protected equipment may be used here. If Zone 1 occurs in a closed room, Zone 1 extends to the entire room **(Friedl and Keckstein, 2022)**.

Zone 2
In these zones, an explosive gas-air mixture is not expected to form during normal operation. If such a mixture does occur, it is likely to be very rare and not of long duration (e.g., during maintenance work or in the event of a malfunction). Zone 2 can be found, among other places, at the entry openings and inside the fermenter, and in the case of gas storage tanks, the immediate vicinity of the ventilation openings. In these areas, the measures of zone 2 must be implemented within a radius of 1 to 3 m **(Friedl and Keckstein, 2022)**.

For zones 0 to 2, no ignition sources may occur within the zones. Sources of ignition are, for example, hot surfaces, naked flames or mechanically or electrically generated sparks. Such areas must be provided with warning and information signs. There are also divisions of explosion protection zones for dusts, but these are of less relevance. They usually play a role if solid, dust-like operating aids are used improperly. This can lead to the formation of explosive atmospheres for a short time. Precautions must be taken to ensure safe storage and handling. For example, packaging and containers with operating materials must be tightly closed and the rooms must be adequately ventilated **(Fachagentur Nachwachsende Rohstoffe, 2013)**.

1.7 Potential for improvement

1.7.1 Technical potential for improvement

There is potential for technical improvement in many areas of biogas production **(Theuerl *et al.*, 2019)**. This includes above all the development of special aggregates adapted to the conditions of a biogas plant. There is a need for development, among other things, of separators for low-structure materials such as liquid manure, for drying plants or for ways to remove nutrients from the digestate in a targeted manner. Above all, there is a lack of specialised methods for digestate processing. Most of the methods used so far have been borrowed from other areas of application and therefore do not yet function optimally. However, due to the tightening of fertiliser guidelines, there will be more demand for targeted digestate treatment in the future.

There is also development potential in the development of novel processes for the fermentation of special input materials such as chicken manure or residues from the food industry. So far, these substrates can usually only be used for biogas production when mixed with other substrates.

Table 13: Properties of biogas components (Fachagentur Nachwachsende Rohstoffe, 2013).

		CH_4	CO_2	H_2S	CO	H
Density	kg/m³	0,.2	1.98	1.54	1.25	0.09
Density relative to air	-	0.55	4.53	1.19	0.97	0.07
Ignition temperature	°C	600	-	270	605	585
Explosion range	Vol.%	4.4 -16.5	-	4.3 - 45.5	10.9 - 75.6	4 - 77
Workplace exposure limit (MAC value)	ppm	n. s.	5000	10	30	n. s.

1.7.2 Biological improvement potential

There is potential for improvement in biology mainly in the adaptability of microorganisms to certain milieu conditions to increase process stability. Increasing the tolerance to pH-value fluctuations and to high ammonium concentrations would be particularly useful here. This would allow the increased use of poultry droppings. This can be obtained cheaply as a residual material from poultry farming, but quickly causes inhibitions in the fermenter due to its high nitrogen content. It would also be economically favourable to improve methane formation at lower temperatures. This would reduce the necessary heating capacity and more heat could be sold **(Wu *et al.*, 2019)**.

1.7.3 Potential for organisational improvement

There is potential for organisational improvement mainly in the legal framework for the construction of biogas plants. In recent years, the number of relevant directives and laws has risen sharply. This often leads to confusion even among the specialised authorities and thus hinders approval procedures. In addition, it is becoming increasingly difficult to find companies for individual plant components that can comply with all directives under the given conditions. An example of this is the construction of tanks with an approved system for leakage detection when the local groundwater level is high. Often, compromises must be made in terms of function, which are usually associated with additional costs.

Another point in need of improvement is the feed-in tariff for the electricity produced. Without state subsidies, electricity production in biogas plants is not economically viable. However, by being able to provide electrical energy on demand and with a very short lead time, the biogas sector makes an important contribution to grid stability in regions with a high density of wind and solar plants. In addition, methane emissions can be avoided massively when cattle or pig manure is used. On the other hand, the legal future is often unclear, especially for old plants. At the same time, the national implementation of the EU Directive for Renewable Energies (Directive 2009/28/EC) imposes further fundamental obligations on biogas plant operators.

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02

Technological description

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Abstract:

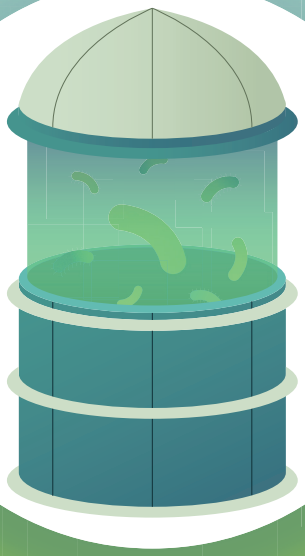
Waste disposal and green waste: In Germany, there are almost 700 plants to produce biogas from waste, and many more throughout Europe. Depending on the composition, the substrate properties of waste vary greatly. Technical challenges often exist in the processing of the waste. Special legal requirements also must be taken into account. Advantages, on the other hand, include low-cost purchasing and, in some cases, very high gas yields.

Agriculture and Livestock: Due to the widespread use of biogas plants that are operated with animal residues and energy crops, many findings on the technical requirements have been documented. In plants for the utilization of energy crops, care must be taken to ensure that the DM content and space load are not selected too high; the retention time should be about 60 days. If mainly animal residues are used, too high DM contents and overloading are usually not to be feared. The retention time can be chosen to be shorter than in other plants, often about 40 days are sufficient.

Successful biogas production relies on optimizing fermentation processes through careful management of technical parameters and the integration of advanced automation and control technologies, ensuring stable, efficient, and safe energy generation.

The primary goal of fermentation is to produce biogas for electricity and heat generation in an effective way, through:

- Operational Management Techniques
- Viscosity and Dry Matter Management
- Automation and Control Technologies



- Optimizing the space load, retention time, and methane concentration
- Monitoring and adjusting parameters like pH and temperature
- Controlling the dry matter content, viscosity and avoid foam formation
- Using of sensors, power feed-in systems, emergency power supplies...

PRIMARY OUTCOMES

- OPTIMIZED BIOGAS PRODUCTION
- ENHANCED PROCESS STABILITY
- IMPROVED SAFETY AND EFFICIENCY

2.1 The goals of fermentation

The goal of fermentation is usually the production of biogas for the generation and sale of electricity and heat. Even in plants where the biogas is upgraded to biomethane for injection into the natural gas grid, the production of biogas is the primary goal. Accordingly, the substrates are selected and used in such a way that the desired amount of biogas can be produced at the lowest possible cost. Downtime due to process disturbances and fluctuating gas production is to be avoided, and stable gas production is to be aimed for. The quality of the produced gas is also important, for use in combustion engines (CHP) the biogas must have a methane content of at least 45%. Also, the hydrogen sulphide content should not be too high, otherwise the biogas plant will be damaged.

An important side effect of the utilization of cattle or pig manure implants is the avoidance of methane emissions that would result from storage and direct spreading (Massé *et al.*, 2008).

However, in the case of plants for the anaerobic treatment of residual materials and waste, the focus is sometimes actually on waste disposal. In these cases, the production of biogas is only a welcome side effect that makes the process more economical.

2.2 Technical parameters of operation management

The following parameters can be used to obtain detailed information about the processes taking place in a biogas plant. These must be recorded, collected, and evaluated on a plantspecific basis. They serve to avoid process disturbances, to recognize trends, to increase plant safety and to optimize plant operation. A distinction can be made between control parameters for early detection of process disturbances (for example, volatile organic acids, FOS/TAC ratio, redox potential, hydrogen in the gas and liquid phases) and control parameters for assessing the process state (for example, substrate composition and quantity, gas production and composition, toxicity, fermentation temperature, DM content and viscosity of the fermentation mixture, and pH value (Drosg, 2013).

2.2.1 Space load

The space load (**figure 40**) indicates the amount of organic DM (oDM) in kilograms that can be fed to the digester per working volume per unit of time (Fachagentur Nachwachsende Rohstoffe, 2013).

Formula 1: Organic loading rate B_R

$$B_R = \frac{\dot{m} * c}{V_R * 100} \; [\text{kg oDM m}^3\,\text{d}^{-1}]$$

- $\dot{m}$ = Quantity of substrate supplied per unit of time [kg/d]
- c = Organic matter concentration [% o DM]
- V_R = Reactor volume [m^3]

The mass of the fed substrates is normally recorded by the feeder via built-in weighing elements in the case of solid substrates, or via flow meters upstream of the pumps in the case of liquid substrates. The DM content in the substrate must be continuously determined by random sampling, this also serves quality assurance purposes. Mobile measuring devices are usually available for this purpose at biogas plants. There are different balance limits for the room load. One level is the gas-tight, insulated fermentation tank, another is the total system (the sum of the working volumes of all levels) and with or without the inclusion of material recirculation. Depending on the reference variables, different space loads result. To be able to compare different biogas plants, it is recommended to specify the space load for the total system without material recirculation (Drosg, 2013). Which space load is chosen for the plant depends on the corresponding conditions. In general, an operator will choose the space load as high as possible to operate his plant effectively. Limits are set by the existing plant technology (performance of pumps, agitators and other aggregates), the maximum permitted biogas production (usually determined with the permit, together with the maximum amount of substances used), the time the substrates needed in the fermenter to be degraded (retention time) and the load capacity of the microorganisms. As a result of the material's transport through the fermenter, these are discharged with the fermentation residue. If more microorganisms are discharged than can be in the same period of time, their number decreases and with it the biological activity in the fermenter. Since the methane-forming archaea in the microbiological community

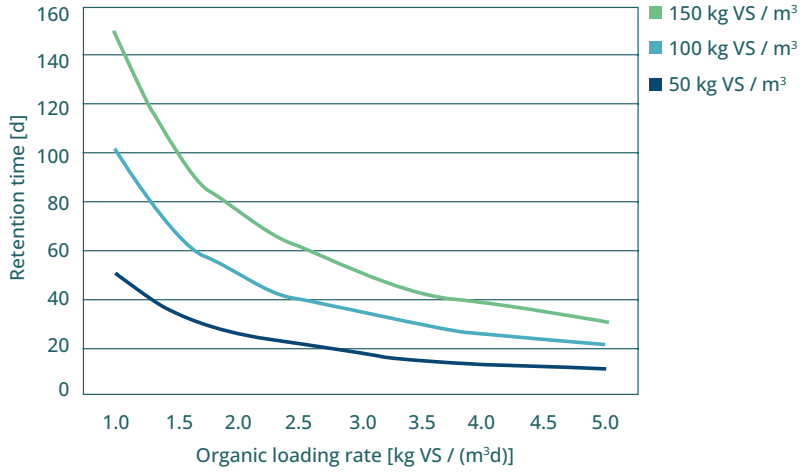


Figure 40: Correlation between organic loading rate and hydraulic retention time for various substrate concentrations (Adapted from Fachagentur Nachwachsende Rohstoffe, 2010a).

of a fermenter are usually the organisms that grow the slowest, methane formation then declines. Due to the lack of degradation of the acidic degradation products of the preceding biological processes, the pH then begins to decrease, further inhibiting the activity of the organisms. This is referred to as tilting of the fermenter. If the substrate supply (and thus the discharge of microorganisms from the fermenter) is not stopped immediately, the process is irreversible, and the fermenter must be restarted over a long period of time. How far the room load can be increased without tipping is a matter of experience. Here, operators can approach an optimal value in small steps over a long period of time (several months). The room load can also be specified in dimensions other than kg oDM m^{-3} d^{-1}. For example, the mass of volatile fatty acids (VS) can be used instead of the DM content kg VS m^{-3} d^{-1}. The adjustment of the space load is adjusted at the end via the added substrate quantity per time **(Schlegel *et al.*, 2008)**.

2.2.2 Retention time

The hydraulic retention time is the period that a substrate remains in the digester on a calculated average and is directly dependent on the space load **(Joutovsky *et al.*, 2004)**.

Formula 2: Hydraulic retention time

$$\tau = \frac{V_R}{\dot{V}} [h]$$

» τ = HRT= Hydraulic retention time

» V_R = Reactor volume [m^3]

» V = Volume of substrate fed per unit time [m^3/ h]

The composition of the substrates has a high impact on the required hydraulic retention time. Likewise, the temperature has a significant influence on the HRT. The dependence between temperature and residence time can be seen in **figure 41**. At higher temperatures the residence time decreases, at lower temperatures it increases. In the initial phase, the relative

gas yield increases sharply and flattens with increasing residence time. The residence time is selected so that the gas yield is as high as possible. The relationship between space loading and residence time is shown in **figure 41**. For a constant substrate composition, as the space load increases, more input is added to the fermenter and the residence time decreases. The reactor contents are constantly exchanged, and the residence time should be selected so that no more mi-

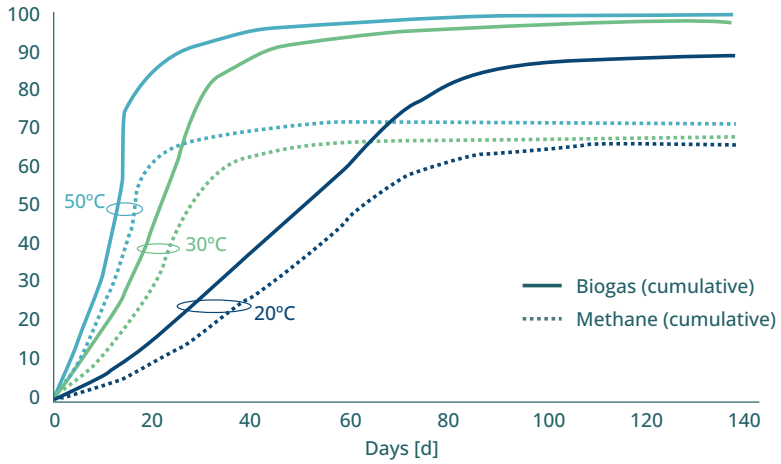


Figure 41: Relative biogas yields, depending on temperature and retention time (Adapted from Out, 2020).

croorganisms leave the fermenter via the effluent mass than can regrow in it. If the residence time is too short, the microorganisms cannot decompose the introduced substrate, which reduces the gas yield. For this reason, the residence time must be adapted to the substrate used. If the amount of substrate added and its composition are known, the reactor volume can be calculated for a given residence time **(Fachagentur Nachwachsende Rohstoffe, 2013)**.

2.2.3 Productivity, yield and degree of degradation

If the gas production is related to the fermenter volume, this corresponds to the methane productivity. It is defined as the quotient of the daily gas production and the reactor volume, and thus provides information about the effectiveness **(Schlegel *et al.*, 2008)**. The productivity can refer to the biogas (P_{biogas}) as well as to the methane (P_{CH4}).

Formula 3: Methane productivity

$$P_{CH4} = \frac{\dot{V}_{CH4}}{V_R} \left[Nm^3 * m^3 * d^{-1}\right]$$

» V_R = Reactor volume $[m^3]$

» V_{CH4}= Methane production $[Nm^3 / d]$

If the gas production is related to the input substances, this results in the yield. It is defined as the quotient of the amount of gas produced, and the organic matter added **(Fachagentur Nachwachsende Rohstoffe, 2013)**. It provides information on the efficiency of methane production from the substrates feed into the digester. As an individual parameter, the yield is not very meaningful since the effective load of the fermenter has no influence in this respect. For a better understanding of the process, the yield should therefore always be stated together with the space load **(Schlegel *et al.*, 2008)**.

Formula 4: Methane yield

$$A_{CH4} = \frac{\dot{V}_{CH4}}{\dot{m}_{oDM}} \left[Nm^3 * m^3 * d^{-1}\right]$$

» V_{CH4}= Methane production $[Nm^3 * d]$

» $\dot{m}_{oTS}$ = Added organic dry matter [t / d]

The degree of degradation η_{oDM} describes the efficiency of utilization of the substrates used **(Schlegel *et al.*, 2008)**.

Formula 5: Biomass degradation rate

$$\eta_{oTS} = \frac{oDM_{sub} * m_{in} - oDM_{Abl} * m_{out}}{oDM_{out} * m_{in}} * 100\ [\%]$$

- m_{in}= Mass of fresh mass added [t]
- oDM_{out}=organic dry matter content of the fermenter effluent [kg/t]
- m_{out}=Mass of the fermentation residue [t]

2.2.4 Gas composition, gas volume measurement and gas analysis

The gas composition of biogas can be taken from **table 14**. Like the gas production, the gas composition depends very much on the load of the digester and the composition of the substrates used. If the gas production or methane content decreases with a constant feed, this indicates a disturbance or inhibition of the processes. Biogas quantity measurement and biogas composition measurement are mandatory on biogas plants and are best suited for process control, as they are measured continuously and react relatively quickly.

Table 14: Composition of biogas (Fachagentur Nachwachsende Rohstoffe, 2013).

Gas	Concentration
Methane (CH_4)	50 - 75 Vol.-%
Carbon dioxide (CO_2)	25 - 45 Vol.-%
Water (H_2O)	2 - 7 Vol.-% (20-40 °C)
Hydrogen sulphide (H_2S)	20 - 20,000 ppm
Nitrogen (N_2)	< 2 Vol.-%
Oxygen (O_2)	< 2 Vol.-%
Hydrogen (H_2)	< 1 Vol.-%

Gas production at biogas plants is not always measured directly by a flow meter. It can also be determined indirectly by the filling level of the gas storage tanks and the output and running time of the CHP units. Since their gas consumption is known, this is sufficient to infer the amount of gas produced. The filling level of the gas storage tanks is important to be able to control the operating times of the CHP units. In double diaphragm roofs, the level is often determined by means of a tensioning cable with a rod and angle sensor **(Lossi and Pütz, 2021)**. Another method is to use a tension cable with a pulley, measuring weight and proximity sensors **(Lossi and Pütz, 2021)**. These mechanical systems have proven themselves in practice and offer the advantage of eliminating ignition sources within the roof. Other systems for detecting the height of the gas storage membrane are based on ultrasound or distance measurement with lasers. In practice, these systems have proven to be more vulnerable and less reliable. In some cases, the gas volume flow is measured before entering the gas control section in the CHP unit. To obtain good measurement results, the gas should already be dried. Thermal probes are used for the measurement. Mechanical volumetric flow meters are less suitable due to the moisture in the gas. The gas analysis is carried out either with mobile gas measuring instruments for individual monitoring of the containers, or it is carried out in a central recording before entering the gas control line. The stationary gas measuring instruments usually record the parameters CH_4, CO_2, O_2 and H_2S.

The methane concentration is the most important component of biogas for the evaluation of the operation management. It represents the combustible fraction of biogas and directly influences the calorific value. The higher the proportions of carbon dioxide and nitrogen, the more the calorific value of the gas is reduced. The gas composition and thus the methane content is mainly dependent on the substrate used; the composition can only be influenced to a limited extent by controlling the process. The methane con-

centration depends on the process parameters such as the fermentation temperature, the load condition of the reactor and the hydraulic retention time as well as on process disturbances and procedures such as biological desulfurization (Weiland, 2010).

2.2.5 Fermentation temperature

Fermentation is an exothermic process, nevertheless, to maintain a defined temperature level, the fermentation tanks must be equipped with heating. The temperature must be measured during the fermentation of the substrates. A rapid change of the temperature results in a continuous disturbance of the process biology, due to which the temperature is not suitable as a variable for process control. The temperature inside the fermenter has a strong effect on the environmental conditions. With an increasing temperature, the balance between ammonia and ammonium shifts towards ammonia. This has an inhibitory effect on the acetoclastic methane bacteria and thus has negative consequences, especially when processing protein-rich substrates. Therefore, the temperature of the anaerobic degradation processes must be monitored. Temperatures below 40°C slow down the biology within the fermenters, the retention time increases, at temperatures above 50°C fewer bacterial strains are present, and the overall process becomes more prone to failure (Song *et al.*, 2004). Temperature sensors are placed at different heights in the fermenter.

2.2.6 Dry matter content and viscosity of the fermentation mixture

The DM content and viscosity are important parameters with regard to the mixing, pumpability and gas discharge of the digested sludge. If the gas bubbles in the digester cannot escape, this results in foam formation. The DM value does not correlate directly with viscosity, yet it is monitored in many plants (Krieg and Fischer, 2001). Direct measurement of the viscosity of the fermentation substrates is very difficult, but it can be measured indirectly via the power input of the agitators. This is possible because the power input of the agitators depends, among other things, on the viscosity of the mixture to be agitated. Normally, the agitators are controlled via a fixed speed or via a predefined time interval. Automatic control of the agitators is state of the art. Nevertheless, manual intervention is also necessary, for example if the viscosity is too high, it must be mixed with fermentation substrate with a very low DM content, with process water from the biogas plant or fresh water. The fermenter is fitted with sight glasses, which are used for visual inspection of the fermenter contents. Associated with this is the problem of foam formation. This is the result of reduced surface tension, which is caused by surface-active substances (Koch *et al.*, 2017). The exact causes of foam formation in a biogas plant are often not known. It occurs more frequently, for example, when spoiled silage is used. It is possible that surface-active intermediates or bacterial groups in interaction with increased gas production are the cause. Foam can clog the gas lines and increase the pressure in the fermenter, which can trigger the overpressure protection or even destroy the roof (Fachagentur Nachwachsende Rohstoffe, 2013). The state of the art in digesters is the monitoring of the filling level and, to some extent, the formation of foam. Pressure sensors, conductivity sensors and occasionally infrared sensors are used for this purpose. It is controversial whether measurement of foam formation is useful; these can create a false sense of security. In the event of a biological overreaction occurring and the foam sensor responding, only a few minutes remain before foam enters the gas sampling system. In extreme cases, even the foil roof can tear open. If there is no foam sensor, charging processes that lead to an overdrive of the biogas plant must be avoided (Lossi and Pütz, 2021).

2.2.7 FOS/TAC

The FOS/TAC determination is a titration test for determining the quotients of acid concentration and buffer capacity in the fermentation residue **(Lili *et al.*, 2011)**. The FOS/TAC value therefore provides knowledge about the degradation performance of the fermenter. FOS stands for Volatile Organic Acids, unit [mg/l acetic acid equivalents] and TAC for Total Inorganic Carbonate (alkaline buffer capacity, unit [mg $CaCO_3$/l]. The value is a measure of the acidification risk of a plant. With the help of the FOS/TAC, process disturbances can be detected at an early stage and a reaction can be taken if necessary **(Reinhold, accessed 04.11.2021)**. The determination is carried out in external laboratories and not in the biogas plant. However, a contract with an appropriate laboratory for sampling and control is not unusual.

As the percentage of organic acids in a digester increases, the methane-producing bacteria are inhibited. With decreasing pH, the inhibition effect increases (at pH >7.4). However, the pH value is only suitable for accurate process analysis to a limited extent, since the acids in the fermenter are initially buffered. In contrast to the pH value, the FOS/TAC value detects the presence of the buffer **(Postel *et al.*, 2009).**

The determination can be done by manual titration or by a titrator. A measurement is carried out in the first step by drawing a representative fermentation substrate sample. In a further step, the sample is freed from coarse components (e.g., by means of a filter or a centrifuge). A defined amount of substrate is taken and immersed in an electrode. The sample is placed on a magnetic stirrer and permanently homogenized. Now titration with 0.1 N H_2SO_4 follows until a pH value of 5 is reached. The acid consumed is recorded. This step is repeated until a pH value of 4.4 is reached. The value can then be calculated according to formula 8 **(Reinhold, accessed 04.11.2021)**.

Formula 6: Determination FOS

Formula 7: Determination TAC

$$TAC = \frac{20 * FP_1 * f_{H_2SO_4} * 250}{\text{sample size}}$$

Formula 8: Determination FAS/TAC Ratio

$$FOS/TAC = \frac{FOS}{TAC}$$

- FP_1= mixed end point up to pH 5.0
- FP_2= mixed end point up to pH 4.4
- 1,66 = free organic acids
- 0.15 = free organic acids
- 500 = free organic acids
- 250 = free organic acids
- f_{H2SO4} = titer from c (H_2SO_4)=0.05 mol/L

$$FOS = \frac{20 * ((FP_2 - FP_1 * 1{,}66 - 0{,}15) * f_{H_2SO_4} * 500}{\text{sample size}}$$

A FOS/TAC ratio of 0.3 to 0.4 is considered normal in practice, but this value is fluctuating in practice because it is plant-specific; there is a strong dependence on the substrate composition. In Energy crops plants, the value is between 0.4 and 0.6 with stable process control **(Singlitico *et al.*, 2017)**.

The smaller the amount of acid required until the pH value of 5.0 is reached, the lower the buffer capacity of the substrate. If there are few organic acids in fermenter, the more acid addition is needed to reach a pH of 5.0. The higher the basic buffer, the more acid is needed to get to a pH of 5.0. A high starting pH above 7.9 is indicative of increased NH_4-N. A high FOS value means that there are more organic acids in the digester, the higher the FOS value, the more acid is needed to get from a pH starting at 5.0 to a target pH of 4.4. Not only is the FOS/TAC ratio required for accurate interpretation, but the individual trends for FOS and TOC must also be considered for accurate analysis. For example, if the FOS/TAC ratio is thought to be less than 0.5,

it may be that the organic acid content is too high, and they are merely well buffered (Lossie and Pütz, 2021). **Table 15** gives an overview of the FOS/TAC ratios, their significance, and the resulting measures.

Table 15: The relationship between the FOS/TAC value and the condition of the plant, as well as the measures required to balance the acing of the plant (Lossie and Pütz, 2021).

FOS/TAC Value	Meaning	Measures
>0.6	Plant heavily overfed	Stop feeding
0.5 - 0.6	Plant overfed	Restrict feeding
0.4 - 0.5	Plant heavily loaded	Increase observation
0.3 - 0.4	Plant utilized to capacity	Keep feeding as is
0.2 - 0.3	Plant hungry	Slowly increase feeding
<0.2	Plant very hungry	Increase feeding rapidly

2.2.8 pH value and volatile fatty acids

The optimum pH environment for the microorganisms across all process stages is between 6.4 and 8. Within this operating window, good gas yield and gas composition is produced (Li *et al.*, 2008).

The pH value is also measured on site by instructed operators using common pH measuring instruments. The value is only suitable to a limited extent for an accurate process assessment; the buffer properties of the fermentation mixture cause a sluggish reaction of the pH value (Lindner *et al.*, 2015). Nevertheless, the pH value is an important value to record. The recording of the measured values shows tendencies and trends, which can be used to draw conclusions about other parameters such as ammonium and ammonia. Prolonged standing or sample transport changes the pH value of the sample; on-site pH values are usually somewhat lower than those determined in the external laboratory (Li *et al.*, 2008).

The biological processes are strongly dependent on the pH value, for optimal methane formation it lies in a range between 7 and 7.5, formation of biogas is also possible outside this window. In a single-stage system, the pH value automatically levels off; the bacterial groups act here as a self-regulating system. In multi-stage systems, the pH value at the hydrolysis stage is between 5 and 6.5, as the acid bacteria are at their optimum here. In the methane-forming phase, it is again in the neutral range, due to the buffer capacity of the medium and the degradation activities. By means of the pH value, the dissociation equilibria of metabolic products such as ammonia, organic acids and hydrogen sulphide can be controlled. Because of the buffering capacity of the medium (hydrogen carbonate and ammonium), the pH value remains stable. If the pH moves outside the optimum, this is an indication of a serious process disturbance, immediate action should be taken. Volatile fatty acids are an intermediate product of fermentation. At high concentrations, they have a process-inhibiting effect. If the individual acids are analysed, this can be used to describe the state of the process. Acetic acid should ideally be present in the low single-digit gram range per litre. The concentration of propionic acid should be well below this, and other fatty acids should be lower by a power of ten. Individual fatty acids are determined chromatographically. The acids dissociate as a function of pH in an aqueous solution. The fatty acids investigated are acetic acid, propionic acid, iso-butyric, butyric, iso-valeric, valeric and carboxylic acids (Fachagentur Nachwachsende Rohstoffe, 2010b). The individual organic acids are expressed in mg/l, and the acetic acid equivalent is also calculated. This is a sum parameter; the different weights of the fatty acids are uniformly calculated to the weight of the acetic acid. The acetic acid equivalent and propionic acid are the indicators used for process

evaluation. If the total acid value is less than 1 g/l and the propionic acid content is less than 200 mg/l, the process is stable. If the values exceed 3 g/l and 300 mg/l for propionic acid, the degradation process is not optimal and there is a malfunction **(Fachagentur Nachwachsende Rohstoffe, 2010b)**. In the case of intermittent feeding with large quantities, the acid concentration increases and if the same total quantity is distributed over several smaller doses the acid concentration remains low. If the load is too high due to incorrect feeding, not all long-chain fatty acids can be broken down. This leads to an accumulation of propionic and butyric acids. In the case of overfeeding or fluctuating substrate contents, an accumulation of acetic acid occurs, this is associated with a decrease in methane content. The non-degradation of acetic acid is associated with a decrease in the activity of methanogenic bacteria **(Li *et al.*, 2008)**.

2.3 Process variants

2.3.1 Dry matter content

For substrate characterization, the amount, concentration, and composition of the substrate must be known. For the concentration, parameters such as dry substance content (DM) and organic dry substance content (oDM) are specified. For liquid substrates, the chemical oxygen demand (COD) parameter is also added, and another parameter is the total organic carbon (TOC) value. For practical purposes, only the DM and the oDM value are relevant. The oDM degradation rate indicates how much of the added organic DM was degraded during the fermentation process.

When determining the DM value, a sample of the substrate to be fed is taken. The DM value of substrates is usually measured by operators on site. Often, several substrates with different DM content are used; the DM content of the substrate mixture is decisive, but not the DM content of the individual substrates **(Streicher *et al.*, 2016)**.

To determine the oDM value, the sample is calcined at 500°C. The loss of mass is called organic mass loss. The loss of mass is called organic dry matter. The oDM parameter is not very meaningful in terms of degradability or expected biogas production. When the sample is heated, volatile substances are evaporated and are therefore no longer available for analysis. This leads to deviations in the estimation of the gas potential, especially in the case of highly acidified substrates. A residue is left behind when the gas is burned up; this represents the proportion of inert ingredients in the substrate **(Fachagentur Nachwachsende Rohstoffe, 2010a)**. The oDM content cannot be determined on site and is therefore less used by operators to assess the substrate **(Weißbach, 2008)**.

Process differentiation based on DM content: From a biological point of view, a strict division of the processes into wet and dry fermentation is misleading; the microorganisms require a liquid medium for their growth and survival in any case. There is no precise definition of the boundary between wet and solid-state fermentation, but guide values have become established in practice. A DM content of up to approx. 12% in the fermenter is referred to as wet fermentation; the fermenter contents are still pumpable under these conditions. If the DM content increases to 15-16%, this process is referred to as solid matter fermentation or dry fermentation, and the material is generally no longer pumpable **(Streicher *et al.*, 2016)**. The rule of thumb is not always applicable, there are some substrates (e.g., dispersed food waste or poultry manure) with finely dispersed particle distribution that are still pumpable at a DM content of up to 20%. Other substrates, such as fruit peels, are in stackable form at a DM content of 10% **(Lossie and Pütz, 2021)**. For stable process control, the DM content of the substrate used should remain constant; if it increases too much, the substrates must be further liquefied; if the DM content falls, it must be counteracted with appropriate substrates with a high DM content. The higher the DM content, the higher the gas production. **Table 16** shows an overview of guide values for mass decomposition and DM content after fermentation for different

Table 16: Guide values for mass degradation and total solid content after fermentation for different individual substrates (Reinhold, accessed 04.11.2021).

	Unit	Cattle slurry	Pig slurry	Stable manure	Dry chicken manure	Corn silage	Corn-Cob-Mix	Grain
DM	%	8	6	25	45	32	65	86
VS	%	80	80	80	75	95	95	98
Mass reduction	%	2.3	2.3	11	19	24	55	75
VS reduction	%	33	46	52	58	70	79	81
DM-Content after fermentation	%	5.9	3.8	14.5	14.5	10.8	14.7	17.4

individual substrates. In any case, however, the agitation and conveying technology must be adapted to the DM content in the fermenter **(Weißbach, 2008)**.

2.3.2 Number of process stages

One stage refers to the process-specific biological environment-hydrolysis or methanation phase **(Lossie and Pütz, 2021)**. If the fermentation process takes place in one system at one temperature and pH under identical process conditions, this is a single-stage process. If two fermentation tanks are used with separate hydrolysis and methanation, this is two-stage process control. The phase denotes the process tank independent of the biological stage **(Nasr *et al.*, 2012)**. In a single-stage system, there is no spatial separation of the phases (hydrolysis, acidification phase, acetic acid and methane formation). For two-stage or multi-stage processes, spatial separation of the phases is aimed at different vessels. For two-stage processes, the hydrolysis and acidification phases take place in separate external vessels. A two-phase or three-stage biogas plant consists, for example, of a hydrolysis tank, fermenter, and secondary fermenter.

In the case of continuous processes, there are three relevant variants. A gas-tight sealed receiver is not considered as an independent phase **(Lossie and Pütz, 2021)**.

1. **Flow-through process (see figure 42):** downstream of the digester is an open digestate storage tank. This variant is two-phase because different temperatures prevail in the preliminary pit and the fermenter. The open digestate storage does not count as fermentation, since gas formation should already be completed in the fermenter to allow open storage.
2. **Storage process:** The digestate storage system also serves as a post-digester to increase the retention time in the gas-tight system. This variant is three-phase and two-stage.
3. **Combined flow-through storage process (see figure 42 & 43):** this combination consists of a digester and a gas-tight, covered digestate storage. Since the digestate storage still captures biogas, this is considered another stage.

Flow-through method: In the past, most plants were operated according to the flow-

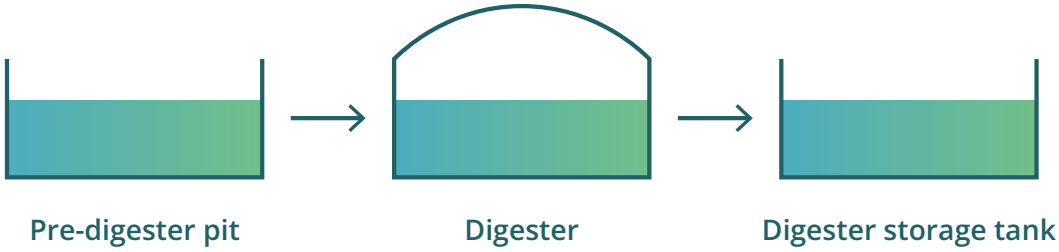


Figure 42: Schematic of the through-flow process (two-phase, two-stage) (Fachagentur Nachwachsende Rohstoffe, 2010b).

Figure 43: Schematic of the combination throughflow (two-phase, three-stage) (Adapted from Fachagentur Nachwachsende Rohstoffe, 2010b).

through method. The substrate is pumped or conveyed from a storage tank into the fermenter. The identical quantity that is fed to the fermenter in this process enters the digestate storage via displacement or withdrawal. The digester is permanently filled and is only emptied if required for maintenance tasks. This process has good and uniform gas production, as well as good digester utilization. Short-circuit flow can occur under unfavourable conditions, but this is usually prevented by the arrangement of feed and discharge. There is a risk of methane emissions in an open digestate storage system.

Combined flow-through and storage process: In this process, digestate storage is also covered. Biogas is produced in the digestate storage facility, which can be collected and utilized. The digestate storage system acts as a storage facility for gas and digestate. The storage facility is downstream of a flow-through fermenter. This process allows for uniform gas production and is state of the art.

For all three processes, regular feeding must take place in small quantities. This applies in particular to single-phase operation without a hydrolysis stage. Intermittent feeding leads to an overload of the fermenter's biology. Multi-stage operation allows material to be recycled from the secondary digester to the digester. This may be necessary if the methane fermentation phase be additionally stabilized **(Lossie and Pütz, 2021)**.

Discontinuous feeding: Discontinuous processes are usually operated in two stages. The two stages consist of a box fermenter and percolate storage. A fermenter battery usually consists of four units, whereby quasi-continuous operation is achieved. While one digester is being fed, the remaining digester units are in operation. To feed a fermenter, the percolate regime is set beforehand **(Nasr *et al.*, 2012)**.

Discontinuous processes can be divided into single-phase and two-phase variants.

1. **Solid-state fermentation single-phase:** the substrate in the box unit is inoculated and strongly percolated so that it enters the methane phase as quickly as possible. The methane phase is permanently given in the percolate storage **(Lossie and Pütz, 2021)**.
2. **Two-phase solid matter fermentation:** Here, there is no inoculation of the fermented material. As a result, mainly hydrolysis/acidification is created in the pit fermenter. The percolate reservoir thus represents the only methane phase **(Qian *et al.*, 2016)**.

2.3.3 Temperature control

In practice, there are two main temperature ranges: mesophilic and thermophilic. In practice, these are operated at temperatures of 36°C to 44°C (mesophilic) and 50°C to 58°C (thermophilic). **Figure 44** shows the relative gas production as a function of the fermenter temperature of mesophilic and thermophilic bacterial strains. The optimum temperature for many bacterial strains involved in the fermentation process is between 30 - 40°C. In this temperature range there is a higher gas production, a higher species diversity and lower concentrations of inhibitory ammonia. The thermophilic temperature range is above the optimum for hydrolysis and acidogenesis and is an optimum for aceto- and methanogenesis. If harmful germs are to be killed, the thermophilic mode should be used. Compared to the mesophilic mode, the thermophilic mode has a better biogas yield, but only a slightly better methane yield can be achieved. Thermophilic operation is more susceptible to failure because it is subject to instabilities. This mode of operation has a lower number of different microorganisms compared to mesophilic operation. The thermophilic mode is more susceptible to temperature fluctuations, especially when the temperature is lowered. However, the higher the temperature, the faster the substrate turnover occurs only within certain limits, as other factors limit it. **Table 17** shows the temperature control in a multistage operation. Which combination is used, depends mostly on the substrate composition. The most used combination is mesophilic/mesophilic, which is used for slurry and most raw plant materials. Components with thermophilic operation are used for substrates with increased fibre content, such as green waste or vegetable waste **(Chae *et al.*, 2008)**.

Table 17: Overview of temperature control for multistage operation (Postel *et al.*, 2009).

Fermenter	Post fermenter	Advantages	Disadvantages
Mesophilic	Mesophilic	Stable biology	Organic degradation takes longer
Thermophilic	Mesophilic	Fast structure decomposition, better flowability in further process chain	Biology susceptible, as fermenter biology most sensitive
Mesophilic	Thermophilic	Stable biology with slightly increased gas yield and good flowability from secondary fermenter	Increased ammonia content in the biogas stream puts a strain on the CHP engine (see below)
Thermophilic	Thermophilic	Increased gas yield (but with somewhat lower methane content)	Vulnerable operation, usually less energy available for external CHP use.

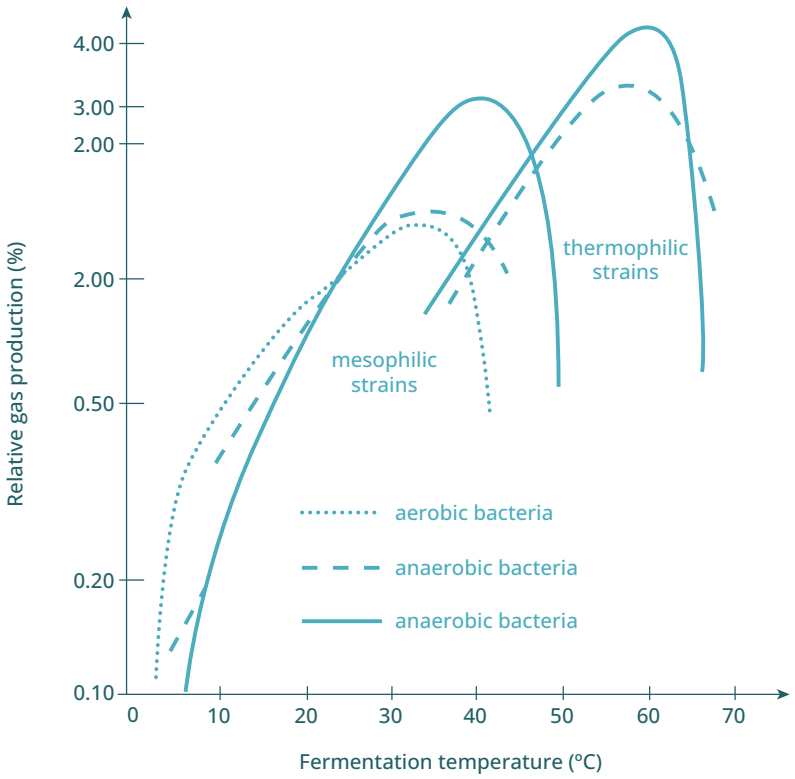


Figure 44: Fermenter temperature vs the relative gas production rate (Adapted from https://www.unilibenergy.com/biogas/).

The secondary fermenters or digestate stores are usually not heated, but secondary fermenters are often equipped with thermal insulation. Thermophilic operation increases the proportion of trace gases such as H_2S and N compounds in the biogas stream. As the temperature increases, the NH_3-NH_4-N equilibrium shifts towards ammonia. Mesophilic operation results in less organic degradation for the same residence time; this can be compensated by longer residence times.

The thermophilic mode of operation reduces the risk of technical failure, this is due to the lower viscosity of the digestate. This protects the pumps used as well as the valves. However, this is only the case if all plant components involved are appropriately designed for higher temperatures. However, there is an increased risk of foam formation, which can escape via the safety equipment.

2.3.4 Auxiliary materials/ additives

Normally, relatively monotonous substrate is used in biogas plants when using energy crops, which can lead to deficiency symptoms as well as to the accumulation of inhibitors. A mix of different energy crops and the use of manure can minimize the need for the use of fermentation aids. The use of fermentation additives depends on the substrate used, fermentation conditions, and other factors. Additives are substances added to the fermentation process with the aim of positively influencing it. All substances or working materials added to the fermenter to promote the microbiological degradation processes, which are not substrates, are auxiliary substances. The fermentation aid itself has no biogas formation potential or

this is negligible. Fermentation aids can be of organic or inorganic composition (including algae preparations, trace elements for supplying the microorganisms, enzymes for hydrolysis) **(Messtechnik für die Biogasanlage..., accessed 10.11.2021)**.

Two applications can be defined for the use of fermentation aids, on the one hand the one-time addition in the event of a malfunction and on the other hand the prophylactic-preventive application. Practical experience has shown that instabilities in the fermentation process can occur if liquid manure and solid manure are not used. This can be caused by a lack of micronutrients as well as the presence of inhibitors. In practice, this is counteracted by using fermentation aids. There are different reasons for using them, e.g., the specific methane yield is too low [m^3/kgoDM], failure to achieve higher digester load [kgoDM/ (m^3 * d)], reduction of hydrogen sulphide, high viscosities, and insufficient mixing, foaming and floating layer formation. The examples listed are indicative of a process upset. Prompt laboratory analysis allows the operator to intervene quickly and efficiently. The symptoms can be traced back, for example, to an accumulation of volatile organic acids, a shift in the acid spectrum towards higher-value fatty acids, an increasing FOS/TAC value, an increased concentration of ammonia. Also, causal factors can be an insufficient degradation efficiency as well as an increasing solids content in the fermenter **(Messtechnik für die Biogasanlage..., accessed 10.11.2021)**.

These examples illustrate that, in addition to the efficiency of the biogas plant (gas yield with short retention time), the ongoing operating costs are also a reason for using auxiliary materials. These can be used to reduce the energy required for the agitators, and the cost of desulfurization can also be reduced. If the benefit of fermentation aids exceeds the expense, their use is recommended. **Table 18** shows typical fermentation aids with examples and the areas of application. A clear subdivision of the individual substances is difficult in that they often have several modes of action at the same time.

Legal regulations apply to the use of auxiliary substances; too high an addition of these can lead to the fermentation residue no longer being allowed to be discharged, for example **(Landwirtschaft, B. B, 2018)**.

Trace elements: The microorganisms involved in anaerobic fermentation require nutrients, the so-called bulk elements, to maintain metabolism and for their own reproduction. These bulk elements are: Hydrogen (H), Carbon (C), Nitrogen (N), Oxygen (O), Phosphorus (P) and Sulphur (S) (12). Also, sufficient sodium (Na), potassium (K), calcium (Ca), iron (Fe) and magnesium (Mg) must be present **(Drosg, 2013)**. To maintain their metabolism and to produce enzymes, microorganisms need so-called trace elements. These are involved in enzymatic conversions, in the formation of co-factors, in redox reactions and other processes in which living organisms are involved. Trace elements in the biogas reactor include nickel (Ni), cobalt (Co), molybdenum (Mo) and selenium (Se). But also, other metals, such as copper (Cu), zinc (Zn), manganese (Mn), tungsten (W), or vanadium (V) and nonmetals such as boron (B) **(Drosg, 2013)**. If a plant is operated with slurry or manure, enough trace elements are available to the biogas plant, but a deficiency can still not be excluded. Biogas plants that use only corn silage have long-term trace nutrient deficiencies. These deficiency situations occur with mono fermentation of energy crops. Trace elements usually come from the substrate, but are often not available in required amounts, so the additional supply of trace elements is the rule in practice. Biogas plants with a very high slurry input, good management and low space loading can do completely without the supplementation of micronutrients. Trace element supply is a function of the biocenosis in the digester and is determined by technical, physical and chemical conditions. Poorly soluble precipitated sulphides in the fermenter, for example, can reduce the concentration of trace elements by several orders of magnitude. Agitators can release trace elements, which leads to an increase in the concentration of trace elements. The trace element contents added must be determined by an external laboratory 97 service provider and must be

Table 18: Typical fermentation aids with examples and areas of application (Fachagentur Nachwachsende Rohstoffe, 2010b).

Type of fermentation additive	Examples	Definition
Trace elements	Iron, cobalt, nickel, zink, etc.	Trace elements are chemical elements, that are necessary for the optimal growth of microorganisms.
Ion exchanger	Zeolites, clay minerals	Reduce the concentration of potentially inhibitory/ toxic fermenter ingredients
Microorganisms	Hydrolytic cultures	Completion of the existing biocenosis organisms that optimize the process (speed, stability) or faster adaptation to new substrate compositions or changed enable boundary conditions
Enzymes	Cellulase, amylase, protease, xylanase	Enzymes break down polymers and improve suspension properties, increase the degradation rate and support microbial activity.

repeatedly validated if the process is stable. Proper use of trace elements can accelerate fermentation, results in a more stable process, and greater space loads and biogas yields can be achieved. Overdosing can have an inhibitory effect on the fermentation process; too many elements have a toxic effect on the biocenosis **(Drosg, 2013)**.

Enzymes: These are proteins that are involved in degradation processes as biological catalysts. In normal operation, these enzymes are produced by the microorganisms; with an optimal fermenter operation, the new enzyme formation is ensured. According to the manufacturer, the addition of enzymes improves the degradability of plant components, which should be broken down more quickly. This is said to improve the productivity of the gas process. By adding enzymes, a faster and more intensive digestion of the biomass is to be achieved, the viscosity is to be reduced, which lowers the stirring energy costs. Furthermore, a faster degradation of cellulose and hemicellulose is to be achieved, floating layers in the fermenter are dissolved. Massive piles of solids from grain, hay, grass silage and solid manure are to be better dissolved and liquefied, floating layers in the final storage are to be avoided with enzymes. Practical studies had shown that there can be significant effects with substrates rich in water and through the application of enzymes. Enzymes do not have a protein structure and are therefore subject to bacterial degradation; for a lasting effect, appropriate preparations must be continuously added to the process. **(Bochmann *et al.*, 2007)**.

Additives to reduce the hydrogen sulphide concentration: Gaseous hydrogen sulphide is formed in the biogas process from organic compounds of sulphur, which are reduced to hydrogen sulphide by microorganisms. Increased levels of organic sulphur components are present in canola and rapeseed products and in protein-rich substrates such as grain, food waste, pig manure, and poultry manure. Hydrogen sulphide affects many microorganisms and can inhibit methane formation in the digester. Sulphide precipitation binds trace elements in the fermenter, which are thus no longer available to the microorganisms. By using the auxiliary substances iron (II) and iron (III) salts such as $FeCl_2$, $FeSO_4$, $FeCl_3$ and $Fe(OH)_3$, a targeted precipitation of sulphide is possible so that it does not enter the biogas as hydrogen sulphide **(Meegoda *et al.*, 2018)**.

Additives for the reduction of the ammonia concentration: Ammonia occurs in low

concentrations in biogas. It is formed during the breakdown of proteins and nitrogenous compounds such as urea or uric acid in animal excrement. To reduce the ammonia concentration, mineral substances are added as additives. These bind excess ammonium ions. Molecular sieves and clay minerals, which can fix ammonia by ionic forces, are also used for the sorption of ammonia. In this process, cations such as potassium, calcium or magnesium are replaced from the lattice structure and bound to ammonium as in an ion exchanger. The ammonium remains bound to the solids and is thus removed from the system. If these additives are used incorrectly or too frequently, sink layers may form, the effectively usable fermenter volume decreases as a result, and there may also be an abrasive effect on the agitators **(Drosg, 2013)**.

Minerals and buffers (pH stabilizers): Methane formation requires a stable pH value. The pH value is influenced by the base and carbonate concentration or the lime-carbonic acid balance, as well as by the ratio of ammonium to ammonia. If excessive amounts of carbonic acids are formed in the fermentation process, acidification of the fermenter contents may occur. To counteract this, buffering substances such as sodium hydrogen carbonate or combination preparations with trace elements and carbonates can be used. These are intended to safeguard the pH value against over acidification **(Drosg, 2013)**.

Floating layer remover: Floating layers (see **figure 45**) can form on the surface of the liquid in fermenters, secondary fermenters or in the digestate store. Floating layers are foamy, dense to solid deposits that may result from lack of mixing, incomplete decomposition of the organic mass, high space loading or hydraulic short circuits. Using enzymes and algae extracts, etc., long-fibered substrates can be better degraded. The hydrolytic degradation should be faster, and the viscosity should be reduced, which leads to an improvement of the stirring performance. As a result, floating layers can be dissolved more easily **(Drosg, 2013)**.

Defoamer: One of the most common malfunctions in biogas plants is the formation of foam. It can lead to blockage/clogging of the pipelines and thus to severe damage to the plant. Pipelines can become fouled, sensors can be disturbed, and gas lines can become clogged. In the worst case, tank leaks can occur. Foam is an important indication that the operation of the biogas plant is faulty. Foams occur when substrates with a high protein content, such as grain, potatoes or dry poultry manure, are fermented. Fats can be released, which are hydrophilic and collect at the liquid surface. There they stabilize formed foam by binding hydrophobic membranes around the air bubbles. Increased acid formation within the fermenter can also contribute to foam formation. Natural oils such as rapeseed oil and silicone oils as well as poly alcohols are used as defoamers. These affect the surface tension of the liquid and change the properties of the bubble formation. In the case of foam formation, the contents of the fermenter are insufficiently stirred and the feedstock and nutrient exchange at the surfaces of the fermentation mixture is reduced. This results in a lack of nutrient supply to the microorganisms, and their cell density and growth rate are negatively affected. The physical gas discharge from the liquid medium is also impaired, which can lead to inhibition or process breakdown **(Drosg, 2013)**.

Figure 45: Large foam bubbles in the fermenter formed by the use of sugar beet (Kliche and Lebuhn, 2017).

Microorganisms: The addition of microorganisms is intended to accelerate biogas production, increase degradation and improve gas yield. The load capacity of the biogas

plant, or more precisely the space load, is to be increased with high-performance bacteria (Drosg, 2013). The addition of special externally bred species also has its drawbacks. These have to show a high performance and face the selection pressure within the biocenosis. However, they can displace existing species in the process. If the species do not grow naturally on a sufficient scale, they must be added again and again (Messtechnik für die Biogasanlage..., accessed 10.11.2021).

Capillary carbon: Plant carbon is not to be understood here as a fertilizer, it is a carrier substance and serves the periodic storage of essential nutrients. It provides habitats for microorganisms. Two types of charcoal can be distinguished, one is charcoal with a large surface area, which favours the growth of bacteria. On the other hand, charcoal with a high capillary density, which particularly ensures effective material flows and substrate supply (Franke, accessed 10.11.2021). Capillary coal consists to a large extent of pure carbon and thus forms an ideal habitat for microorganisms, which can live and multiply there. The pH value of the charcoal is between 8 - 8.7 and thus provides an ideal growth habitat for methane-producing archaea. The structure of the coal ensures pressures in the pores and capillaries, which guarantee the substrate supply for the microorganisms. Salts can be introduced into the pore structure and serve as nutrients. In the acetogenic phase, the carbon suppresses the sulphate-reducing bacteria and promotes the denitrifying bacteria. This promotes the formation of acetic acid, which generates a higher methane yield (Franke, accessed 10.11.2021).

2.4 Automation and control technology

2.4.1 Sensors

In practice, the process of biogas production is focused on a few essential parameters in order to reduce the effort of evaluation as well as the acquisition and maintenance costs. Some values such as filling levels, gas composition or power consumption and power generation are recorded online and evaluated without loss of time. Other parameters such as the FOS/TAC value, DM and oDM values are recorded discontinuously, and in some cases determined and evaluated in external laboratories. Values determined off-line and in external laboratories are prone to errors; inaccuracies occur due to sample collection and transport, making precise and timely process control difficult.(Anleitung zur Ermittlung des FOS/TACs, accessed 03.11.2021). Sensors must often be used in areas where explosive gas mixtures may occur. These sensors must then be suitable for this and must also be installed accordingly. In addition, the sensors must be resistant to high humidity and corrosion. Measuring devices with moving parts in the biogas stream are susceptible to malfunctions due to contamination. Special requirements also apply to all sensors that must trigger safety shut-downs. For example, level sensors that must shut down the filling of the tank when a maximum level is reached. Depending on local regulations, these probes must be connected by cable and trigger shutdowns via relays. Other sensors can communicate directly with the plant control system via BUS systems. Some important process control parameters and their measurement are explained below.

Input quantity and substrate composition: For solids, it is advisable to weigh them, for example by means of scales embedded in the floor on which, for example, loaded machines are weighed before and after unload-

ing. Pressure sensors are used for feeding systems such as the solids feeder. Flow measuring devices can be used on the pipelines for the introduction of liquid substrates, and the volume added can also be determined using level measuring devices. Inductive and capacitive sensors are mainly used for flow measurements, ultrasonic and thermal conductivity sensors are also used less frequently.

DM measurements in the fermenter with microwave spectroscopy (figure 46): Mean-while, there are compact and robust microwave-based measuring systems that have been developed specifically for determining the DM content in biogas plants. These can record the individual or total DM content of the substrates involved. There are also versions that are specifically designed for digester installation. Usually mounted in a guard, the sensor measures through a Plexiglas front and there is no direct contact with the fermentation substrate. Microwave radiation passes through a dielectric window of its shroud and detects a representative sample volume. Solids can be detected in water because their relative dielectric constant is much lower (about 2 to 10) than that of water (about 80). Reflective microwave arrays are used for DM measurements. An electromagnetic wave with very low energy is radiated into the material from an antenna and reflected. The reflected wave is detected again, and the reflected portion depends on the dielectric properties of the material under investigation. This provides information on the water or dry substance content **(Henkelmann *et al.*, 2010)**. The method is not very susceptible to interference, changes in pH values or conductivity have no effect, the microwave DM sensors are available in a wide variety of designs, can be retrofitted and have long-term stability.

Figure 46: Microwave DM sensor MWDM PP tube in a waste digestion plant (Göller, accessed 2021).

Mobile DM measurement: This measurement is performed gravimetrically. The measuring device has a heated balance for this purpose. A sample of the material to be measured is weighed in the moist state and then automatically dried until the weight is constant. The water content is calculated from the difference in mass. This method cannot be operated continuously but has the advantage that the device is inexpensive and easy to operate and can also measure substrates and fermentation residues. These values are also important for the operation management.

Level measurements: To detect the amount of energy present in the gas storage tank, the pressure, temperature, gas composition and storage volume must be known. A variety of sensor types exist for level detection in the fermenter, these can be hydrostatic pressure sensors, also distance measurements to the surface can be determined by ultrasound or radar.

Way rope sensors (figure 47) are sensors for length, displacement, and position determination. Mechanical level gauges are often used in double diaphragm accumulators to

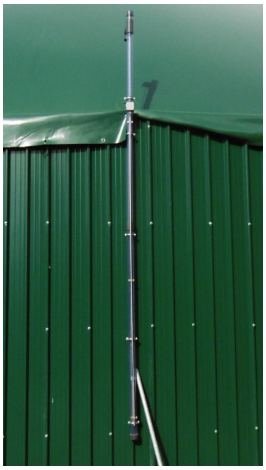


Figure 47: Image of a wey rope sensor for detecting the gas level. Source AEV Energy, biogas plant Ansprung (Germany).

determine the height of the inner gas diaphragm. The filling level of the gas accumulator can be determined by the distance between the gas accumulator diaphragm and the weather protection diaphragm. Draw-wire sensors attached to the gas storage membrane are used for this purpose. The cable is guided through the storage membrane and ends in a measuring tube via deflection pulleys. If the diaphragm lifts, the change in the sensor's travel is registered. Magnets are installed at the end of the cable, and these switch via installed reed contacts in the measuring tube. The output signal, which is proportional to the displacement, is converted into a level. This technology is simple but relatively robust and reliable.

Rod probes, measure the electrical conductivity between metal rods. Depending on the number of bars installed, different measuring points can be detected. They are often used for leakage detection and shutdown in case of overfilling. Leakage probes are used to detect leaks in tanks containing liquids hazardous to water. On contact with an electrically conductive liquid, the integrated electronics react, and a permanently emitted signal is interrupted. This can result in an acoustic or optical signal. Overfill sensors are mostly self-sufficient and not coupled in a bus system. Rod probes can only determine discrete levels. Other systems are used for continuous measurement.

Pressure probes can continuously and reliably measure liquid levels. For this purpose, the probe is installed near the bottom of the tank. This technology is widely used to determine the levels in tanks of biogas plants.

Radar sensors are also suitable for continuous level measurement. The advantage of radar sensors is their maintenance-free operation due to non-contact measuring methods and the fact that they provide exact measurement results independent of the process conditions. The instrument emits a continuous radar signal via its antenna. The signal is reflected by the medium and received by the antenna as an echo. The frequency difference between the transmitted signal and the received signal is proportional to the distance. This depends on the level and can therefore be determined. Radar probes can be used more flexibly than pressure probes. They can also detect foam formation.

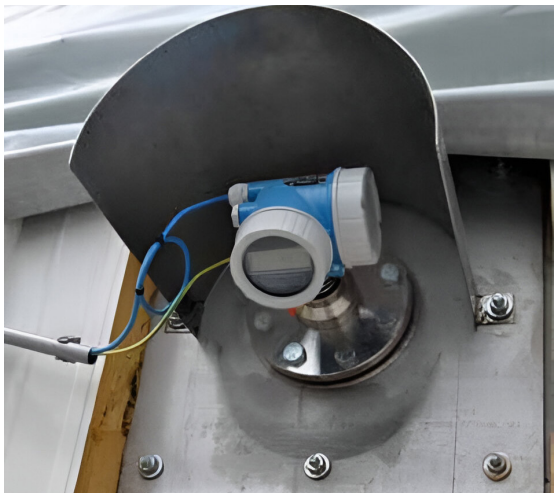


Figure 48: Radar measuring device for level measurement (Micropilot FMR52 from Endress+Hauser) (Messtechnik für die Biogasanlage..., accessed 10.11.2021).

Ultrasonic measurements based on the transit time principle offer another non-contact method. A sensor emits ultrasonic pulses, they are reflected by the surface and detected by the sensor. The required transit time is a measurement of the distance travelled in the empty part of the tank **(Arbeitsgemeinschaft Landtechnik, accessed 08.11.2021)**. This value must be subtracted from the total height of the tank, from this the level can be calculated. Radar uses electromagnetic waves, while ultrasound uses mechanical waves that propagate at the speed of sound. Ultrasonic level sensors are insensitive to changes in density and viscosity. Foam, turbulence, vapor and haze can affect measurements. Other optical sensors such as laser measurement have problems with steam and particles and are therefore not generally recommended.

Table 19: Overview of advantages and disadvantages of different sensors for gas volume measurement (Drosg, 2013).

Sensor type	+	-
Ultrasonic flow meters	» Good results at low pressure » No moving parts » Very reliable even at changing process conditions	» Long straight measuring distance needed (15 times the diameter)
Fluidistor oscillator	» No moving parts » High accuracy » Low cost » Easy handling, exchange and cleaning	» Complex calculation to norm cubic meters » Error of 1.5% » Sensitive to vibrations in the biogas caused by e.g., piston compressors
Turbine flow meters	» Robust technology	» Deposits can become problematic » Moving parts
Vortex flow meter	» No moving parts » High durability » Resistant to corrosion » Low pressure loss	» Sensitive to disturbances in flow » Long straight measuringdistance needed (30 times the diameter)
Dynamic pressure probes	» Long durability » Dirty gas has little influence » Pressure fluctuations have no negative effect on accuracy	» Works better at higher gas pressure » Large calibration effort » Error of 1.5-5% » For calculation of Nm^3 the density (gas composition) is needed » Long measuring distance needed
Thermal flow meters	» Easy handling » Good for mobile applications » Direct measurement of Nm^3/ mass » Exact Measurement also at pressure fluctuations	» No dirty biogas measurement possible » Measurement error of 3-5% (increases rapidly if gas is dirty) » Extremely sensitive to humidity » Long straight measuringdistance needed » Calibration once a year
Diaphragm gas meters / bellows gas meters	» Simple and cheap » Direct volume measurement » Robust technology	» Corrosion, fouling or deterioration of gas meter by biogas components and particles » Increased utilization time decreases accuracy of measurements » External calibration and maintenance

Biogas production: A change in gas composition or gas volume can be an indicator of process imbalance. Accurate measurement of biogas volume is a technical challenge because biogas is composed of different gases, is saturated with water vapor, and may also contain particles. In addition, biogas is produced at low pressure. Gas metering systems should be located so that they are easy to remove and clean. There are many different types of sensors available for gas meters, these and their typical advantages and disadvantages are listed in Table 19 **(Arbeitsgemeinschaft Landtechnik, accessed 08.11.2021)**. Flow sensors are used for both gas flow and substrate flow. Special requirements apply if the determined values must be used for billing purposes. When biomethane is fed into the natural gas grid, the meters are provided by the grid operator.

Gas composition: In many biogas plants, online measuring devices are installed to record the gas composition, but portable measuring devices are also used. Figure 49 shows a stationary analysis station from Binder Engineering GmbH. It detects CH_4 and CO_2, which are measured with infrared or thermal conductivity sensors. In the vast majority of cases, H_2S and O_2 are determined by electrochemical sensors. Oxygen is also measured by paramagnetic sensors. H_2S is measured less frequently than other parameters, this increases the lifetime of the sensors. Measurement of biogas composition is not necessary for modern CHP units, they are self-regulating, however, biogas composition is a useful parameter to monitor the processes in a biogas plant. For example, a decrease in methane content may indicate an overload, and an increase in H_2S may indicate process instability. The table shows different measurement methods or gas sensors for the analysis of biogas. Methane, carbon dioxide and oxygen are indicated in vol.-%, hydrogen sulphide is usually indicated in ppm.

Figure 49: Flexible modular gas analysis station (COMBIMASS® GA-s hybrid premium; Binder Engi-neering GmbH) (Binder Engineering GmbH, accessed 09.11.2021).

Table 20: Suitable gas sensors for the analysis of biogas (Franke, accessed 10.11.2021).

Measurement method	CH_4	CO_2	O_2	H_2S
Thermal conductivity sensor	X	X		
Spectrometer valence electrons (Near infrared)	X	X		
Spectrometer valence electrons (UV light)				X
Spectrometer Molecular Vibrations	X	X		
Electrochemical gas sensors			X	X
Paramagnetic sensor			X	

Temperature: Biogas digesters require a stable process temperature; this is necessary for high performance of the microorganisms. Pt100 T probes are mainly used to measure the temperature. They must cover a temperature range of 20 - 60°C to be able to measure in the psychrophilic, mesophilic, and thermophilic fermentation environment. Due to measurement inaccuracies, it is recommended to use several temperature sensors in different local areas. Pt-100 measurement sensors are very accurate temperature sensors, all parts are made of stainless steel, they are characterized by their robust mechanical design and low cost.

Electricity meter/power feed: An electricity meter is a measuring device that records the energy supplied and the instantaneous power of a consumer. The measured values are given in kilowatt-hours for energy and in kW for power. An electricity meter in a biogas plant evaluates not only the kilowatt-hours delivered to the grid operator, but also the own consumption and the most important electrical consumers of a biogas plant are the CHP, agitators, solids input, solids and slurry pumps, drives, the support air blowers, and pumps. The average own power consumption is between 6 - 12% of the generated electrical energy. The energy consumption of aggregates can be measured at various points and is evaluated by the plant control system.

2.4.2 Power feed-in

Grid connection point and transfer station: The electrical energy generated in the CHP unit can be fed into the low-voltage grid (usually 400 V) or medium-voltage grid (7 - 25 kV (after conversion in the transformer)). The power is fed into the grid at the grid connection point, where the operator's power grid merges with that of the power supplier. At the point of connection to the grid, the energy fed in and taken out must be measured and protective devices must be installed for disconnection in the event of a fault. These installations form the transfer station

.

Telecontrol technology, control specifications of the grid provider: In most cases, the transfer station must be included in the communication of the plant control system since the grid provider must be able to throttle the power of the plant in case of emergency or to take it off the grid completely. In simple cases, the transfer station contains a radio receiver for this purpose, which acts on the CHP unit through a direct wired connection and regulates it in stages (30%, 60%, 100%). The connection to the CHP unit is then made via a switching contact without the system controller being interposed. This ensures that the control from the grid provider always has priority over other controls. In new buildings, the grid provider usually communicates with the plant control system and the CHP unit via a BUS system. The advantage is that the grid provider receives important information for grid operation, such as available power or available runtime (for flexibly operating plants). Disadvantage is a considerable financial expenditure for the installation of this remote-control technology. The grid provider determines which method is used. Regardless of the grid provider's ability to intervene in power generation via remote access, general compliance with various characteristic curves is often required by the grid provider. Such characteristics can, for example, control the reactive power fed into the grid as a function of the power or the grid voltage. Depending on the control concept, such characteristic curves can be stored directly in the CHP controls or in the plant control system.

Protection concept: During the planning phase of the power feed-in, a protection concept must also be drawn up. It regulates the separation of the generating unit (CHP) and the power grid from the grid of the generating plant in the event of a fault (overcurrent, undervoltage and overvoltage, frequency protection, shutdown if the contractually regulated power is exceeded, shutdown if the reactive power is too high, etc.). Basically, the protection takes place in two stages. The higher-level protection disconnects the entire plant from the power grid (this usually also means a power failure on the entire plant). The subordinate protection disconnects only the generating unit

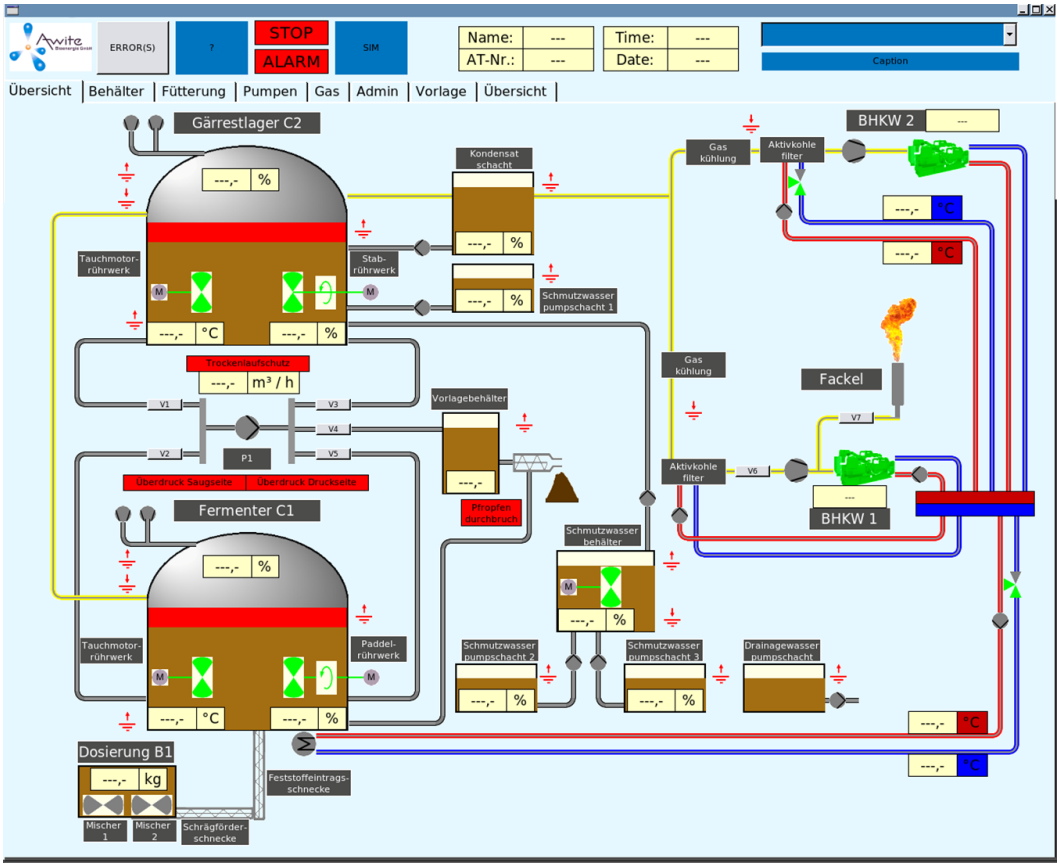


Figure 50: Example of a plant visualization (AEV Energy).

from the mains. The protection functions must be parameterized in such a way that the subordinate protection trips first.

2.4.3 Plant control

The plant control system handles the communication between the individual plant components, aggregates, and their control. It essentially consists of electrical components such as frequency converters for speed-controlled motors, relays for switching and the programmable logic controller (PLC) on which the programming is stored. Usually, the PLC has a visualization of the plant on which the most important parameters can be read, and many values can be set. An example for a plant control is shown in **figure 50**.

The basis of the control is the programming of the PLC. Here, the running times of the stirrers, the feeding quantities of the tanks, the running times, and the schedule of the CHP and much more are set. In the event of malfunctions, for example the failure of a pump, the PLC must register this and issue an error message. Usually, this error message is also forwarded as a text or voice message to stored telephone numbers. In addition to the control tasks required to maintain operation, the system controller must also perform safety shutdowns. A special feature here is that these switches do not run via the PLC, but the corresponding relays are controlled directly. This concerns, for example, the starting of the gas flare when the maximum gas storage level is reached or the switching off or switching on of pumps when min. or max. filling levels are reached. This is intended to increase process reliability.

2.4.4 Emergency power supply

Prolonged power failures can lead to process disruptions and dangerous situations. Therefore, various aggregates must be able to be supplied with emergency power within a short time. As a rule, emergency power generators are used here which are started manually or automatically in the event of a power failure. Basically, an automatic emergency power supply is always necessary if it is not ensured 24 hours a day that the emergency power supply can be started manually within a short time. The period in which the emergency power supply must run depends on the technology installed on the biogas plant. The time must be chosen in such a way that no dangerous situations can occur. The most important functions are listed below.

The **gas emergency flare** and the associated gas blower must be integrated into the emergency power supply to ensure the combustion of biogas if the gas storage is full. Since the CHP cannot run without a connection to the power grid, the gas flare is the only way to utilize the gas during power outages. If the gas flare does not work, the gas flows out via the over-pressure protection. In this case, explosive areas are created around the overpressure protection.

The **plant control system** must also remain functional in the event of power failures to be able to send fault messages and continue to execute the most important controls. Unlike the other equipment, the PLC must be supplied with power without interruption. For this purpose, additional battery storage units are installed for the PLC for a bridging time of about 10 - 120 min.

The **support air blowers** of double diaphragm gas storage tanks must also be supplied with emergency power. Without running blowers, the supporting air flows out of the space between the gas storage foil and the weather protection foil and the weather protection foil is no longer tightly stretched. As soon as wrinkles form, the membrane becomes very susceptible to gusts of wind. In extreme cases, this can lead to rupture of the gas accumulator.

Especially in plants working with a high DM content or a viscous substrate, the **agitators** must be included in the emergency power supply. Otherwise, the gas bubbles in the substrate cannot escape properly without agitation. This can quickly (approx. 20 min) lead to large increases in volume of the fermenter contents. The fermenter “boils over”. This applies to plants that work purely with corn silage or grain silage and a DM content of 10% or more. In other plants, the problem is less acute. In plants for the exclusive fermentation of liquid manure, the problem does not exist at all.

Which other parts of the plant still need to be supplied with emergency power depends on the plant in question. This can concern, for example, heaters on parts at risk of frost, pumps that protect against overfilling and various other installations.

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03

Economical and ecological aspects

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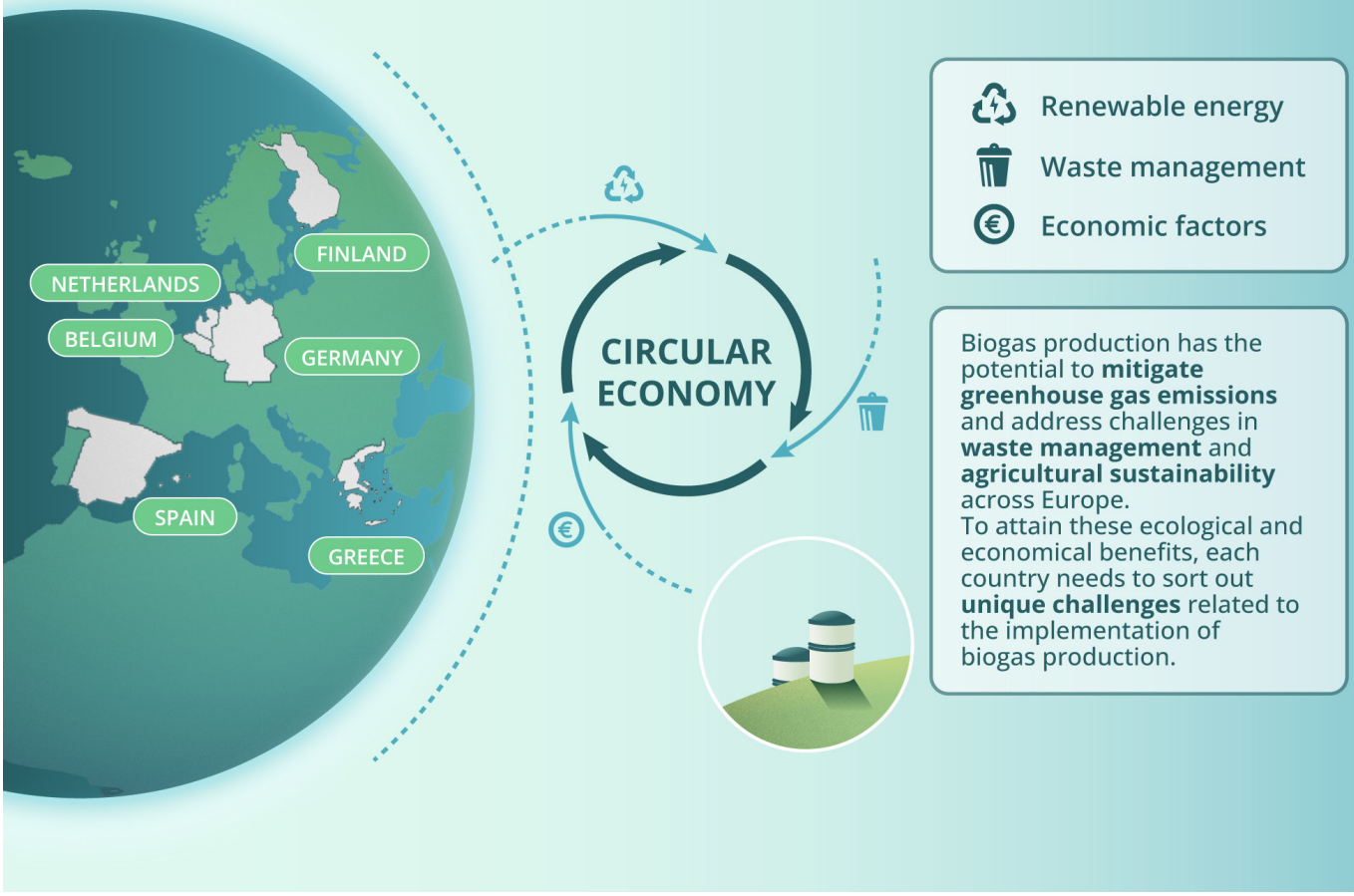
Abstract:

From an economic perspective, anaerobic digestion (AD) is a valuable process that generates a renewable energy source with the production of biogas as the energy carrier. It also contributes to waste management and adds value to organic waste as the feedstock for the process. Biogas is a mixture of gases, mostly carbon dioxide (CO_2) and methane (CH_4), which is the most valuable compound since its combustion releases energy that can be used to produce electricity and heat, or as a replacement for natural gas. In addition, the digestate, which is a co-product of the process, can be used as a fertilizer in the agronomic industry. In this way, AD is a powerful tool to promote circular economy systems, producing a sustainable energy source and recycling organic waste that returns to the economic circuit in the form of fuels and fertilizers. From an environmental perspective, biogas production through AD can help to mitigate four important environmental concerns of the 21st century: climate change, overuse of synthetic fertilizers, methane emissions by landfilling of organic waste and waste management. AD produces a renewable energy source (biogas) with a net balance

of zero-carbon release into the atmosphere. This positive effect on the reduction of greenhouse gas (GHG) emissions is even enhanced considering that if waste is not treated, it is also a source of GHG, mostly methane. Also, it is a sustainable management practice for the enormous amounts of waste that are constantly generated due to human activities, while most of the current practices are not sustainable at all. Finally, the use of the digestate as organic fertilizer allows the recycling of nutrients, especially nitrogen and phosphate, with further positive impacts on the environment, and helps to promote agroecology and bioeconomy.

Biogas production in Europe, driven by renewable energy policies, fosters a sustainable bioeconomy within the circular economy framework. Each country adopts distinct strategies and faces unique challenges in promoting biogas production.



3.1 The Economic Biogas Framework in Europe

The production of biogas and biomethane has been growing constantly worldwide as well as in Europe, and incentives for the use of this renewable energy have been gaining importance within public policies. According to the Renewable Energy Directive, by 2030, 32% of all energy used in the European Union (EU) must come from renewable sources, including biomass, bioliquids and biogas. In addition, European countries must establish a national action plan with the objective of defining the share of energy from renewable sources as well as establishing procedures for the reform of planning, pricing scheme and access to electricity networks, promoting energy from renewable sources **(Biogas3, 2016)**.

More generally, incentives in the biogas sector seek to implement a sustainable bioeconomy that represents the renewable segment of the circular economy, capable of transforming organic waste into valuable resources and creating innovations and incentives to help reduce waste and improve organic waste treatment **(Communication, 2018)**.

As a product, biogas constitutes an economy generator centre in Europe, especially because it is considered a consumer of a significant supply chain, presenting concrete economic results in the form of energies, carbon credits and energy efficiency, which constitute economic revenues in the biogas economy. The demands of this economy are prerequisites for biogas generation to be implemented, such as projects, environmental licensing, regulation, technical training, and others. In addition, the biogas sector moves a supply chain which is necessary for a biogas plant to be installed and operated, such as engines, generators, controls, biodigesters, filters, pipes and several other parts, components, and processes of industrial origin, which drive commerce and specialized services. The economic results that come from the biogas economy are considered direct, such as electric, thermal, automotive energy, and digestate applied for self-consumption and for the sale of surplus. Indirectly, these are supplemented, for example, by obtaining emission credits for the reduction of greenhouse gas emissions and environmental protection through the reduction of organic pollution and energy efficiency **(Fagerström *et al.*, 2018)**.

However, according to current trends, biogas production still depends on subsidies to attract investors and establish substantial scale. In the EU, there is still no specific policy on biogas, biogas solutions are addressed in various policy documents and directives related to renewable energy and bioenergy such as the Waste Directive (Directive 2018/851; Directive 2008/98/EC), EU Bioeconomy Strategy **(Communication, 2018)**, Renewable Energy Directive (Directive 2018/2001; Directive 2009/28/EC) and Landfill Directive (Directive 1999/31/EC), but there were no major initiatives aimed directly at the development of biogas. Currently, some new strategies are emerging in which biogas solutions play a central role. As an example, the EU Methane Strategy **(Communication, 2020)** emphasizes the importance of biogas production to reduce methane emissions from the agriculture, waste and energy sectors, and the Energy System Integration Strategy **(Communication, 2020)** pronounces the mobilization of residual resources for energy production and replacement of fossil gas with renewable gases. However, despite the various incentive schemes to stimulate the economic development of the biogas sector like certification systems, feed-in tariffs, and investment support, the implementation process depends on several factors, including national market conditions, energy prices, tax regimes, economic policy, technical, institutional, sociocultural, and environmental factors **(Nevzorova and Kutcherov, 2019)**.

The advantage is that biogas solutions offer many benefits in different sectors of the economy and can contribute to a sustainable, circular economy. However, the many functions and externalities of biogas solutions also make the assessment more complex. **(Gustafsson and Anderberg, 2022)**.

3.2 Exemplary biogas frameworks of selected countries

3.2.1 Finland

Finland is a prominent country in terms of the implementation of renewable energies (Lyytimäki *et al.*, 2018), and also has a growing development in the biogas sector. However, due to the high costs regarding the conversion of biogas into biomethane, the country has a much lower biomethane production compared to biogas. Thus, in order to support biogas and biomethane production, Finland has developed new tariff and tax systems in which both biogas and biomethane are exempt from excise duties in all end-use applications (EBA 2021; Winquist *et al.*, 2021). Nevertheless, the taxation of biomethane as a vehicular fuel is now under discussion.

Currently, the country has a biogas and biomethane production potential of 10 TWh, with a theoretical potential of 25 TWh (Biokaasu2030) and with the purpose of implementing improvements and increasing production. The Finnish biogas sector set a target of reaching an annual production of 4 TWh by 2030, which was confirmed by the government in September 2021 (EBA, 2021).

Due to the different biogas incentive strategies used by the government, the number of biogas plants increased considerably, reaching more than 38 plants between 2011 and 2021 (EBA, 2021). Presently, Finland has a total of 113 plants that use biomass from different sources such as landfills (34 plants), urban solid waste (26 plants), agriculture (25 plants), sewage treatment sludge (19 plants), and industrial biogas (9 plants) (**figure 51**). In addition, the urban organic waste and sewage sludge plants are responsible for presenting a greater amount of biogas production (EBA, 2021). However, despite the biomass from the agricultural sector presenting the highest generation potential, only a small part is treated in biogas plants (Huttunen *et al.*, 2018).

In 2020, Finland reached a production of 878 GWh of biogas, and it is estimated that approximately 60% of the biogas produced is used as thermal energy for heating or sold directly as raw biogas, while the other part is consumed in cogeneration. (EBA, 2021).

As well as the biogas sector, despite slower growth, the biomethane sector presents a promising development due to the support policies that have been implemented in re-

Figure 51: Development of biogas production (GWh) (left); and development of the number of biogas plants (right), (Adapted from EBA, 2021).

cent years by the Finnish government, and mainly due to Finland's national biogas action plan published in 2020, which describes all the measures and actions that will support the sector until 2024 **(EBA, 2021)**. This can be observed between 2011 and 2021, when the number of biomethane plants increased in the country from 1 to 22, reaching a production of 109 GWh in 2020, with most biomethane plants using solid urban waste as a source of biomass **(EBA, 2021)**.

However, although the number increase of biomethane plants, currently, only 40% of plants are connected to the biogas grid. This is because only the southern part of the country has a gas network enabling direct injection into it.

In general, due to the great political incentive to promote biogas for the use of transport fuel, the production of biomethane is growing. Thus, from 2022 it will be part of the national biofuel delivery obligation, giving a stable perspective to increase the production and use of biomethane by 2030 **(EBA, 2021)**.

Influence of the biogas sector on GHG emissions: According to the National Inventory Report, the total greenhouse gas emissions in 2020 was 47.8 million tonnes of carbon dioxide equivalents (Mt CO_2 eq.). However, compared to the base year (1990) and 2019, emissions decreased by 33% (23.4 Mt) and 9% (5 Mt) respectively.

Regarding the different sectors responsible for gas emissions in Finland, it is clear that the energy sector is considered the most significant source among them (**table 21**) with a variation in CO_2 emissions according to the economic trend, the supply structure of energy, and weather conditions (**Finland. 2020 National Inventory Report (NIR) | UNFCCC**). However, over the years, due to some changes related to the level of electricity imported annually, the consumption of energy based on fossil fuels and the growth in the use of renewable energy, greenhouse gas emissions have decreased considerably **(IEA, 2021)**.

Table 21: Finnish greenhouse gas emissions and removals (Mt CO_2 equivalent) (NIR, 2020).

Sector	Base year	1990	1995	2000	2005	2010	2011	2012	2013	2014	2015
					Mt CO_2 eq.						
Energy	53.4	53.4	55.3	53.7	53.7	60.2	52.8	47.5	48.1	44.3	40.6
Industrial processes and product use	5.3	5.3	4.9	5.2	5.6	4.8	4.7	4.6	4.4	4.2	4.4
Agriculture	7.5	7.5	6.7	6.6	6.5	6.7	6.5	6.4	6.5	6.6	6.6
Waste	4.7	4.7	4.6	3.8	2.8	2.6	2.5	2.4	2.3	2.2	2.1
Indirect CO_2 emissions	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
TOTAL	71.2	71.2	71.8	70.2	69.9	75.7	67.9	62.4	62.8	58.6	55.0

Sector	2016	2017	2018	2019	2020
			Mt CO_2 eq.		
Energy	43.3	40.9	42.1	38.9	34.3
Industrial processes and product use	4.7	4.6	4.6	4.4	4.1
Agriculture	6.7	6.6	6.5	6.6	6.6
Waste	2.0	1.9	1.8	1.8	1.7
Indirect CO_2 emissions	0.1	0.1	0.1	0.1	0.1
TOTAL	57.9	55.1	56.2	52.8	47.8

In 2020, emissions from the energy sector fell by 12% to 34.3 Mt CO_2 eq. from the previous year. Emissions were 36% below the 1990 level and 51% below the 2003 level **(Finland. 2020 National Inventory Report (NIR) | UNFCCC)**

This way, renewable energy consumption has been growing steadily in the country as well as the development of the biogas sector, representing 44.6% of the total consumption of final energy. In 1990, the share of renewable energy was only 18%, showing that the increase in the use of renewable energy was the main reason for the decrease in gas emissions, despite the growth in total energy consumption **(NIR, 2021)**.

3.2.2 Spain

Spain started to support renewable energy in 1997, through the "General Electricity Law 54/1997"; however, the country still has a very strong dependence on energy imports, dominated by oil and Natural Gas **(IEA, 2019)**. With the implementation of a national policy mechanism that provides payments and long-term contracts to renewable electricity producers called the Feed-in Tariff (FIT), biogas production has increased considerably in the country **(EBA, 2021; Del Rio, 2008)**. Unfortunately, according to the EBA 2021 report, the records referring to the number increase of biogas plants do not reflect reality, as many of the plants that already existed in the past were not registered due to lack of data. However, the growth in the number of biogas plants in Spain is believed to have largely occurred between 2000 and 2004, mainly with the use of sewage and sanitary landfills for biogas production. In addition, after the enactment of Royal Decree-Law 1/2012, which eliminated all incentives aimed at generating electricity from renewable sources, the commissioning of new plants decreased dramatically **(EBA, 2021; Flórez and Isabel, 2017)**. **Figure 52** shows biogas production and the reported number of biogas plants in Spain during the last decade.

Furthermore, the Royal Decree Law 413/2014 establishment, which regulates the calculation of feed-in tariffs (FIT) for the electricity production from biogas together with the hydrocarbons tax applied to biogas (0.65 €/GJ), helped to further reduce activities in the biogas sector **(Romero-Rubio and de Andrés Díaz, 2015; Ministerio de Industria,**

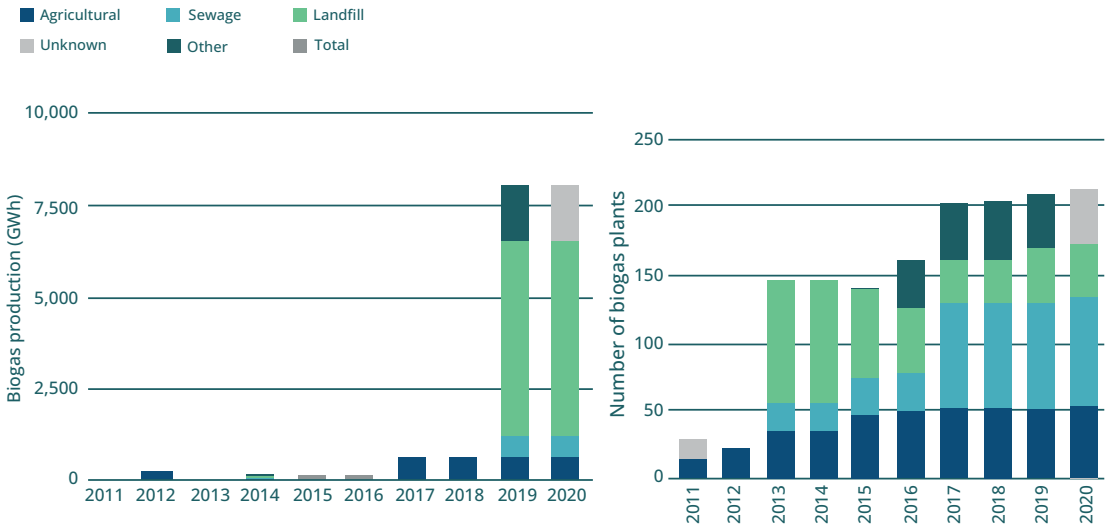


Figure 52: Development of biogas production (GWh) (left); and development of the number of biogas plants (right) in Spain (Adapted from EBA, 2021).

Energía y Turismo, 2014). Currently, Spain has no incentive for new projects related to biogas production. However, there are still projects regarding to the biogas generation, which are not encouraged by the potential for selling biogas, but rather they are driven mainly by the needs of waste treatment and/or by the prospect of providing energy for private consumption (EBA, 2021). Today, Spain has 210 active biogas plants, the majority coming from sewage treatment plants, followed by agriculture, landfills and others. With this number of biogas plants, the country has a total biogas production capacity of 836 MW, corresponding to 318 MW of installed electrical capacity (EBA, 2021). Spain also has three biomethane plants, with two of them coming into operation in 2021 (EBA, 2021). In addition, due to the incentive associated with private initiatives (R&D) and partly funded by the EU (LIFE, H2O2 and CEF2), several pilot-scale projects are growing. Currently, the Spanish Ministry of Ecological Transition and Demographic Challenge (MITTERD) has approved the first Law on Climate Change and Energy Transition of Spain in the first half of 2021, where it commits the country to reduce emissions by 23% until 2030, comparing to the levels of 1990 (BOE, 2021). With this achievement, the government has the possibility to approve mechanisms to support renewable gas and its injection into the gas grid. The law also approves the guarantees registration of origin (GOs) for renewable gases injected into the natural gas network (BOE, 2021). This way, with more support and encouragement of the government, Spain can exceed 100 TWh per year if every type of raw material available is exploited, increasing biomethane production (EBA, 2021).

Influence of the biogas sector on GHG emissions: According to the NIR report (2021), total greenhouse gas emissions (GHG) in Spain estimated for 2019 were 314,528.5 kilotons of CO_2 equivalent (CO_2-eq). This represents a reduction of -5.6% in relation to the estimated emissions for the year 2018. Moreover, it constitutes +8.5% in relation to the base year 1990 and -28.9% in relation to the year 2005. In Spain, the gases that showed the highest emission rates in 2019 were CO_2 (80%) and CH_4 (12.2%) (**tables 22 and 23**). However, comparing the different sectors, all of them suffered a drop in GHG emissions, the main being related to electricity generation (-27.7%), to the commercial and residential sector (-8,6%), and the industrial sector (-1.2%) (NIR, 2021).

In general, the evolution of CO_2 and CH_4 emissions in Spain over time responds to a four phase pattern fundamentally linked to variations in economic growth, population, and energy consumption in Spain since 1990 (IEA, 2021; NIR, 2021). However, the

Table 22: CO_2 emissions: absolute values, temporal variation and ratios (NIR, 2021).

	1990	2005	2010	2015	2018	2019
CO_2 (kt CO_2-eq)	231.194	369.681	283.873	271.694	269.713	251.498
Variation % vs. 1990	100%	159.9%	122.8%	117.5%	116.7%	108.8%
CO_2 / INV (CO_2-eq)	79.7%	83.6%	79.3%	80.6%	80.9%	80.0%

Table 23: CH_4 emissions: absolute values, temporal variation and ratios (NIR, 2021).

	1990	2005	2010	2015	2018	2019
CH_4 (kt CO_2-eq)	36.647	40.924	39.462	38.177	38.566	38.493
Variation % vs. 1990	100%	111.7%	107.7%	104.2%	105.2%	105.0%
CH_4 / INV (CO_2-eq)	12.6%	9.3%	11.0%	11.3%	11.6%	12.2%

decrease in emissions was mainly marked by the reduction in emissions from electricity production (-27.7%), due to the greater production of renewable energies, such as wind, photovoltaic, solar thermal and biomass, which increased +9.4%, +19.0% and +16.8% respectively, and the decrease in the use of coal in electricity production (-66.0%) **(IEA, 2021; NIR, 2021)**. The energy sector accumulates a total GHG reduction of -6.6% **(NIR, 2021)**. This represents a significant reduction in GHG emissions compared to the increase in the renewable energy use, showing that with incentives through public policies, and the appropriate treatment of organic solid waste significantly influences the biogas sector growth and the preservation of the planet.

3.2.3 Netherlands

In the biogas market, the Netherlands is one of the countries that has stood out significantly throughout the world due to the implementation and upgrading of biogas plants to large-scale biomethane plants, presenting strategies well established by the government in partnership with the private sector **(IEA, 2021; (Winquist *et al.*, 2021)**. According to **Kwant, 2003**, the Netherlands had its first support for encouraging the renewable energies use in 1995 with the Green Funds certification. However, the biogas sector only showed significant growth in 2006, 3 years after the introduction of the "Environmental Quality of Electricity Production" Feed-in-Premium (FIP) which helped to support the implementation of new plants **(EBA, 2021)**. In addition, with the goal of stimulating the production and cogeneration of renewable energy, the SDE (Stimuling Duurzame Energie) was created. This was later updated to the SDE+ and SDE++ which are currently being used to encourage sustainable growth and the circular economy through subsidies from the government **(Netherlands, 2022; IEA, 2021)**. As a result, there was an increase in the number of biogas plants (260 in 2020) and biomethane plants (60 in 2020), showing the importance of implementing efficient laws driven by the government **(EBA, 2021)**. Thus, the Netherlands produced 2,439 GWh of biogas in 2020, of which approximately 927 GWh of electricity was generated (**figure 53**) **(EBA, 2021)**.

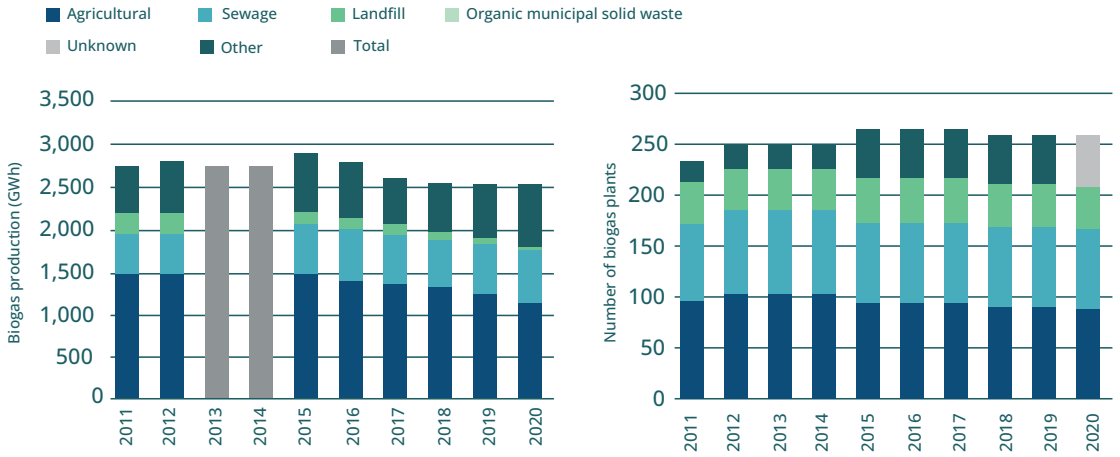


Figure 53: Biogas production in the Netherlands: development of biogas production (GWh) (left); and development of the number of biogas plants (right) (Adapted from EBA, 2021).

Due to government incentives for biogas production, the biomethane market has also grown significantly, making the Dutch market in this sector one of the pioneers in Europe **(Winquist *et al.*, 2021)**. The Netherlands currently has its own national renewable gas registry operated by Vertogas, the green subsidiary of Nerderlandse Gasunie, since 2009, and registration and certification via Vertogas is mandatory for renewable gas producers in the country (Verto-gas). Because of this incentive, the number of biomethane plants grew from 21 in 2016 to 60 plants in 2020, with agricultural substrates as the most used feedstock, followed by industrial waste. Furthermore, in 2020 the Netherlands reached a production of 2,166 GWh of bio- methane **(EBA, 2021)**. Today, it is known that out of the 60 biomethane plants in the Netherlands, 53 are connected to the gas grid, 4 plants produce Bio-CNG on site and have no connection to the grid, one produces Bio-LNG, and the others do not have record **(IEA, 2021; EBA, 2021)**.

However, it was pointed out in 2020 that the production of 402 GWh of biomethane which were used in transport representing practically 1/5 of the biomethane produced in the same year. Due to the large investment in the production of vehicular biofuel, seven new Bio-LNG plants are planned for the period 2021-2024 with a total capacity of 1.5 TWh per year **(IEA, 2021; EBA, 2021)**.

Influence of the biogas sector on GHG emissions: Another important factor related to the growth influence in the Dutch biogas sector is the significant reduction in greenhouse gases (GHG). According to **NRI (2021)**, the energy sector is responsible for 83% of total GHG emissions in the Netherlands, being considered the most important source of GHG emissions. However, in 2019, total GHG emissions in the Netherlands were estimated at 180.7 Tg CO_2-eq. This is 18% lower than the 220.5 Tg CO_2-eq. reported for the base year in 1990 (**figure 54**).

In the period 1990-2019, emissions mainly of carbon dioxide (CO_2) and methane (CH_4) decreased by 5.6%, and 45.9%, as well as nitrous oxide (N_2O) and fluorinated gases (F-Gases) 54.9% and 75.9%, respectively.

However, despite the use of fossil fuels has been decreasing and the amount of energy from renewable sources has been increasing, natural gas (44%) and oil (36%) are still the most important energy sources in the Netherlands **(IEA, 2021)**. Renewable energies accounted for 181 PJs in 2019 (8.7% of total energy use in the Netherlands).

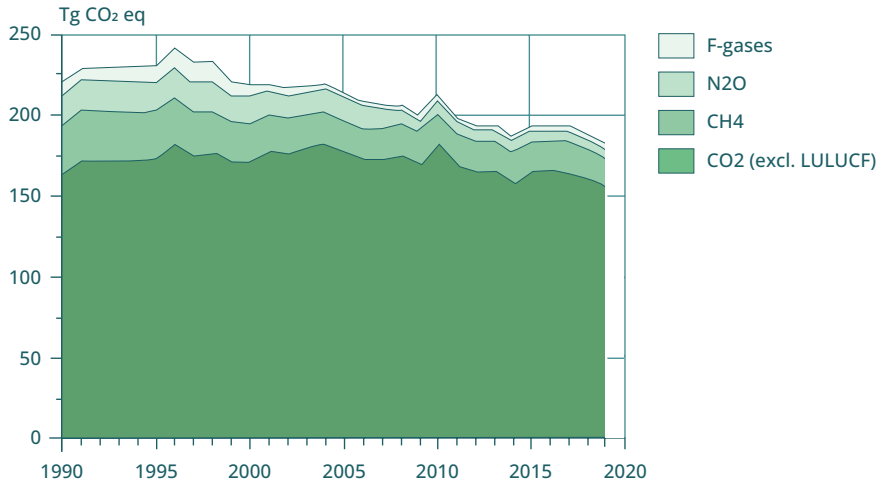


Figure 54: Greenhouse gases: emission levels and trend, 1990-2019 (Adapted from NIR, 2021)

3.2.4 Belgium

Before 2001, the biogas sector in Belgium was stagnant and consisted mainly of plants operated in landfills or sewage treatment located in the region of Brussels, Wallonia and Flanders **(EBA, 2021)**. However, after the introduction of the Green Certificate Scheme project by royal decree, the biogas sector grew considerably **(Marchal *et al.*, 2007)**. The Green Certificate determined the electricity share from renewable sources delivered to users connected to the distribution network (from 2% in 2002 to 6% in 2006), and a fine of 75 euros per certificate for non-compliance with the quota corresponding to 1 MWh **(EU Comission, 2018)**. Between 2015 and 2018, due to technical reasons, several small biogas plants had to be closed in the Flanders region, but with the effort of the sector to solve this problem, the growth rate of plants grew again in 2019 (**figure 55**) **(EBA, 2021)**. Currently, Flanders and Wallonia are the regions that most biogas produce in Belgium, with 134 and 55 plants in operation. In 2020, the country reached a production of 2700 GWh resulting in the generation of 1075 GWh of electricity **(EBA, 2021)**.

In addition to biogas plants, interest in biomethane production increased in the country after the installation of the first biomethane plant in the Flanders region, which helped to encourage policy changes across Belgium **(IEA, 2021)**. In Wallonia, the system to support the use of green energy has changed to include the biomethane production, for which the company can receive a government subsidy by reducing the use of fossil fuels. Furthermore, Wallonia has taken on the "Plan wallon Energie Climat", which is the Wallonia regional climate plan to help achieve its 2030 targets for biogas development. A voluntary registration system for renewable gases was also created by the association of gas distributors in Belgium (Gas.be) to help with the incentive in the sector (Biogaz). In September 2021, Belgium had the registration of the first Bio-CNG produced in Wallonia, however a second plant is being built in Flanders **(EBA, 2021)**.

Currently, six biomethane plants are in operation in Belgium, where two are in the Flanders, and four in the Wallonia region. Of these six plants, three are operating with agricultural substrates, one producing biomethane from sewage sludge, one using organic municipal solid waste and an industrial biomethane plant. It is estimated that today Belgium reaches about 17% of its biogas and biomethane production potential **(EBA 2021)**.

Influence of the biogas sector on GHG emissions: As well as the other countries, the biggest contribution to GHG emissions in Belgium comes from the energy sector. In 2019,

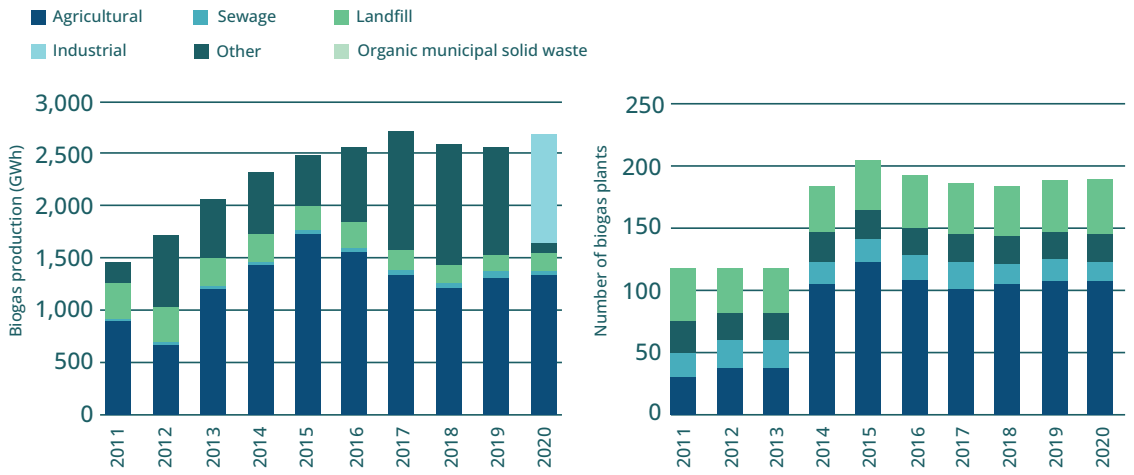


Figure 55: Biogas production in the Belgium: development of biogas production (GWh) (left); and development of the number of biogas plants (right) (Adapted from EBA, 2021).

the sector contributed 74% to total GHG emissions. Mainly, CO_2, CH_4 and N_2O emissions come from the energy sector. However, since 1990, emissions from this sector have declined by around 17% **(NIR, 2021)**.

Another largest source of greenhouse gases is agriculture with 8%, where emissions from this sector arise mainly from CH_4 and N_2O. However, since 1990, emissions have fallen by 19%. In 2019, the waste sector contributed around 1.2% where emissions arise from CO_2, CH_4 and N_2O and originate from waste incineration, solid waste disposal on land and wastewater treatment. Emissions from this sector have steadily declined and are 69% below the 1990 level since 2019. According to the National Inventory Report from Belgium, the total net emissions have decreased by 18.8% since 1990.

This way, it is evident that the emissions decrease in the energy, agricultural and waste sectors in Belgium is due in part to technological improvements, the shift from solid fuels like coal to gaseous fuels (natural gas) and the use of renewable energies, such as the biomass for biogas and biomethane production, as well as the proper treatment of urban solid waste using anaerobic digestion.

3.2.6 Greece

Greece began to explore energy using biogas in the early 1980s. The main raw material was animal waste and food-processing industries waste. However, due to a lack of adequate legislation, financial incentives and public awareness, the projects ended up being forgotten **(Markou *et al.*, 2017)**. From 2000 to 2010 the country started to produce biogas again, which was dominated by plants that used sewage and sanitary landfills as a source of substrate. Between 2011 and 2020 there was an increase in biogas production from 543 GWh to 718 GWh, most of which came from Athens and Thessaloniki **(EBA, 2021; IEA, 2021)**. With the introduction of Feed-in Tariffs (FiTs) in its Renewable Energy Act in 2010, fees for landfill-based biogas plants were up to 120 €/MWh, while for agriculture-based biogas plants they were up to 220 €/MWh, which resulted in an increase in agricultural biogas production **(EBA, 2021; Markou *et al.*, 2017)**. Later, with the increase of the maximum tariff rates (FiT) to 129 €/MWh (landfill biogas) and 225 €/MWh (agricultural-based biogas), Greece further increased the number of plants, mainly biogas plants using agricultural substrate, reaching a production of 392 GWh in 2020 related to this type of plant **(Markou *et al.*, 2017)**. Due to these changes,

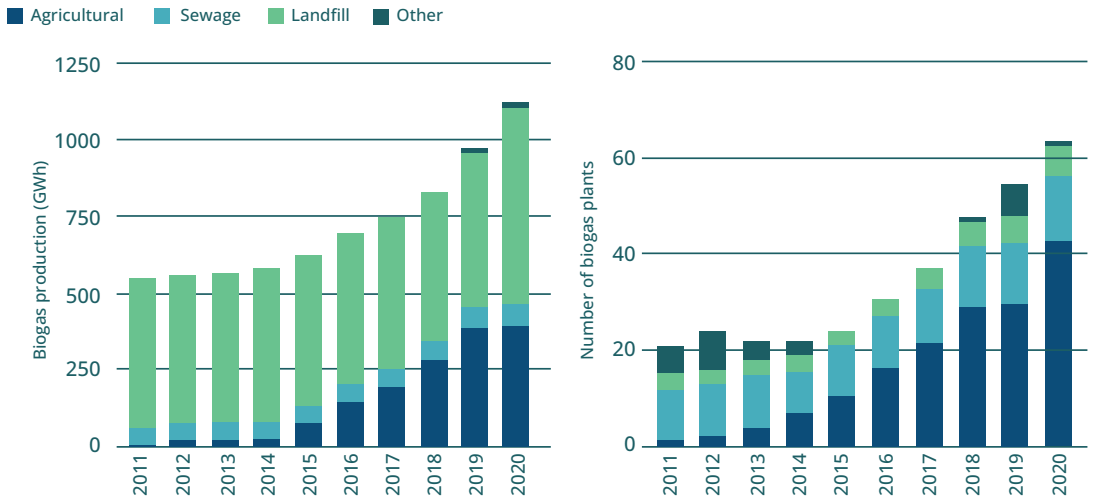


Figure 56: Biogas production in the Greece: development of biogas production (GWh) (left); and development of the number of biogas plants (right) (Adapted from EBA, 2021)

that year the country increased by 42 plants with a total production of 1,126 GWh (**figure 56**) of biogas, from which 428 GWh of electricity were generated **(EBA, 2021)**.

However, biomethane production has lagged due to lack of incentive through public policies. It is estimated that there will be no significant growth within the next 2 years in this area. In order to have and improvement in this sector, the government must encourage through tariffs (FIT), for example, with generous prices for production as well as facilitating the injection of biomethane into the gas network **(Rutz, 2021)**.

Influence of the biogas sector on GHG emissions: According to the National Inventory Report from Greece, in 2019 GHG emissions totaled 85.63 Mt CO_2-eq, showing a reduction of 17.10% compared to 1990 levels. Carbon dioxide emissions represented 76.77% of total GHG emissions in 2019 and decreased by approximately 21.20% compared to 1990. Methane emissions accounted for 11.70% of total GHG emissions in 2019 and decreased by 9.29% compared to 1990.

The energy sector represented 71.50% of total GHG emissions and decreased approximately 20.51% in 2019 compared to 1990 levels. This is due to the improvement in living standards, due to economic growth, the significant growth of the services sector and the introduction of natural gas into the Greek energy system for the period 1990 to 2007.

Emissions from the waste sector have decreased by around 0.52% since 1990. Improved living standards have resulted in an increase in waste generation and therefore in emissions since 1990. However, the increase in recycling along with exploration of the biogas produced limits the increase in methane emissions **(NIR, 2021)**. In this way, it is possible to realize the importance of the biogas sector development for the reduction of negative environmental impacts and the economic improvement of the country.

3.2.7 Germany

Germany is considered the leading country both in Europe and in the world regarding production of biogas and biomethane **(EBA, 2021)**. With around 11,000 biogas plants and 242 biomethane plants currently installed, it is responsible for more than half of the total primary energy produced from biogas. This fact is largely explained by the large state incentive about plantations for the energy production from biomass **(WBA, 2021; Zlokower, 2019; EBA, 2021)**.

With several laws encouraging the biogas and biomethane production, Germany was one of the first countries to implement a subsidy for the renewable electricity generation, which was later called the Renewable Energy Sources Act (EEG or Eneuerbare Energien Gesetz) and came into force in 2000 **(Büsgen and Dürrschmidt, 2009)**. This document regulates the connection of energy production units from renewable sources to the distribution network, as well as the purchase, transmission and payment of energy by the network operator. These differ according to the type of renewable energy, conversion technology and production unit capacity. After the implementation of this law, biogas production in Germany tripled **(EBA, 2021)**. However, over time after the updates of this law, remuneration rates became less generous causing the growth rate of the number of biogas plants to decrease **(Matschoss *et al.*, 2019)**. Furthermore, with the abandonment of Feed-in Tariffs (FIT) that helped to boost the biogas market. Germany implemented a tendering system (EEG-2017) that focuses electricity production on a market-driven rationale **(Thomas, 2019)**. With this change, biogas production has decreased, dropping to 71 TWh in 2020 compared to a production of 81 TWh in 2017 (**figure 57**) **(EBA, 2021)**.

However, despite the decrease in biogas production, installed electrical capacity in the German biogas industry continues to grow yearly due to the flexibilization of Germany's biogas plants, which shows the adaptation of these facilities to generate power in a more flexible and responsive manner. This is crucial for integrating variable renewable energy sources into the energy system **(Lauer *et al.*, 2016)**. Flexibilizing biogas plants can lead to cost savings compared to conventional power plants, making them an economically viable option for Germany's energy landscape **(Lauer, Leprich and Thrän, 2020)**. Currently, the latest EEG updates have prioritized smaller biogas plants that use higher proportions of manure, biowaste and food waste, focusing more on ecological sustainability and waste management **(Thomas, 2019)**.

Germany is one of the largest producers of biomethane in the world with 242 registered plants and more than 11 TWh of biomethane production in 2020. After changes in German legislation, the increase in the number of plants has slowed down in recent years, making France overtake Germany as the fastest growing country in the biomethane sector with 306 plants registered in 2021 **(EBA, 2021)**.

Among the biomethane plants registered in Germany, 197 use agricultural substrate, which is the main pillar of the German biogas sector **(EBA, 2021)**. The main end-use application of biomethane in Germany is the electricity generation, followed by use for vehicle fuel (Torrijos, 2016). In addition, most biomethane plants in Germany in 2020 were

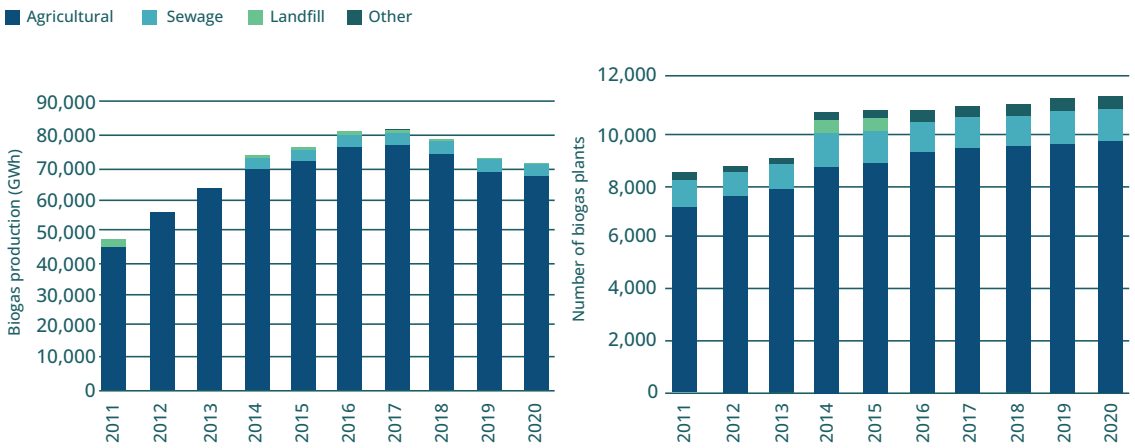


Figure 57: Biogas production in the Germany: development of biogas production (GWh) (left); and development of the number of biogas plants (right) (Adapted from EBA, 2021).

connected to the gas grid, of which two were connected to the distribution grid and 104 to the transmission grid (EBA, 2021).

It is estimated that around 1000 GWh of biomethane produced in 2020 was used as transport fuel, representing around 9% of total production. Due to the implementation of the Federal Pollution Control Act, which requires fuel companies to reduce their carbon foot-print, the incentive to produce vehicular fuel from biomethane has increased. Germany, together with Italy and the Netherlands, is expected to become a leader in the European production of Bio-LNG (EBA, 2021).

The largest plant under development in Europe is in Germany, which will have a production capacity of 1,500 GWh/year and is expected to start operating in 2022. In addition, the number of Bio-CNG and Bio-LNG filling stations in Germany also is increasing rapidly. Currently, of the 810 CNG stations in operation, 533 supply Bio-CNG (EBA, 2021). In addition, to encourage the production of vehicular fuel through renewable sources, in October 2021, the country obliged producers and suppliers of fossil fuels and non-sustainable biomass to purchase a CO_2 Emission Certificate for each ton of CO_2 that they release (New CO_2 emissions tax in Germany). Thus, making Bio-LNG a much more attractive, ecological and economical option.

Influence of the biogas sector on GHG emissions: Among other factors, considering the policies to encourage the renewable energy use and the biogas sector development in Germany, it is possible to notice a significant reduction in the levels of GHG, mainly CO_2, which represents the largest share of greenhouse gas emissions (NIR, 2021). The CO_2 are being mostly released through the fossil fuels combustion, and CH_4, released predominantly by livestock, fuel distribution and landfills. **Figure 58** shows a reduction of approximately 35.1% in total GHG emitted compared to the base year (1990), which represents an approximate reduction of 32.4% in CO_2 and 58.2% in CH_4 in 2019 (NIR, 2021).

The observed reduction is largely due to structural changes in the energy sector, as well as climate and price effects. Furthermore, in recent years, coal has been increasingly supplanted by natural gas, which has lower specific CO_2 emissions in electricity generation, and the share of renewable energies in electricity generation has largely increased considerably, mainly the energy produced through the biogas sector, which has gradually influenced the reduction of CO_2 and CH_4, respectively (NIR, 2021).

3.3 Ecological and economic impacts of the biogas industry

In this section, we ask which are the main environmental issues that can be mitigated with anaerobic digestion (AD) and biogas production. We state the current situation of the identified issues, together with a theoretical description that helps to understand the basic aspects of each one. Also, we ask how AD and biogas production can take profit of these issues as an opportunity to increase its adoption as a technology for renewable energy production, sustainable waste management, and the production of organic fertilizer. We continue with a description of the concepts of circular economy and bioeconomy, again with AD of organic matter as a pivotal approach that can foster these production and economical systems. Finally, we explain the life cycle assessments (LCA) and how can they be implemented for AD approaches and projects.

3.3.1 Current Status

An ecosystem can be defined as a biological system constituted by live organisms (biotic factors) and the physical medium that they share and where they live in (abiotic factors).

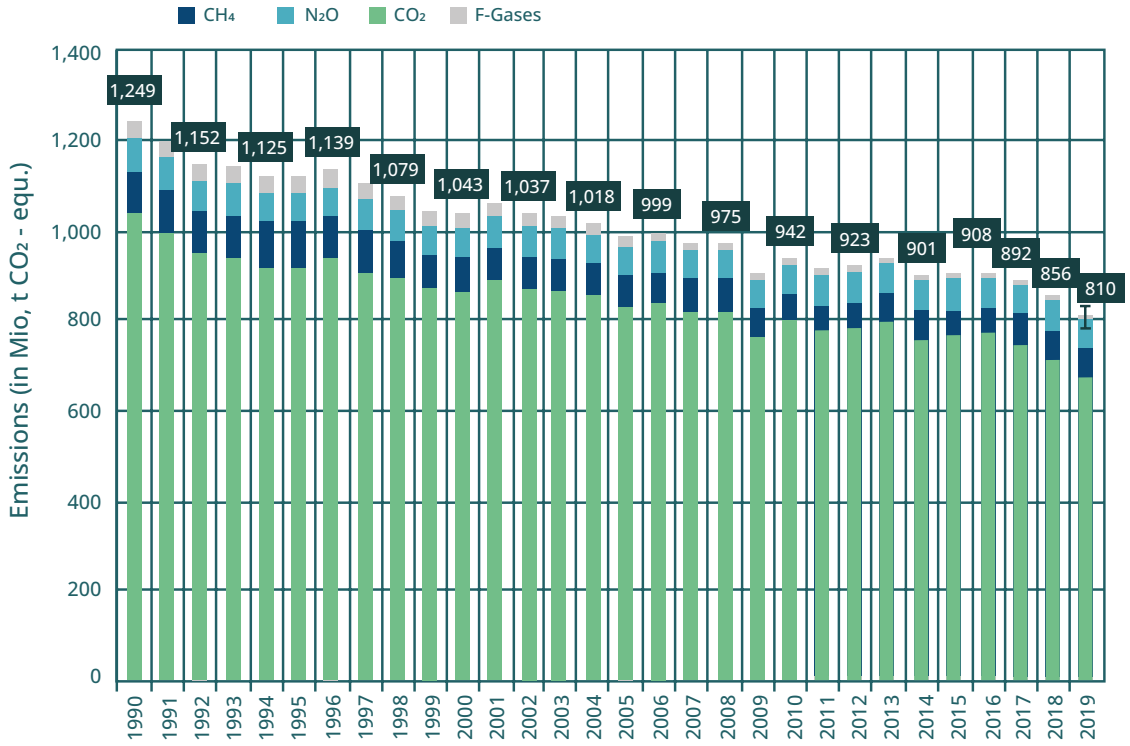


Figure 58: Development of greenhouse gases in Germany since 1990, by greenhouse gases. (Adapted from NIR, 2021)

From the point of view of a single species, the environment is defined as the biological, physical and chemical components of the ecosystem with which this species interacts. The lack of awareness in the modern era about the human species as a biological factor of the ecosystems, has led to the underestimation of the consequences of human actions over the rest of the biotic and abiotic factors, this is, over the environment. These environmental consequences are also translated into economic consequences.

In the Resolution of October 15th, 2019, the General Assembly of the United Nations recognizes that in the actual world *"(...) inequalities in wealth, incomes and opportunities are increasing in and between countries. Biodiversity loss, environmental degradation, discharge of plastic litter into the oceans, climate change and increasing disaster risk continue at rates that bring potentially disastrous consequences for humanity"*. In the same resolution, the United Nations reaffirm the commitment to the 2030 Agenda. In this Agenda, the 17 Sustainable Development Goals, which guide policies to achieve a sustainable development, were coined. Sustainable development is defined as the development capable of satisfying today´s needs without compromising the capacity of future generations to satisfy their own needs. To achieve it, it is fundamental to harmonize three basic elements: economic growth, social inclusion and protection of the environment.

AD of organic matter is a technology that can contribute to these basic elements of sustainable development. From an environmental perspective, AD provides a sustainable energy source, which is biogas or biomethane. It also provides a sustainable source of organic fertilizer which is the digestate known as the organic matter that remains after the anaerobic digestion. In the same way, it provides a powerful tool to mitigate methane emissions. And of course, AD is a sustainable waste management approach. The characteristics of biogas production are important for considering this approach as a promoter of circular economies, revalorizing waste from many sources.

3.3.2 Energy

Global energy demand has grown rapidly due to rising world populations and the consumerist lifestyle. Globally, energy consumption reached 1,500,000 TWh in 2010, and is estimated to be 2,340,000 TWh by 2040 **(Sawatdeenarunat *et al.*, 2015)**. Fossil fuels such as coal, oil and natural gas are the most used energy carriers providing more than 80% of global energy consumption **(Sawatdeenarunat *et al.*, 2015; Hijazi *et al.*, 2016; Energy Statistics Pocketbook, 2022)**. These resources are limited in supply (non-renewable), have adverse effects on the environment due to the emission of greenhouse gases (GHGs), particulate matter into the atmosphere, and also due to accidents that provoke leaks to the water and soil **(US EIA, 2013)**. Unconventional fossil sources and new techniques, for example hydraulic fracturing, are becoming economically viable, but these alternatives entail even increased risks to the environment and reduced energetic yield **(Hijazi *et al.*, 2016)**.

According to the Fifth Assessment Report of the IPCC **(Stocker *et al.*, 2013)**, more than 50% of the observed increase in global mean surface temperature is very likely caused by the anthropogenic increase in GHG emissions, the main being CO_2. Fossil fuel sources (coal, oil and natural gas) account for 84% of total emissions of CO_2 **(Friedlingstein *et al.*, 2020)**.

Climate system and climate drivers: The climate system is the group of five components of the Earth: the atmosphere (air), the hydrosphere (water), cryosphere (ice and permafrost), litosphere (upper rock layer of the Earth) and biosphere (living organisms). Some authors consider the anthroposphere as a sixth component due to the significant effect of human actions over the climate system. These components interact by exchanges of energy and matter. The interaction determines what we define as the climate. The climate is the statistical description in terms of mean and variability of the relevant parameters of a specific place over a period, from months to thousands or millions of years. The parameters considered for the definition of climate consists of temperature, humidity, pressure, wind, and precipitations. A climate change refers to a change in the mean and/or variability of one or more than one of these parameters that persists for an extended period, generally decades or more **(Stocker *et al.*, 2013)**.

The climate system receives energy from the sun as solar shortwave radiation (SWR) and, in much less quantity, energy from the earth´s nucleus and energy of tidal waves from the moon. Half of the SWR is absorbed by the earth's surface, 20% is absorbed by the atmosphere, and 30% is reflected by the earth's surface (albedo) and by aerosols, clouds, and gases in the atmosphere. The earth surface also emits energy, which is in the form of longwave radiation (LWR, or infrared radiation). Changes in the outgoing LWR result from changes in the temperature of the surface or the atmosphere, or by changes in the emissivity of LWR from either the atmosphere or the Earth surface. The net balance between incoming and outgoing energies is called the energy budget of the earth **(Stocker *et al.*, 2013)**.

Climatic parameters can change due to internal variability of the six components of the climate system, by external substances or processes that affects the earth´s energy budget. These so-called climate-change drivers can be natural or anthropogenic, and affects the climatic parameters by changing the energy and matter fluxes of the earth. This is quantified by radiative forcing (RF). RF is a measure of the net change in the energy balance in response to an external perturbation. Positive values of RF means that the energy fluxes changed leading to warming of the earth surface, while negative values of RF leads to surface cooling. For example, "atmospheric CO_2" is a climate driver. An increase in atmospheric CO_2 concentration leads to a positive RF, which means an increase in surface temperature. But, in the case of the climate driver "atmospheric aerosols", an increase in atmospheric aerosol concentration leads to a negative RF, which means a decrease in surface temperature **(Stocker *et al.*, 2013)**.

Atmosphere, GHGs and carbon cycle: The atmosphere is the mass of gases that surrounds the earth. It is mainly composed of diatomic nitrogen (N_2; 78%), diatomic oxygen (O_2; 21%), argon (Ar; 1%) and carbon dioxide (CO_2; 0.04%). Also, an important component is water vapor with a range from 0.1-5%. In a second place are neon (Ne; 18ppm), helium (He; 5ppm), krypton (Kr; 1ppm), diatomic hydrogen (H2; 0.5ppm), xenon (Xe; 0.09ppm), methane (CH_4; 0.04ppm). In addition, there are other gases as traces (CO, N oxides, HNO_3, NH_3, H_2O_2, formaldehyde, CS_2, SO_2, O_3) **(Baird and Cann, 2014)**. The atmosphere is divided altitude-wise into strata: troposphere (from sea level to 15km; 85% of the mass of the atmosphere), stratosphere (15km to 50km; here is the ozone layer), mesosphere (50km to 85km) and thermosphere (85km to 120km) **(Baird and Cann, 2014)**.

The main climate-change drivers in the atmosphere are greenhouse gases, ozone, clouds and aerosols. They act affecting the radiative properties of the atmosphere absorbing incoming SWR emitted from the sun or absorbing outgoing LWR emitted from the earth's surface. Absorption of SWR reduces the incoming heat, but absorption of LWR, after being reemitted in all directions, adds heat to the lower layers of the atmosphere and to the earth. This is the greenhouse effect **(Stocker *et al.*, 2013)**.

As stated above, more than 50% of the increase in surface temperature can be explained by anthropogenic GHGs that accumulate in the atmosphere. The RF for the emissions of CO_2 from 1750 to 2011 is 2.29 W m^{-2}. The increase in the atmospheric concentration of CO_2 that resulted from anthropogenic emissions is the largest contributor of positive radiative forcing **(Stocker et al., 2013)**.

Well-mixed GHGs represent the gaseous phase of global biogeochemical cycles, which can be defined as the complex flows and transformations of the chemical elements between the different components of the climate system (atmosphere, ocean, land, ice, living beings) by biotic and abiotic processes. The carbon cycle accounts for the stocks and fluxes of carbon compounds, for example CO_2 and CH_4.

Carbon stocks are usually measured in relation to the mass of carbon atoms in the different carbonated compounds. In this way a common measure is a gigatonne of carbon (GtC) which equals a petagram of carbon (PgC) which equals 10 e15 g of carbon **(Friedlingstein *et al.*, 2020)**. In the atmosphere, the main carbon compound is CO_2 at a concentration of 390 ppm (corresponding to 828 PgC). Other carbon gases are methane CH_4 (3.7 PgC) and carbon monoxide CO (0.2 PgC). In addition to traces of hydrocarbons, carbon black aerosols and volatile organic compounds can be found **(Baird and Cann, 2014; Friedlingstein *et al.*, 2020)**.

Carbonated compounds cycle between stocks which are located in all the components of the Earth. The exchange of carbon between different stocks is indicated as a quantity of carbon mass per year (i.e. GtC/yr or PgC/yr). Carbon compounds in the lithosphere and cryosphere are stocked in fossil reserves (gas, coal and oil), in the permafrost (frozen land) and in the soils; carbon compounds in the atmosphere are mostly stocked as CO_2 and CH_4, but also as other volatile compounds. Carbon compounds are stocked in the hydrosphere as dissolved inorganic carbon, as well as in the ocean surface sediments; and carbon compounds in the biosphere are stocked in the marine or land biota **(Stocker *et al.*, 2013; Friedlingstein *et al.*, 2020)**.

The carbon atoms are transferred from one stock to another. If the above-mentioned stocks are grouped in land (cryosphere, lithosphere and land biota), ocean (marine biota and hydrosphere) and atmosphere categories, and if only natural carbon fluxes are considered, there is a balanced efflux-influx of carbon between land-atmosphere, land-ocean and ocean-atmosphere groups. However, anthropogenic actions disturb this balance. Mostly by the huge extraction of carbon from fossil fuel reserves. The combustion of fossil fuels generates GHGs that are delivered to the atmosphere. The increased amount of CO_2 in the atmosphere leads to an increased uptake of CO_2 from the ocean. Within this situation, the net balance

of 130 carbon due to anthropogenic causes is -365±30 PgC/yr in fossil fuel reserves, +240±10 PgC/yr in the atmosphere and +155±30 PgC/yr in the ocean **(Stocker *et al.*, 2013)**. There are other carbon fluxes caused by anthropogenic actions but are not considered here due to the much lower impact in the carbon cycle.

Carbon stocks can be classified into two domains, defined by the amount and rate of carbon transfer to or from each stock. The fast domain is composed of stocks that exchange a high quantity of carbon compounds at a fast rate. The slow domain is composed of huge carbon deposits in rocks and sediments that are characterized by exchange times of more than 10k years. The natural carbon exchange between both domains is relatively small (less than 0.3 PgC/yr) and can be assumed to be constant over time. It is caused by volcanic emission events (CO_2), chemical weathering, erosion and sediment formation on the ocean floor **(Stocker et al., 2013)**. Anthropogenic actions can be considered, in fact, to transform the fossil fuel reserves from a slow-domain stock into a fast-domain one, delivering high quantities of CO_2 to the atmosphere. The use of fossil fuels since the industrial age has resulted in a huge transfer of carbon stocks accumulated in the slow domain to the fast domain, mainly in the atmosphere.

Environmental effects: The use of fossil fuels as a source of energy impacts the territories in many ways because of either the extraction, the transport, or the use of the fuels. The main and most dramatic impact is on the climate system, which entails effects over the five components of the climate system. The IPPC 5 Report describes the changes in temperature, the changes in energy budget and heat content, changes in circulation, changes in the water cycle and cryosphere, changes in sea level, changes in extremes, and changes in the biogeochemical cycles **(Stocker *et al.*, 2013)**. The global mean surface temperature has certainly increased since the late 19th century, and with a steady increase in warming during for each of the last three decades, the 2000s decade has been the warmest. It is also virtually certain that globally the troposphere has warmed, and the stratosphere has cooled since the mid-20th century. The same high confidence is regarded to the virtually certain warming of the upper ocean (above 700m) since late 19th century. Improved sampling technologies also allows to state that likely the ocean from 700-2000m has warmed since 1957 to 2009, and that ocean from 3000m to the bottom has also warmed.

It is also virtually certain that the Earth has gained significant energy from 1971 to 2010, with more energy from the Sun entering to the climate system than exiting the atmosphere.

The atmospheric circulation (e.g. winds) and oceanic circulation (e.g. currents) have also suffered from changes. It is likely that atmospheric circulation features have moved poleward since 1970s, with a widening of the tropical belt, a poleward shift of storm tracks and jet streams, and a contraction of the northern polar vortex. The variability in major ocean circulation systems is also strongly evidenced, for example, it is very likely that the subtropical gyres in the North Pacific and South Pacific have expanded and strengthened since 1993.

The water cycle describes the continuous movement of water through the climate system in its liquid, solid and vapour forms, and storage in the reservoirs of ocean, cryosphere, land surface and atmosphere. The movement of fresh water between the atmosphere and the ocean can influence oceanic salinity, which is an important driver of the density and circulation of the ocean. It is very likely that regional trends have enhanced the mean contrasts in sea surface salinity since the 1950s: saline surface waters in the evaporation-dominated mid-latitudes have become more saline, while fresh surface waters in rainfall-dominated tropical and polar regions have become fresher. There is also very high confidence that the Arctic Sea ice extent decreased over the period 1979-2012. There is very high confidence that glaciers world-wide are persistently shrinking, while snow cover extent has decreased in the NH_4.

The increased uptake of heat by the oceans led to its warming which in turn led to the expansion of the ocean water and the increase in the sea level. This is enhanced by the high transfer to the ocean of water currently stored on land, particularly from glaciers and ice sheets.

There is also evidence that climate change led to changes in extremes. For example, it is very likely that the number of cold days and nights has decreased, and the number of warm days and nights has increased globally between 1951 and 2010. It is also likely that since 1950 the number of heavy precipitation events over land has increased in more regions than it has decreased.

Finally, the global biogeochemical cycles have also suffered from changes due to effects related to climate system drivers. We will take about methane cycle in following headings.

Opportunities for biogas industry: Even though the combustion of CH_4 molecules produces the same number of CO_2 molecules ($CH_4 + 2\ O_2 \rightarrow CO_2 + 2\ H_2O$), the overall effect on global warming is smaller because methane effect as GHG is 28 times higher than CO_2 **(Hijazi *et al.*, 2016)**. Besides, the carbon source of biogas or biomethane belongs to a fast domain of the carbon cycle because it is generated from biomass that obtained the C atoms and compounds from the atmosphere (plants) or the biosphere (animals). So, contrary to fossil fuels, there is no net increase in CO_2 molecules in the atmosphere because the emitted CO_2 molecules are balanced to molecules that were transferred from the atmosphere to the biosphere.

3.3.3 Artificial mineral fertilizers

According to the Food and Agriculture Organization of the UN, the cultivated area worldwide was 4.8 billion hectares (ha) in 2019 **(FAO, 2021)**. This agricultural land is used in two thirds as meadows and pastures, and one third as cropland. Although agricultural land decreased since 2000, on average there is a steady increase by 0.1% since 1961, with a peak in the 1990s. In spite of this, cropland area per capita decreased globally between 2000 and 2019 as population increased faster than cropland. This can be explained by an increase of 53% in primary crop production from 2000 to 2019 (9,4 billion tonnes in 2019).

However, this was not due to increased global workforce employed in agriculture. Indeed, this figure decreased from 1050 million people in 2000 to 874 million in 2020. On contrary, the increased production is explained by an increased yield due to developments in irrigation, increase in pesticide use (+36% from 2000 to 2019) and increase in fertilizer use **(FAO, 2021)**. The same report states that the use of inorganic fertilizers worldwide in 2019 was 190 million tons of nutrients, of which 57% corresponds to nitrogen (N), while the rest corresponds to phosphorus (P) and potassium (K). This number represents an increase of 40% (54 million tons) of the fertilizer use since 2000, which is explained by the increase in fertilizer use per crop area, from 91 kg/ha in 2000 to 122 kg/ha in 2019, of which the N:P:K ratio is 70:28:24. Europe accounts for the 12% of the total fertilizer use. The main nutrient is N, representing 60% of the European fertilizers. Fertilizer use per area in Europe is 80kg/hectare, which represents a 12% increase since 2000 **(FAO, 2021)**.

The nitrogen budget of the soil represents the difference between the amount of N added with synthetic and organic fertilizers and the amount of N taken away by livestock and crop production during their biomass growth. While a negative budget indicates that more N is being taken from the soil than added with fertilizers, which impacts yield and the health of the soils, a very positive budget indicates that excess fertilizers are being used, which leads to environmental and human health issues.

Nitrogen cycle: While the fourth element in the biosphere is nitrogen, dinitrogen gas (N_2) is the main compound in the atmosphere. All

N-containing species except N_2 are called reactive nitrogen species (Nr). These compounds are the N sources that support cellular metabolism and growth **(Stein and Klotz, 2016)**.

The N cycle is composed by Nr compounds together with N_2, spanning nine different oxidation states of N atoms, which shows the complexity of this biochemical cycle. Roughly, there are three types of chemical reactions in the N cycle: N fixation, denitrification, and nitrification. N fixation is the reduction of N_2 gas to ammonia (NH_3) or ammonium (NH_4^+). These species are the only ones that can be assimilated by cells, which is when N interacts with C atoms making N organic compounds. Nitrifications are the reactions that oxidize NH_3 or NH_4^+ to nitrite (NO_2^-) or nitrate (NO_3^-). Denitrification are the reactions that transforms NO_2^- to less oxidized compounds, such as nitric oxide NO and nitrous oxide N_2O, and finally to the dinitrate gas N_2 **(Stein and Klotz, 2016)**.

In the primordial atmosphere, abiotic reactions dominated the nitrogen cycle and provided oxidized forms of N (NO_2^- NO_3^-) and also reduced forms of N (NH_3 NH_4^+), the lasts being essential for biological assimilation **(Stein and Klotz, 2016)**. Before the industrial age, the interchange between N_2 in the atmosphere to the rest of the Nr species was dominated by terrestrial and marine microorganisms via biological fixation of N_2 to ammonia, and in a much smaller extent, by NO_x production by lighting events **(Fowler *et al.*, 2013; Stocker *et al.*, 2013)**.

Since the industrial era and specially since the 20th century´s Green Revolution, three anthropogenic sources of Nr have greatly increased Nr creation: the Haber-Bosch process to create NH_3 from atmospheric N_2 and H_2 for fertilizer and for industrial inputs; the cultivation of legumes and other crops capable of biological N fixation; and the combustion of fossil fuels, which converts atmospheric Nr from fossil fuels and N_2 into nitrogen oxides (NO_x) emitted into the atmosphere and redeposited on the land surface or in the oceans **(Stocker *et al.*, 2013; Stein and Klotz, 2016)**.

In 2010, global nitrogen fixation from atmospheric N_2 to terrestrial and marine ecosystems was 413 Tg N as estimated by **Fowler *et al.* (2013)**. Of this Nr, 210 Tg N were fixed by anthropogenic causes, either intentionally or unintentionally. The main anthropogenic causes were fertilizer production through Haber-Bosch process (120 TgN) and agricultural fixation by legumes and grasses (60 TgN), with 30 TgN produced unintentionally by combustion. The main natural cause was the marine biological fixation of 140 TgN, followed by terrestrial biological fixation of 58 TgN and 5 TgN fixed by lightning **(Fowler *et al.*, 2013)**.

The Nr that is denitrified to N2 is between 100-280 TgN/yr depending on different authors. The remaining N (difference between fixed and denitrified) is: emitted as NO or N_2O from soils to the atmosphere (5 and 13 TgN/yr respect.) or as N_2O from the oceans to the atmosphere (5.5 TgN/yr); emitted as NH_3 from terrestrial ecosystems and oceans to the atmosphere (60 and 9 TgN/yr respect.); wet and dry deposited as NO_x to terrestrial surfaces and oceans (70 and 30 TgN/yr); and buried in oceans (20 TgN/yr) **(Fowler *et al.*, 2013)**.

The concept of nitrogen cascade illustrates how an atom of Nr circulates along the biogeochemical pathway, with different effects to the environment and human health, until it is denitrified again to nonreactive N_2. For example, a N cascade can start with the use of fertilizer, transforming non-reactive N from atmospheric N_2 molecules to Nr (NH_3) by Haber-Bosch process. From the 120 TgN fixed by Haber-Bosch, 80% is used as agricultural fertilizer and 20% as feedstock for industrial processes. Half the Nr fertilizer applied to agroecosystems is incorporated into crops harvested for human and livestock consumption. The other half is transferred to the atmosphere as NH_3, NO, N_2O or N_2, or deposited as NO_x to terrestrial surfaces and oceans **(Fowler *et al.*, 2013)**.

Environmental effects: As stated above, when Nr creation rates are higher than the rates of removal by denitrification or assimilation by cell metabolism, the remaining Nr is

available in the atmosphere and in marine and terrestrial ecosystems and is either accumulated or dispersed by hydrologic (leaching and runoff) and atmospheric transport processes. **(Galloway *et al.*, 2003; Erisman *et al.*, 2013)**.

The environmental effects of the excess of Nr are varied. Emissions of N_2O are the third GHG source in importance, together with CO_2 and CH_4; the global balance of the effect of Nr on terrestrial radiation, this is in global warming, is 0.24 Wm^{-2}. N_2O also degrades the stratospheric ozone layer. NO_x species produce O_3 (Ozone) and nitrate aerosols increasing smog and the haziness of the troposphere. NH_3 leads to the production of nitrate aerosols. Together with S, Nr contributes to the acidification of lakes, rivers, and streams, followed by loss of biodiversity. Nr increases productivity in forests, grasslands, and waters, which can lead to eutrophication and reduced biodiversity. In coastal ecosystems, Nr is considered one of the biggest pollution problems, again leading to eutrophication, hypoxia, and loss of biodiversity. Increased soil Nr changes the rate of decomposition of soil organic matter and therefore affects the emission of CO_2; it also affects plant productivity, either increasing it because of the greater availability of Nr, but also decreasing plant availability due to volatile organic compounds and Nox-mediated tropospheric O_3. Increased NO_x, aerosols, tropospheric O_3, and nitrates in drinking water also negatively impact human health provoking respiratory illnesses, cancer and cardiac diseases **(Galloway *et al.*, 2003; Leach *et al.*, 2012; Stocker *et al.*, 2013)**.

Opportunities for biogas industry: Nitrous oxide (N_2O) emissions from fertilizer application and manure management account for 5% of total Europe GHG emissions (210 $MtCO_2eq$) **(Bacenetti *et al.*, 2013)**. Although Europe is the only region where the N soil budget declined (by 5%) from 2000 and 2018, it is still considerably high, with a budget of 48kg/ha. This budget was made from an input of 90.7kg/ha of N and an output of 42.9kg/ha. Of these 90.7 kg/ha of input, 51.4 kg/ha correspond to synthetic fertilizers, 24.8 kg/ha correspond to manure, and 0.7 and 4.8 kg/ha correspond to atmospheric deposition and biological fixation respectively **(FAO, 2021)**.

The organic agriculture differs from conventional agriculture in that it promotes the avoidance of synthetic fertilizers and pesticides. Fifteen of the top 20 countries with the highest ratio of organic:conventional agriculture is in Europe. This means that the region has emphasized the importance of organic agriculture. However, the percentage of organic agriculture from the total agricultural area is still low with a 3,4% in Europe **(FAO, 2021)**.

Digestate as a by-product from biogas production via AD can substitute synthetic fertilizer and be used as an organic fertilizer to boost the adoption of organic agriculture practices in Europe **(Hijazi *et al.*, 2016)**.

However, there are many things that must be addressed before adopting digestate as a fertilizer. For example, the digestate from animal manure may contain high concentrations of elements such as copper and zinc (micro-nutrients supplemented in animal feed), and direct application of the digestate to agricultural land may result in phytotoxicity. Under such conditions, the digestate must be diluted with irrigation water before being applied to the field **(Sawatdeenarunat *et al.*, 2015)**. Also, open storage of digested residues is considered a hot spot of emissions in biogas systems. If the CH_4 produced during digestate storage is not properly recovered, thus the released CH_4 may worsen GHG emissions **(Sawatdeenarunat *et al.*, 2015; Hijazi *et al.*, 2016)**.

3.3.4 Methane emissions

Climate warming is a global phenomenon caused mainly by the abundance of well-mixed GHGs in the atmosphere. Among the GHGs, the most important is CO_2. However, methane emissions (CH_4) have a significant contribution to global warming. Methane, as well as CO_2, are compounds that are integrated into the so-called carbon cycle above explained. At normal pressure and temperature conditions, CH_4 is a gaseous compound, so most of it is located at the atmosphere. There, the concentration depends on the ratio among sources:sinks.

Methane cycle: In the methane cycle, there are much fewer sinks than sources. Indeed, 90% of atmospheric CH_4 is eliminated by the oxidation with hydroxyl radicals (OH), mostly at the troposphere. The OH radicals are made after the photolysis of O_3 in the presence of water vapour and are then eliminated by reaction with CO, CH_4 and non-methane volatile organic compounds. The remaining methane is lost by photochemistry in the stratosphere amd perform reaction with atomic chlorine (Cl), atomic fluorine (Fl) and excited atomic oxygen (O(1D)), by photochemistry in the marine boundary layer and by oxidation in soils. This last methane sink is due to methanotrophic bacteria that can oxidize methane in unsaturated oxic soils and consume it as an energy source **(Saunois *et al.*, 2020)**.

As stated above, there are many sources of CH_4. They can be classified based on the process that creates CH4, or based on if the process is provoked by natural or anthropogenic causes. The processes that can create CH_4 can be biogenic, geological, or pyrogenic. Biogenic methane is a result of the AD of organic matter performed by communities of microorganisms. Geological processes that emit CH4 are for example volcanic eruptions, gas reserves, perma-frost or methane hydrates accumulated mainly in the ocean that can emit CH_4. Pyrogenic processes emit CH_4 because of the incomplete combustion of fossil fuels or biomass **(Stocker *et al.*, 2013)**.

Combining both of the classifications, **Saunois et al. 2020** describe three anthropogenic categories: **(1)** agriculture and waste accumulation, **(2)** fossil fuels, and **(3)** burning of biomass and biofuels; and eight natural categories: **(1)** wetlands, **(2)** other inland water systems, **(3)** onshore and offshore geological sources, **(4)** termites, **(5)** wild animals, **(6)** oceanic sources, **(7)** terrestrial permafrost and hydrates, and **(8)** vegetation.

The anthropogenic fossil fuel related methane emissions are due to the exploitation, transportation and usage of coal, oil and natural gas. The composition of natural gas is mostly methane. The extraction, transportation or use of gas can all contribute to methane emissions. The same accounts for shale gas, which emits in a similar amount as natural gas. Also, for example in coal mines, high quantities of CH_4 are pumped out to maintain a CH_4 percentage of 0,5% inside the mine. While in some countries this CH_4 is used as fuel, in many countries it is still emitted to the atmosphere. However, the highest source of anthropogenic CH_4 is agricultural and waste accumulation, mainly by livestock production, and followed by rice cultivation, landfills, and wastewater treatment. The livestock emissions are due to domestic ruminants whose digestive system, particularly the rumen, provides methanogenic archaea with stable temperatures (39ºC), optimum pH (6.5 - 7.6) and a constant plant matter flow. These archaea produce methane that is released mostly through the mouth of the animals (87%) or absorbed in the blood system. The rest of the CH_4 is emitted through the rectum of ruminants. Manure decomposition is another important source of CH_4 when manure is treated in liquid or slurry forms. If manure is deposited as a solid, aerobic conditions produce no CH_4, but it produces N_2O which has a larger warming impact than CH_4. Flooded fields for rice cultivation are another important source of anthropogenic CH_4. Finally, landfills and wastewater handling produce CH_4 according mostly to the type of waste and to the amount of degradable organic material respectively. In some landfills, however, CH_4 is used to generate heat instead of just ventilating it to the air. And for example, Europe counts with laws for the regulation of landfilling. Finally, biomass and biofuel CH_4-emissions are 90% produced by anthropogenic causes compared to a 10% of natural fires **(Stocker *et al.*, 2013; Saunois *et al.*, 2020)**.

The natural sources of CH_4 are even more varied, as stated above. Wetlands are ecosystems where soils or peats are saturated with water, or where surface inundation dominates the soil biogeochemistry. The anaerobic conditions lead to CH_4 production. Other inland water systems can also produce CH_4, such as lakes, ponds, reservoirs, streams, or rivers. Also, there are important onshore and offshore geological sources of CH_4 emissions produced in the earth crust that reach the atmosphere through tectonic

faults and fractured rocks. Just like livestock, wild ruminants also emit CH_4 when they degrade plant biomass in their digestive system through AD. There are even important emissions from insects that generate CH_4 in 136 their guts. The most representative are the termites. There are also oceanic sources of CH_4, both in coasts and in open ocean, for example, CH_4 production from marine sediments, in situ production in the water column, leaks from geological marine seepage and also emission from the destabilization of marine hydrates. Marine CH_4 hydrates are ice-like crystals formed under specific temperature and pressure conditions that comprise a potential important source of CH_4, but without a significant relevance yet. The permafrost, which is frozen soil below 0ºC, can generate direct and indirect CH_4 emissions. The direct ones are due to the release of CH_4 contained in thawing permafrost, which was created before the last glacial era and was then trapped as temperatures went down. Indirect emissions rely on methanogenesis induced when the organic matter is released from thawing permafrost. This source is projected to be more important as warming climate thaws more permafrost. Finally, vegetation can produce small amounts of CH_4, either through abiotic photochemical processes induced by stress, acting like straws releasing CH_4 from soils, or providing suitable environments for methanogenic microorganisms, especially in the stems **(Stocker *et al.*, 2013; Saunois *et al.*, 2020)**.

Since beginning of industrial era in 1750 to 2011, atmospheric CH_4 levels grew exponentially by a factor of 2.5, from 0.7ppm to 1.8ppm. The 5th intergovernmental panel for climate change stated, with a very high statistical confidence level, that this increase was due to anthropogenic causes. In fact, satellite measurements show that there are higher CH_4 concentrations in places where anthropogenic influence is high, for example in urbanized or agricultural areas. This effect can also be detected downwind of urbanized or agricultural areas **(Stocker *et al.*, 2013)**. Actual measurements indicate that anthropogenic causes explain 50-65% of total emissions. And top-down approaches estimates that for the period 2008-2017, there was an atmospheric growth of 18.2 $TgCH_4$/year **(Stocker *et al.*, 2013; Saunois *et al.*, 2020)**.

Environmental effects: Although exposure of hydrocarbon mixtures can have some adverse effects on humans, methane emissions are not considered relevant for direct health issues. However, the global warming potential of CH_4 as a GHG is estimated to be 28-36 times higher than CO_2 in a 100-year lapse. In this way, it is the second major component among anthropogenic GHG **(Paolini *et al.*, 2018)**.

The RF associated to CH_4 emissions are estimated to 0.97 Wm^{-2}, while for the same period, total GHG and CO_2 RF are 3 Wm^{-2} and 1.68 Wm^{-2} respectively. This reveals the considerably high effect of CH_4 as GHG **(Stocker *et al.*, 2013)**. Almost a quarter of the RF associated to CH_4 is not due to a direct consequence of CH_4 itself, but due to its reaction with O_2 that produces OH radicals and then water. Stratospheric water vapour also acts as a highly potent GHG **(Stocker *et al.*, 2013)**.

In Europe, CH_4 emissions from enteric fermentation, manure management and rice cultivation produces 5% of total Europe GHG emissions (195 $MtCO_2eq$) **(Bacenetti *et al.*, 2013)**.

Opportunities for biogas industry: Biogas production avoids the emission of CH_4 to the atmosphere from organic matter going through AD. However, there remains spots of avoidable emissions. For example, the open storage of not-fully digested residues or flaring excess biogas **(Hijazi *et al.*, 2016)**. MICRO4BIOGAS plans to develop new designs of bioreactors for an improved AD of organic matter.

3.3.5 Waste management

The European Union defines waste as “an object the holder discards, intends to discard or is required to discard”. There are many types of waste, each one with also many forms to be managed. Some types of waste are agricultural waste, chemical waste, con-

struction and demolition waste, food waste, green waste, wastewater, sewage, organic municipal waste, etc.

Considering municipal waste, annually 2 billion tons of this waste is globally produced. And this is without considering the two other components of organic solid waste, which are agricultural waste and animal waste **(Wainaina *et al.*, 2020)**. The EU countries contribute with approximately 250 millions of tons of municipal solid waste, which leads to an estimate of 482 kg per capita This represents 10% of total waste generated in Europe **(Bourguignon, 2018; Commission, 2020)**.

The level of waste generation in EU countries positively correlates with the gross domestic product per capita, which means that the amount of waste depends significantly on economic development. However, the increasing waste production in EU countries does not correlate with an increase in the adoption of reducing- or reusing-behaviours. This is different for the recycling behaviour, which correlated with the amount of waste generated, showing to be the only positive-behaviour adopted when people try to manage their waste production. So, as standard of living rises, it also does the level of consumption and the amount of waste, which has to be managed properly **(Minelgaitė and Liobikienė, 2019)**.

AD with different residues: Manure as feedstock is a very favourable substrate in terms of environmental impact. Biogas scenarios with manure as input material show lower GHG emissions than their reference systems. The transportation of manure can increase the impact of this feedstock **(Hijazi *et al.*, 2016)**. Animal manure shows more environmental benefits than wastes from food industries and households, with an indirect benefit because of avoided emissions of CH_4 and N_2O from traditional manure storage. Also, the manure digestion can be even improved for an increased methane yield if animals are fed with higher crude protein content food **(Hijazi *et al.*, 2016)**. Manure is a good substrate to be used in co-digestion treatments. For example, co-digestion of animal manure with lignocellulosic residues can be used to biologically pretreat energy crops and remove the amorphous hemicellulose fraction of the biomass structure **(Sawatdeenarunat *et al.*, 2015)**. The co-digestion of energy crops with manure is beneficial for the AD because it increases the yield of biogas, stabilizes the organic matter and decreases the methane emission during storage **(Bacenetti *et al.*, 2013)**.

Wastewater treatment is an energy intensive activity. The treatment of municipal waste water accounts for about 3% of global electricity consumption and 5% of global green-house gas emission. The biological wastewater treatment demands 20-30 kWh energy per person equivalent per year, but the energy recovery via AD of wastewater sludge is only about 15–18 kWh per person equivalent per year. So, utilizing the sludge for energy recovery, waste water treatment plan (WWTP) can achieve energy self-sufficiency only up to approx. 65%. In practice, a typical WWTP can currently offset 20–30% of the energy consumption and green-house gas emission **(Nghiem *et al.*, 2017)**. Traditionally, WWTPs have been designed with the aim of meeting discharge standards to receiving water bodies while waste produced during treatment is destined for landfill or incineration.

An option to make WWTPs more convenient both economically and ecologically is the co-digestion of wastewater sludge with substrates with enough stock availability. For example, wastewater sludge may be co-digested with municipal organic waste. With this, WWTPs could achieve energy-neutrality and reduce the cost of municipal organic waste management while facilitating nutrient recycling. Other possible substrates for this may be food waste from urban areas, organic waste from food processing, market waste, dairy waste, etc. **(Nghiem *et al.*, 2017)**. Co-digestion is also a viable alternative for small WWTPs that require a specific biogas production threshold that can justify the maintenance cost and capital investment of biogas utilization equipment such as combined heat and power unit **(Nghiem *et al.*, 2017)**.

It is estimated that in EU countries, 20% of the food produced is lost **(Commission, 2020)**.

Food waste can be sorted and processed either onsite or delivered in a pre-treated liquid form. In addition to sorting and processing equipment, onsite processing also requires several auxiliary facilities including weighing bridge and even airlock passage to the receiving bay (Nghiem *et al.*, 2017).

Environmental effects: The collection of OSW (organic solid waste) is an environmental concern both in urban and rural areas. Technologies for OSW management are important to tackle environmental issues, to develop sustainable practices, and to forge circular economies. Currently, most of the OSW is treated to be converted in organic fertilizers or is deposited in landfills or incinerated (Wainaina *et al.*, 2020).

EU countries, just like the rest of the countries in the world, have produced increasing masses of wastes for many decades. The most practical solution for waste management in most of EU countries remains landfilling due to technical, economic, and legal reasons. This is true even with the European Union Directives on waste landfills that has introduced specific goals for reducing the volume of disposed waste, strict requirements of landfilling, and landfill sites (Vaverková, 2019).

Landfilling is the worst option in environmental terms: it provokes contamination on soil and water with chemicals that leach from waste, animals can ingest micro plastics, and methane and other pollutants are released to the air. Also, in economic terms, landfilling leads to the loss of valuable resources that could be used for manufacturing other goods (Bourguignon, 2018).

Waste disposal options by landfilling or incineration are expensive and not compatible with the concept of a circular economy. Ongoing leachate management and extensive monitoring for potential groundwater and air pollution are required during active landfill operation and even up to 50 years' post-closure. On the other hand, extensive air pollution control is required for the waste incineration. Incineration of waste materials also results in significant greenhouse gas emission (Nghiem *et al.*, 2017).

Sharma *et al.* (2019) recognizes the positive environmental effects for the recycling (composting, vermicomposting and AD) of agricultural organic wastes and the use of the treated waste (e.g. digestate): improved soil texture and fertility, enhanced crop productivity, GHG´s mitigation and availability of alternatives to agrochemicals.

Opportunities for biogas industry: Potentially, 60 million tons of OSW could be recycled by AD and composting technologies in Europe, which could save one million tons of nitrogen and 20 million tons of organic carbon that are currently lost through landfilling organic waste (Mayer *et al.*, 2019). European countries in average recycle only 5% of the total OSW (Commission, 2020). If a higher portion of OSWs could be recycled and reused, it is estimated that approximately 30% of the chemical fertilizer applied to soil could be replaced (Paes *et al.*, 2019).

EU-28 nations produced nearly 25.38 million tons of wastes through all the activities including economic and households in 2016. It is estimated that global urban waste collection market can reach to an approx. income of U$S 410 billion. Only 25% of this waste is recycled, which means that there is a big economic opportunity (Wainaina *et al.*, 2020). While policy efforts do not seem to succeed in changing personal habits regarding the reduction of waste, AD of municipal waste may be an important tool for the waste management. This is further relevant considering the close link among economic development and waste production. A study that analysed national and regional approaches linked to circular economy models in several European countries identified waste management strategies in almost every of these approaches, and concluded that waste management appears to be critical in this transition (Vanhamaki *et al.*, 2019).

In the final version of this roadmap, we will assess the extent to which AD of organic waste could tackle the currents issues re-

garding waste management. And we will also assess the efficiency of microbial communities developed by MICRO4BIOGAS for degrading different types of feedstock's obtained by several sources of waste.

3.3.6 Economical aspects of biogas industry

In the previous sections it has been analysed the environmental potential of biogas and digestate production by AD. It has been summarized in which way this technology can provide sustainable alternatives for waste treatment, energy production and land fertilizing. However, the environmental challenges also affect the global economy in several ways. New approaches also arise to meet not only environmental goals, but also increasing the profits and decreasing the loses in terms of money. This include the generation of other platform chemicals out of the process.

Two important concepts that emerge as pillars of a sustainable economy are **(1)** circular economy and **(2)** bioeconomy/biorefineries. These concepts will be explained in the following sections. However, in summary they relate to the biogas production because AD can be a pivotal piece for the development of circular economies, valorising waste from different sources; and in the way that AD can be considered as a bioeconomy process, obtaining energy and fertilizer without appealing to fossil-derived resources.

Circular economy: Circular economy is defined as an economic model where the value of products and materials that they contain are valued for as long as possible. It differentiates from the linear economy based on the "take-make-use-dispose" pattern, and considers waste as another resource that can be reintroduced to the production cycle **(Bourguignon, 2018; Vanhamaki *et al.*, 2019)**. The aim of circular economy is the maximization of resource efficiency and minimization of waste production, with the simultaneous effect of keeping resource consumption within planetary limits and reducing production costs. Obsolete products or waste materials are turned into resources for another purpose, thus closing loops and minimizing waste. By adopting circular economy strategies, several European countries are estimated to be able to reduce their national GHG emissions by 70%, while growing their workforce by 4%, decreasing their dependency from resource and energy import **(Nghiem *et al.*, 2017)**.

Two important concepts related to circular economy and waste management are the extended producer responsibility (EPR) and the waste hierarchy **(Bourguignon, 2018)**. The EPR states that producers have the responsibility for collecting used goods as well as sorting and treatment for recycling. The waste hierarchy implies that waste may be treated preferentially by prevention, then by preparing for re-use, then by recycling, then by energy recovery and finally disposal.

Bioeconomy and biorefineries: The European Commission defines bioeconomy as "the production of renewable biological resources and the conversion of these resources and waste streams into value added products, such as food, feed, bio-based products and bioenergy". While some people is strongly optimistic on the potential positive effects of this way of production, others argue that bioeconomy could perpetuate the linear economy model **(Stegmann *et al.*, 2020)**.

In this way, the European Commission stated that bioeconomy should be coupled to sustainability and circular economy, coining the concept of circular bioeconomy. In turn, the bio-based circular economy concept refers specifically to the reuse and valorisation of organic wastes and residues **(Wainaina et al., 2020)**. Other options of waste management such as landfilling, and incineration offer very limited possibilities for resource recovery. But in a bio-based circular economy, organic wastes represent a convenient source of resources in terms of energy and nutrients **(Nghiem *et al.*, 2017)**.

A biorefinery system can be defined as the processing of non-fossil-based feedstock like biomass or organic waste through biologi-

cal or chemical unit operations into a wide range of marketable products such as energy, materials, and chemicals. Biorefineries can utilize while valorising a diverse range of renewable feedstock's such as biomass from forestry, agriculture, aquaculture, and waste (Katakojwala and Mohan, 2021).

The classification of biorefineries are based on **(1)** the type of feedstock (biomass, waste, gas, etc.), **(2)** the biocatalyst-organism (bacteria, yeast, fungi, algae, etc.), **(3)** the type of process/technology (fermentation, acidogenesis, methanogenesis, photosynthesis, thermo-chemical, catalysis, etc.), and **(4)** the targeted product(s) or generation (first, second, third and fourth) (Katakojwala and Mohan, 2021).

The major challenges of biorefineries are its acceptance in the existing fossil-based market, the feedstock availability and composition, the volumes to meet market requirement, the resource recovery efficiency, the techno-economic feasibility, and the environmental sustainability. Taking a biorefinery to a commercial scale is an intricate issue because the biobased products face severe competition from petro-chemicals with respect to their market cost, and therefore, consistent processes and competitive products compared to conventional counter parts are needed. Biorefineries shouldn´t only be assessed for their economic competitiveness, but also for their environmental sustainability. That is why life-cycle assessments (LCAs) and techno-economic analysis (TEAs) can help with this (Katakojwala and Mohan, 2021).

Although AD is a widely used renewable bioenergy production technology, biogas production alone may not sufficiently justify the capital and operating costs. AD can rather be integrated into a biorefinery process (Sawatdeenarunat *et al.*, 2015).

The main by-product obtained with biogas is biomethane. This is made by the upgrading of biogas with different techniques. Once converted in biomethane, this product may be used to feed the gas system or as vehicle fuel. The conversion of biogas to transport fuel has recently been implemented in several EU countries including Germany, Italy, and Sweden (Nghiem *et al.*, 2017).

The consortium of microorganisms in the AD of lignocellulosic matter can easily convert the sugar constituents of hemicellulose into CH_4. Recalcitrant lignin and cellulosic fibres stays in the digested residue, which can be treated with hydrolytic enzymes to solubilize monomers that can be used as precursors in the production of diverse products ranging from bioenergy/biofuel (i.e., CH_4, H_2, ethanol and butanol) to organic acids (e.g., succinic acid) and biopolymers (e.g., bioplastic) (Sawatdeenarunat *et al.*, 2015). Successful production of bio-plastic from biogas has been demonstrated at proof-of-concept experimental levels (Nghiem *et al.*, 2017). Regarding to the effluent of lignocellulosic AD, it may be combined with micro-algae production as a nitrogen rich feedstock that may be utilized by algae as a nutrient source. Such produced algae could be utilized for biodiesel production and the residue after lipid harvest could be further fed into the digester for CH_4 production (Sawatdeenarunat *et al.*, 2015).

Incentives: According to Raboni and Urbini (2014) the incentives granted to the producers and users of biogas and biomethane are in general of the following kinds:

- **Renewables Obligations** that support renewable electricity generation by requiring suppliers to increase the generators production through the purchase of tradable Renewable Obligation Certificates.
- **Feed-in Tariffs** which comprises an extra payment for renewable electricity producers, mainly addressed to support small generation plants.
- **Renewable Heat Incentive**, which provides a guaranteed payment for heat used from biogas combustion and all the biomethane injected into the grid of natural gas.

- **Renewable Transport Fuels Obligation** which places an obligation on fuel suppliers to source 5% of their transport fuels from renewable sources by 2014.
- **Various incentives and tax breaks** introduced in a few countries to promote the use of biomethane in transport.

In the 2010s, there was an expansion of AD plants in Europe mostly thanks to the Renewable Energy Directive I (REDI) with feed-in-tariffs scheme in 29 countries **(Bacenetti *et al.*, 2016)**. These incentives aim an economic feasibility of biogas plants at electricity markets **(Saracevic et al., 2019)**.

Quantitative analysis of economic impacts: The economic analysis of AD plants can be evaluated using different indexes. For example, the net present value (NPV) is operationalized by calculating the costs of the plants (negative cash flows) and the plant cash inflows (positive cash flows) for each period considered. The payback period is defined as the time span required to recover the cost of a project or an investment. And the internal rate of return is the discount rate that makes the net present value of an investment zero **(Lovarelli *et al.*, 2019)**.

However, the technique most widely used to analyse economic yields of AD plants are the techno-economic analysis. These assessments can help to predict an economically competitive production process of bio-based products compared to petro-chemical derived products **(Katakojwala and Mohan, 2021)**. During the techno-economic analysis, three areas of anaerobic digestion process are important: (1) unit operations with collection and transport of feed stocks, (2) to provide the treatment facility for production of biogas, and (3) upgrading the bio-energy for various applications as electricity and liquid methane for household cooking **(Saracevic *et al.*, 2019)**.

Opportunities for biogas industry: The reduction of capital and operating costs of AD facilities is promoting the growth of the biogas industry. It is estimated that the cost of production of biogas will reduce 38% by the year 2050 compared to 2015. It the estimated that biogas production worldwide had an average growth rate of 11.2% reaching up to 58.7 billion Nm^3 in 2017 **(Wainaina *et al.*, 2020)**. The biogas market is estimated to reach 50 billion in 2026 **(Waste Management World 2017)**. It is estimated that only from the global urban waste collection market, the income through the AD of this waste can be around 410 billion incomes. However, currently only 25% is recycled **(Wainaina *et al.*, 2020)**.

3.3.7 Economical aspects of biogas industry

3.3.7.1 Life cycle assessment (LCA)

The AD of organic matter is an activity that has many implications in environmental terms, as stated above. However, as any other economic activity, it is important to analyse thoroughly the possible impacts that it may provoke on the environment, either positive or negative.

There are different ways to quantitatively analyse the environmental impact of the AD of organic matter. One of the most used is the so-called Life Cycle Assessment (LCA). LCAs systematically measure the effect of an economic activity based on different environmental parameters. The main focus is the measurement of the resources and energy consumed and the waste emitted into the environment **(Hijazi *et al.*, 2016)**. The analysis contemplates the entire life cycle of a product, from the acquisition of the raw material to the recycling or disposal of the product or the waste of the economic activity **(Bacenetti *et al.*, 2013)**.

Other techniques are also used for the estimation of environmental impact: environmental performance evaluation, environmental impact assessment, risk assessment, etc. **(Wang and Liu, 2021)**. However, two characteristics identify LCA and differentiate it from the previous: LCAs are relative and comparative.

The LCAs are relative to a functional unit. The functional unit is used as a quantitative measure to normalize the data describing the functioning of the system in terms of energy and matter that is used and that is produced **(Ingrao *et al.*, 2019)**. For example, a LCA of a car factory may define one car as the functional unit. In this case, inputs and outputs consumed and produced by the system during the fabrication of one car will be measured.

The LCAs are comparative studies where the environmental burden of two or more scenarios are calculated. Each scenario is assigned a value for each environmental impact category. The overall impact can be used to choose the best option under study **(Timonen *et al.*, 2019)**. In the example of the car factory, considering the same functional unit as above, an LCA could compare a scenario where the entire factory pipeline is managed by humans against another scenario with robot-assisted phases.

Although there is not a unique way to perform an LCA, the general protocol is defined by international standards (ISO 14040). An LCA must adhere to these standards to be compared to other LCAs. According to them, each study comprises four phases: goal and scope definition, Life Cycle Inventory, Life Cycle Inventory Analysis, and final phase of interpretation.

First phase: Goal and scope: The first phase of an LCA is where the goal and the scope are defined. Together with the scope, there may be expressed the reasons for the making of the assessment and the target audience. The scope of the assessment involves the definition of both the functional unit and the boundaries of the productive system.

The definition of the system boundaries means the explicit expression of all the stages that will be considered as part of the activity that will be assessed. Each stage is connected to each other by fluxes of energy, intermediate products, or waste to be treated. For example, the product of one stage may be the substrate for the following unit. Dividing the system in these units facilitates the identification of resource/energy entry points to the system, and waste or products exit points.

The system boundary specification and the stage of a process leads to the definition of the unit processes of the system intended for the study, which act as key-element in assessing the sustainability of a product/process. The system boundaries can be considered from any segment of the product chain (Gate-to-Gate, Cradle-to-Gate, Cradle-to-Grave, Cradle-to-Cradle) to include various stages like biomass cultivation, transport, processing of biomass and products manufacture in biorefinery, transport of products to the consumers, customer use of the products, and product disposal to the environment **(Katakojwala and Mohan, 2021)**.

Second phase: Life Cycle Inventory: The second phase is called the Life Cycle Inventory (LCI). All the data corresponding to input or output spots of matter or energy is compiled for each unit of production **(Hijazi *et al.*, 2016)**. The data may be classified according to headings like **(1)** resource inputs, **(2)** waste outputs, **(3)** other outputs (**table 24**).

Third phase: Life Cycle Impact Assessment (LCIA): The third phase is the Life Cycle Impact Assessment (LCIA). The data of the LCI is analyzed in terms of impact categories to assess which is the effect of each scenario under study over the environment.

The quantification of the impact for any input or output is based on scientific evidence. For example, in the case of Green House Gases (GHGs) CO_2, CH_4 and N_2O, the estimation of the effect of each gas under the Global Warming Potential category is performed normalizing the effect of each amount of gas to the equivalent amount of CO_2, the standard for this category. For example, an amount of N_2O equals 265 amounts of CO_2, and an amount of CH_4 equals 28 amounts of CO_2 **(Hijazi *et al.*, 2016)**.

There are different categories where data from the LCI can be allocated for the impact assessment. Selection of appropriate impact categories that specifically fit the process is

Table 24: Classification of LCI data extracted from *https://ecochain.com/knowledge/impact-categories-lca/.*

	Parameter	Unit	Description
Resource inputs	Primary renewable energy (materials)	MJ	Use of renewable primary energy resources as raw materials
	Primary renewable energy (energy)	MJ	Use of renewable primary energy, excluding renewable primary energy resources used as raw materials
	Primary renewable energy (total)	MJ	Sum of the two values above
	Primary non-renewable energy (materials)	MJ	Use of non-renewable primary energy resources as raw materials
	Primary non-renewable energy (energy)	MJ	Use of non-renewable primary energy, excluding renewa-ble primary energy resources used as raw materials
	Primary non-renewable energy (total)	MJ	Sum of the two values above
	Use of secondary material	kg	Material recovered from previous use or from waste which substitutes primary materials
	Use of fresh water	m^3	Freshwater use in absolute values
	Use of renewable secondary fuels	MJ	Renewable fuel recovered from previous use or from waste which substitutes primary fuels
	Use of non-renewable secondary fuels	J	Non-renewable fuel recovered from previous use or from waste which substitutes primary fuels
Waste outputs	Hazardous waste disposed	kg	Hazardous waste has a certain degree of toxicity that necessitates special treatment
	Non-hazardous waste disposed	kg	Non-hazardous waste is non-toxic and similar to household waste. It consists of inert waste and ordinary household waste
	Radioactive waste disposed	kg	Radioactive waste mainly originates from nuclear energy reactors
Other outputs	Components for re-use	kg	Material or components leaving the modelled system boundary which is destined for reuse
	Materials for recycling	kg	Material leaving the modelled system boundary which is destined for recycling
	Materials for energy recovery	kg	Material leaving the modelled system boundary which is destined for use in power stations using secondary fuels.
	Energy production	kg	Energy exported from waste incineration and landfill

Table 25: List of impact categories (*https://ecochain.com/knowledge/impact-categories-lca/).*

Impact category	Unit	Description
Climate change – total, fossil, biogenic and land use	kg CO_2-eq	Indicator of potential global warming due to emis-sions of greenhouse gases to air. Divided into 3 subcategories based on the emission source: (1) fossil resources, (2) bio-based resources and (3) land use change.
Ozone depletion	kg CFC-11-eq	Indicator of emissions to air that cause the de-struction of the stratospheric ozone layer.
Acidification	kg mol H^+	Indicator of the potential acidification of soils and water due to the release of gases such as nitrogen oxides and Sulphur oxides.
Eutrophication – freshwater	kg PO_4-eq	Indicator of the enrichment of the fresh water eco-system with nutritional elements, due to the emis-sion of nitrogen or phosphor containing compounds.
Eutrophication – marine	Kg N-eq	Indicator of the enrichment of the marine ecosystem with nutritional elements, due to the emission of nitrogen containing compounds.
Eutrophication – terrestrial	mol N-eq	Indicator of the enrichment of the terrestrial eco-system with nutritional elements, due to the emis-sion of nitrogen containing compounds.
Photochemical ozone formation	kg NMVOC-eq	Indicator of emissions of gases that affect the crea-tion of photochemical ozone in the lower atmos-phere (smog) catalysed by sunlight.
Depletion of abiotic resources – minerals and metals	kg Sb-eq	Indicator of the depletion of natural non-fossil resources.
Depletion of abiotic resources – fossil fuels	MJ, net calorific value	Indicator of the depletion of natural fossil fuel resources.
Human toxicity – cancer, non-cancer	CTUh	Impact on humans of toxic substances emitted to the environment. Divided into non-cancer and cancer related toxic substances.
Eco-toxicity (freshwater)	CTUe	Impact on freshwater organisms of toxic substances emitted to the environment.
Water use	m^3 world eq. deprived	Indicator of the relative amount of water used, based on regionalized water scarcity factors.
Land use	Dimensionless	Measure of the changes in soil quality (biotic pro-duction, erosion resistance, mechanical filtration).
Ionising radiation, human health	kBq U-235	Damage to human health and ecosystems linked to the emissions of radionuclides.
Particulate matter emissions	Disease incidence	Indicator of the potential incidence of disease due to particulate matter emissions.

significant (**Katakojwala and Mohan, 2021**; **table 25**). The preferred way to evaluate the energy inputs is to integrate various kinds of impact categories to a solitary weighted environmental impact metrics by employing characterization, weighing and normalization factors which assists to convert emissions and resource consumption into impact categories **(Katakojwala and Mohan, 2021)**.

Last phase: Interpretation: Finally, the last phase of an LCA is the interpretation phase. The results of the LCI and LCIA are discussed together to arrive to conclusions, recommendations and/or decision making according to the goal and scope defined in the first phase.

3.3.7.2 LCAs assessing AD and biogas production (LCA-AD)

First phase: Goal and scope: The usual goal of an LCA-AD is to determine the environmental impact of the production and use of biogas as an energy source **(Hijazi *et al.*, 2016; Saracevic *et al.*, 2019)**.

The functional unit of an LCA-AD depends on the choice of the authors of each study. Commonly, the functional unit which is used to estimate the impact of an AD activity is an amount of energy obtained from biogas. Alternatively, another functional unit that can be defined is an amount of feedstock processed by AD. In this way, possible functional units may be, for example, 1 kWh of electricity produced on a Combined Heat and Power unit (CHP) with biogas as fuel; or 1 ton of processed fresh matter **(Bacenetti *et al.*, 2013)**. Other functional units may be TJ, MJ, m^3 biogas, driving distance, etc **(Hijazi *et al.*, 2016)**.

Like the functional unit, the subsystems of an AD system differ among different LCA-ADs. **Hijazi *et al.*, (2016)** propose that the production of biogas using AD can be divided in four subsystems: feedstock supply, biogas production, digestate utilization and biogas utilization.

For the feedstock supply, the input of the subsystem is the feedstock prior to any treatment or transportation to the biogas plant, and the output is the substrate ready to feed the system of AD. The impact of this phase depends strongly in the type of feedstock. If it is an energy crop, all the steps involved in the cultivation of the crop must be assessed: land preparation, sowing, cultivation, harvest, and transport to the biogas plant. This considerably increase the impact of the use of biogas. Contrary, if the feedstock is composed of residues of the harvesting of a crop dedicated for food, the impact on the environment will decrease, as the feedstock can be considered as waste of another system. The same happens with animal slurry and industrial waste **(Hijazi *et al.*, 2016)**.

The biogas production subsystem is the AD process itself. The output of the previous phase is the input of this respective substrate. Microbial communities present in the feed sludge digest the substrate during the four phases that comprise the AD: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The outputs of this phase are biogas and digestate.

Digestate-use subsystem only considers the impact produced by the storage of digestate, because the use of the digestate is outside of the system boundaries. It may be considered for example for the cropping that will use the digestate as fertilizer.

Regarding biogas-use subsystem, the impact measurement applies to produce a certain amount of energy and/or heat from biogas combustion. The way by which energy stored in CH_4 molecules is recovered will be very important for the impact measurement: if biogas is just combusted for heating or cooking, if it is used to feed a CHP unit, or if it is upgraded to biomethane to feed the gas grids **(Morero *et al.*, 2015)**.

Second phase: LCI: For the inventory of the AD process, data can be obtained from several sources. It is very important that sources are reliable because the quality of an LCA strongly depends on the quality of the LCI **(Bacenetti *et al.*, 2013)**.

For example, **Bacenetti *et al.* (2013)** performed an LCA comparing three biogas plants using different feedstock's: pig slurry, maize silage, or a combination of both. The authors performed surveys in farms and interviews to farmers to assess data on production and transport of maize; also surveys in biogas plants to assess data on pig slurry transport; they monitored biogas plants during a year for the data relative to biogas plant operation, such as biomass, electricity and heat consumed, amount of produced biogas, etc. They also supplemented this information with secondary data obtained from the Ecoinvent database **(https://ecoinvent.org/)**, the biggest LCA database available. Finally, they used the IPCC method to assess the emissions derived from the use of fertilizers **(https://www.ipcc.ch/2019/05/13/ipcc-2019-refinement/)**.

Third phase: LCIA: Environmental sustainability will be assessed based on the impact of a stage or the whole process, on several categories relevant to the AD of biomass, such as resource depletion, acidification, ozone depletion, global warming, human toxicity, eutrophication, and eco-toxicity.

Defining the functional unit and allocation are the major determinant components to assess the environmental sustainability of a biorefinery. This is due to its multi-functional nature, which causes ambiguity in the allocation of burdens to the other products of the system when only comparing the global warming impacts while ignoring other impacts **(Katakojwala and Mohan, 2021)**.

In general, electricity from biogas has lower environmental impacts compared to electricity generation from fossil fuels. While savings in terms of GWP and RC can still be achieved, such biogas systems have higher impacts of AC, EP, and LU in comparison to fossil-based energy systems **(Hijazi *et al.*, 2016)**.

Last phase: Interpretation: The sustainability of any biorefining majorly depends on whether the feedstock is dedicated biomass or alternatively is a residue biomass/waste **(Katakojwala and Mohan, 2021)**.

Regardless of the selection of the feedstock, the following steps are necessary to minimize the negative environmental effects of biogas systems (impact categories that are mostly tackled by each step are stated in brackets) **(Hijazi *et al.*, 2016)**:

- Installing a flare to avoid discharge of biogas to the atmosphere during outages of the combined heat-and-power unit (GWP);
- Covering the storage tank for digested residues and collecting the residual biogas production (GWP, EP);
- Minimizing the parasitic electricity demand of the biogas plant and supplying it from low-emission sources (GWP, RC);
- Utilizing as much heat output from the CHPU as possible to substitute fossil energy carriers (GWP, RC);
- Employing high-efficiency CHPUs, possibly with additional exhaust gas treatment (RC, GWP, AC, EP);
- Checking the biogas plant for leakages on a regular basis (GWP).

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04

Policy of the European Union

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Abstract:

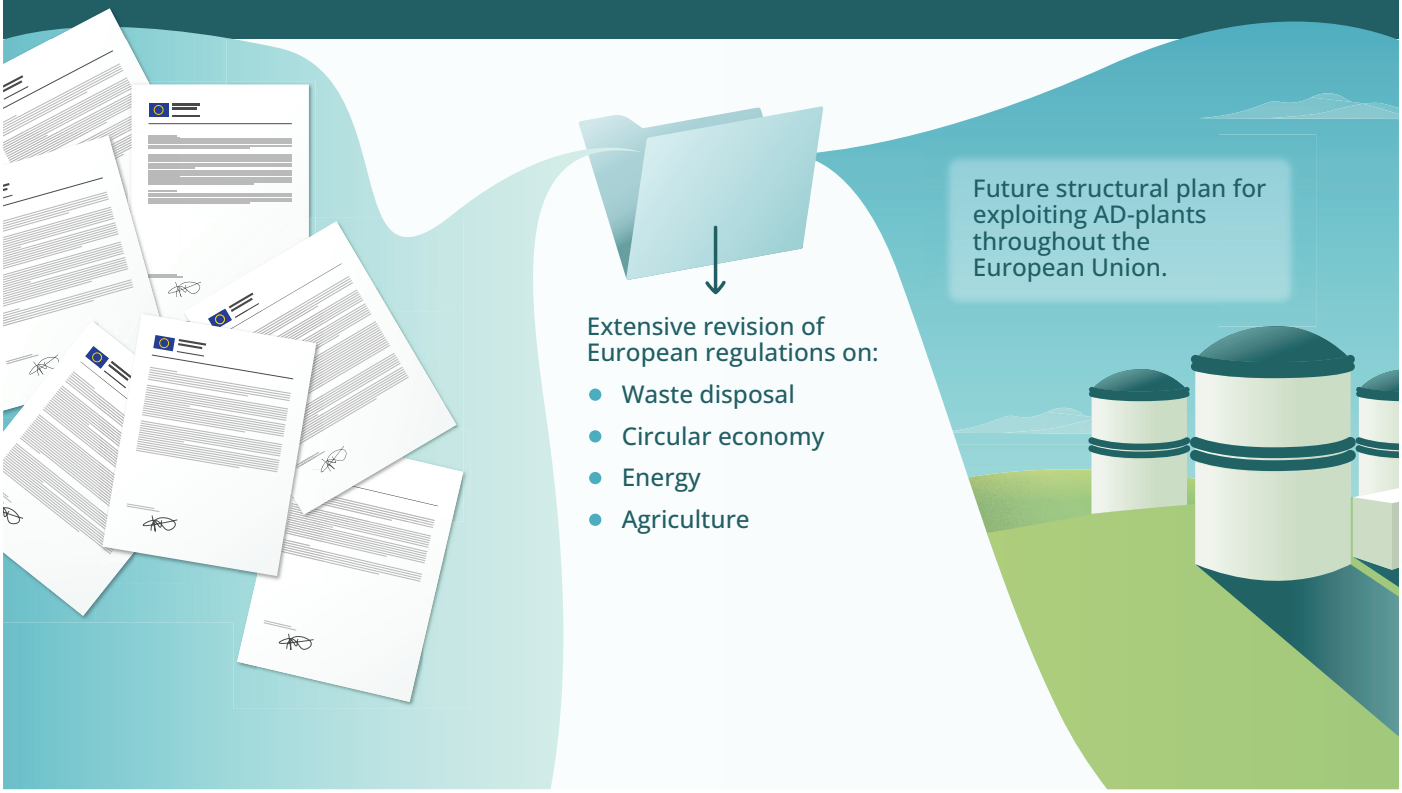
This chapter addresses the main political instruments of the European Union that are intended to promote the growth of renewable energies. In particular, the promotion of biogas and biomethane will be discussed in depth. This section aims to summarize the EUs decisions, directives, regulations, and other actions in order to provide an ideal overview of the political starting position for biogas and bio-methane in the EU.

First, the basics of European policy, especially with regard to climate and revolution, are outlined. The European Green Deal has been at the center of European climate action since 2019. All further additions and procedures to the European Green Deal are presented chronologically below. In addition, sustainability provisions such as the Renewable Energy Directive (REDI & REDII), the Paris Agreement, REPowerEU, the promotion of the bioeconomy, the use of bio-waste for the production of biogas and biomethane are put into context. The individual measures of the European Union or other international associations are arranged chronologically and discussed. However, it must be clarified what decisions, directives, recommendations, opinions and regulations are and what significance they have for the individual countries. This is done in the following section.

Furthermore, all important decisions, directives, regulations and measures that concern the European Union or have been adopted by it with regard to biogas and their significance for the biogas sector are presented. In doing so, the relevant document is examined directly for the keywords related to biogas, anaerobic digestion, organic waste and bioeconomy. The occurrence of the keywords is presented as a quotation and the content of what is written is discussed in detail.

The last section deals directly with the implementation in the individual countries. The implementation of EU law at national level results in some differences between the selected countries. These are highlighted and discussed in this chapter. The selected countries are countries that strongly promote biogas or bio-methane production and thus achieve the expansion of these plants.

The analysis and summary of EU decisions, directives, regulations and other measures regarding biogas production highlights a comprehensive plan to introduce, promote, and exploit anaerobic digestion plants within the European Union.



4.1 European Union policy around biogas and bio-methane

4.1.1 Regulations of the European Union

In European law, regulations are legal acts of the European Union and as such form part of secondary Union law. According to Art. 288 (2) of the TFEU, they are of general application and have a direct effect in the Member States. Regulations are addressed either to the European Union itself, to all Member States or to the citizens of all Member States. If the regulation is to affect only selected Member States or their citizens, it is adopted as a decision, i.e. it is directly binding, or as a directive, which must be transposed into national law. Regulations are adopted according to one of the procedures provided for in the Treaties, depending on the subject matter of the regulation. A distinction is made between legislative acts, Commission implementing regulations and delegated regulations.

Regulations, which are legislative acts, are usually adopted jointly by the Council of the European Union and the European Parliament in accordance with the ordinary legislative procedure, on a proposal from the European Commission. They are published in the Official Journal of the European Union and are available online in the legal information system EUR-Lex. Regulations are numbered with the word "Regulation", the year, a serial number and the symbol "EU".

In summary, regulations set the goals and the concrete means to achieve the results. They do not have to be transposed into national law, which is referred to as the "pass-through effect", and modifications are protected by the "transposition prohibition".

4.1.2 Directives of the European Union

In European law, directives are legal acts of the European Union and as such part of secondary Union law. According to Art. 288(3) of the TFEU, they are binding and not directly applicable, which means that they must first be converted into national law by the Member States. The form in which they are then implemented by the individual member states depends on them, which leaves them a certain amount of leeway. However, there are authorizations and/or obligations that must be complied with and which provide the framework for the directives.

Directives, which are legislative acts, are usually adopted jointly by the Council of the European Union and the European Parliament in accordance with the ordinary legislative procedure, on a proposal from the European Commission. They are published in the Official Journal of the European Union and are available online in the legal information system EUR-Lex. Directives are numbered with the word "Directive", the year, a serial number and the symbol "EU".

In summary, directives set objectives that must be achieved without specifying the means to achieve that result. Member states are required to incorporate the directive's provisions into their national laws within a specified timeframe. This allows for some flexibility in implementation while ensuring a common standard across the EU.

4.1.3 Decisions of the European Union

In European law, decisions are legal acts of the European Union and as such form part of secondary Union law. Alongside regulations and directives, decisions are the third form of EU legislative instrument that have legally binding effects on each individual. Depending on the subject, decisions are adopted through different, treaty-bound procedures. According to Art. 288 TFEU, decisions are binding

in their entirety and may be addressed to specific addressees such as Member States, companies, individuals, or the general public. Decisions are used when a resolution is to be binding but there is no case for the adoption of regulations or directives. This is the case in the following examples Case-by-case decisions, Nominations, Common Foreign and Security Policy, International Agreements, Simplified Amendments to the Treaties.

In this way, decisions are used to react to individual problems and abuses, such as mergers of companies, actions that endanger security, non-compliance with EU directives and regulations, the introduction of hazardous substances and the use of genetic engineering. Decisions are binding on those to whom they are addressed, whether an individual, a company, or a member state. They can be used to address specific cases or issues and are often used to ensure compliance with competition rules or to settle disputes.

4.1.4 Further Political Agreements

The Treaty on European Union states that *"... to ensure the proper functioning and development of the common market, the Commission (...) formulate recommendations or deliver opinions on matters dealt with in this Treaty, if it expressively so provides or if the Commission considers it necessary"* **(European Union 1993)**.

Recommendations: These are part of the secondary legislation of the European Union and thus legal acts of the Union. Recommendations are usually issued by the European Commission. They are defined in Art. 288 TFEU as non-legally binding acts, although some legal consequences are attached to their adoption. Member States are free to decide whether to implement the recommendations. If they implement a recommendation, it is part of the legal order of the Member State, which means that the resulting rights can only be enforced before the courts of the Member State, not before the European Court of Justice. This makes the recommendation to an instrument of indirect action. Similar to the directive, it aims at the development of legislation in the member states, whereby recommendations are not binding.

Opinions: These are part of the secondary legislation of the European Union and thus legal acts of the Union. Opinions are issued by the institutions and other bodies of the Union. They are defined in Art. 288 TFEU as non-legally binding acts, although legal consequences are attached to their delivery. Two important areas in which the submission of opinions is provided for are mentioned below. Procedures for the adoption of legislative acts and infringement procedures.

Framework Regulations: These are regulations that set out broad objectives and establish the framework for further detailed measures to be adopted at a later stage. Member states then implement these measures based on the framework provided.

White Papers and Green Papers: These are policy documents released by the European Commission. White Papers outline proposals for future legislation, while Green Papers are consultation documents that initiate discussions on particular topics. These papers help gather input from member states, stakeholders, and the public before formal regulations are developed.

Impact Assessments: Before introducing new regulations, the EU often conducts impact assessments to evaluate the potential economic, social, and environmental consequences of proposed policies. This helps ensure that regulations are effective and balanced.

Public Consultations: The EU frequently seeks input from citizens, businesses, and organizations through public consultations. These consultations help shape regulations by incorporating diverse perspectives and expertise.

Cooperation and Negotiation: The EU institutions, including the European Commission, the European Parliament, and the Council of the European Union, work togeth-

er to negotiate, draft, and adopt regulations. This involves discussions, amendments, and compromises to create regulations that reflect the interests of member states and the EU as a whole.

Enforcement Mechanisms: The EU also establishes mechanisms to ensure that member states comply with the regulations. This includes the European Court of Justice, which can adjudicate disputes and rule on cases of non-compliance.

Action plans: An action plan addresses a wide range of issues, from environmental sustainability and energy transition to digital transformation and economic development. Each plan includes a set of initiatives, targets, and measures designed to drive progress and achieve desired outcomes within the respective areas.

4.1.5 Directive 2008/98/EC: The Waste Framework Directive (WFD) // November 2008

The Waste Framework Directive (WFD) provides the member states with guidelines for political measures for the transition to a circular economy and in particular for their waste legislation.

1. **Waste Hierarchy:** The WFD introduces a waste hierarchy that prioritizes waste prevention, followed by preparing for reuse, recycling, other forms of recovery such as energy recovery, and safe disposal as a last resort.
2. **Extended Producer Responsibility (EPR):** The directive emphasizes the principle of EPR, where producers are held responsible for the environmental impacts of their products throughout their lifecycle, including post-consumer waste management.
3. **Waste Minimization and Prevention:** The WFD encourages the prevention of waste generation and promotes measures to minimize the environmental impact of waste, including the reduction of hazardous substances in products.
4. **Recycling Targets:** The directive sets a target for recycling and recovery rates for different types of waste materials, aiming to increase the recycling and reclamation of valuable resources from waste streams.
5. **End-of-Waste Criteria:** The WFD establishes criteria that define when certain waste materials cease to be considered waste and become secondary raw materials, facilitating their use in various applications.
6. **Landfill Restrictions:** The directive restricts the disposal of certain types of waste in landfills to prevent environmental contamination and promote more sustainable waste management practices.
7. **Reporting and Information:** Member states are required to provide regular reports on their waste management activities and progress toward meeting the directive's goals. Transparency and access to information are important elements of the WFD.
8. **Illegal Waste Shipment:** The directive addresses the issue of illegal waste shipments between EU member states and non-EU countries, aiming to prevent environmental harm and ensure proper waste treatment.
9. **Cooperation and Coordination:** The WFD encourages cooperation among member states and coordination of waste management practices to achieve a more consistent and efficient waste prevention and management approach.

The aim is to protect the environment and human health by managing waste in the EU. It prioritizes waste prevention, promotes recycling and recovery, establishes responsibilities for various stakeholders, and seeks to minimize the environmental impact of waste generation and disposal, thus ensuring the long-term competitiveness of the EU. This directive is still active **(The European Parliament and**

Council of the European Union 2008).

4.1.6 Directive 2009/28/EC: Renewable Energy Directive I (REDI) // June 2009

Directive 2009/28/EC, also known as the Renewable Energy Directive I (REDI), was adopted by the European Union in June 2009. The aim of the directive is to promote the use of renewable energy sources and to increase the share of renewable energy in the EU's energy mix. The main points of the directive include:

1. **Renewable energy targets:** REDI sets binding targets for each EU Member State to increase the share of renewable energy in its total energy consumption. The overall EU target is to achieve a 20% share of renewable energy by 2020.
2. **National action plans:** Member States are required to develop national renewable energy action plans setting out how they intend to achieve their individual targets. These plans include measures to promote different renewable energy sources such as wind, solar, biomass and hydropower.
3. **Sustainability criteria:** The policy sets sustainability criteria for biofuels and bio liquids to ensure that their production and use have a positive impact on reducing greenhouse gas emissions and protecting biodiversity.
4. **Guarantees of origin:** REDI introduces a system of guarantees of origin to track and verify the origin of renewable energy sources. This helps consumers make informed choices about the sources of their energy consumption.
5. **Cross-border cooperation:** The Directive promotes cross-border cooperation between Member States to develop joint projects and initiatives in the field of renewable energy production and transmission.
6. **Promotion of electricity and heating/cooling from renewable energy:** REDI promotes the use of renewable energy in electricity generation and heating and cooling systems by setting targets and providing support mechanisms for these sectors.
7. **Integration of renewable energy into the electricity grid:** The directive emphasises the need to integrate renewable energy sources into the existing energy infrastructure and grids to ensure stability and reliability.
8. **Reporting and monitoring:** Member States are required to report regularly on their progress towards their renewable energy targets to promote transparency and accountability.

In summary, Directive 2009/28/EC, the Renewable Energy Directive I (REDI), establishes a framework for promoting the use of renewable energy sources in the European Union. It sets binding targets for Member States, outlines sustainability criteria for biofuels and promotes cooperation to achieve a more sustainable and diversified energy mix. This directive was superseded by RED II (The European Parliament and of the Council 2009).

4.1.7 Sustainable Development Goals (SDGs) // January 2016

The Sustainable Development Goals (SDGs, **figure 59**) are a set of 17 global goals set by the United Nations in September 2015 as part of the 2030 Agenda for Sustainable Development and agreed by 197 countries. These include **(1)** No Poverty, **(2)** Zero Hunger, **(3)** Good Health and Well-being, **(4)** Quality Education, **(5)** Gender Equality, **(6)** Clean Water and Sanitation, **(7)** Affordable and Clean Energy, **(8)** Decent Work and Economic Growth, **(9)** Industry, Innovation and Infrastructure, **(10)** Reduced Inequality, **(11)** Sustainable Cities and Communities, **(12)** Responsible Consumption and Production, **(13)** Climate Action, **(14)** Life Below Water, **(15)** Life On Land, **(16)** Peace, Justice, and Strong Institutions, **(17)** Partnerships for the Goals.

Figure 59: Sustainable Development Goals (United Nations 2015).

These goals are intended to pave the way for a future characterised by peace, sustainability, a healthy environment, a stable climate, and environmentally aware people with socio-ecological prosperity. The most important aspects of the SDGs include:

1. **Broad scope:** the SDGs cover a wide range of interconnected issues, including poverty, hunger, health, education, gender equality, clean water, affordable and clean energy, decent work, reducing inequalities, climate action and more.
2. **Universal application:** The goals apply to all countries and take into account the fact that sustainable development is a global responsibility that requires joint efforts by developed and developing countries.
3. **Holistic approach:** The SDGs take a holistic perspective and recognise the need for integrated solutions that balance economic growth, social progress and environmental protection.
4. **Leave no one behind:** The SDGs emphasise inclusivity and prioritise the needs of marginalised and vulnerable populations to ensure that no one is left behind on the path to sustainable development.
5. **Data and monitoring:** The goals are supported by a framework for monitoring progress and collecting data to track progress, identify gaps and adjust strategies as needed.
6. **Partnerships:** Achieving the SDGs requires collaboration between governments, businesses, civil society and individuals. Partnerships are critical for mobilising resources and expertise for effective implementation.
7. **Timeframe:** The SDGs are to be achieved by 2030, which provides a 15-year timeframe for action and progress towards the outlined goals.

The Sustainable Development Goals (SDGs) are a set of 17 global goals adopted by the United Nations in September 2015. They address a wide range of interconnected challenges and provide a comprehensive framework for countries and stakeholders to work together towards a more equitable, prosperous and sustainable world by 2030, so they are still current **(United Nations 2015)**.

4.1.8 Paris Agreement / December 2015

The Paris Agreement is an international treaty adopted in December 2015 under the United Nations Framework Convention on Climate Change (UNFCCC). It aims to address the global challenge of climate change by bringing countries together in a common effort to limit global warming and its impacts. Key points of the Paris Agreement include:

1. **Temperature targets:** The agreement aims to keep the increase in average global temperature well below 2 degrees Celsius above pre-industrial levels and to continue efforts to limit it to 1.5 degrees Celsius. This ambitious target is intended to reduce the severity of climate impacts.
2. **Nationally Determined Contributions (NDCs):** Each participating country submits its own Nationally Determined Contributions (NDCs) outlining its specific climate change mitigation plans, targets and strategies to reduce greenhouse gas emissions. These contributions are regularly updated to reflect increasing ambition.
3. **Transparency and reporting:** The Paris Agreement establishes a robust transparency framework that requires countries to report regularly on their emissions and progress towards their climate goals. This transparency promotes accountability and encourages countries to stay on track.
4. **Adaptation:** The agreement emphasises the importance of improving countries' capacity to adapt to the impacts of climate change. It promotes support for vulnerable countries and the development of adaptation strategies.
5. **Support and finance for climate action:** Developed countries commit to providing financial support and technology transfer to developing countries to help them transition to low-carbon and climate-resilient pathways.
6. **Loss and damage:** The agreement recognises that some climate impacts may not be adaptive and provides a mechanism to address loss and damage related to climate change impacts, including extreme weather events.
7. **Global stocktake:** A global stocktake will be conducted every five years to assess collective progress towards the Agreement's goals and to encourage countries to improve their climate action.
8. **Long-term goal:** The Paris Agreement envisages achieving a global balance between anthropogenic emissions and removals of greenhouse gases in the second half of the century, effectively achieving climate neutrality.

In summary, the Paris Agreement is a landmark international treaty adopted in December 2015 to combat climate change. It outlines a framework for global cooperation, focusing on limiting temperature rise, improving transparency, promoting adaptation and supporting climate action. The agreement represents a collective commitment to tackle one of the greatest challenges of our time **(United Nations Framework Convention on Climate Change 2015)**.

4.1.9 Closing the loop - An EU action plan for the Circular Economy // December 2015

In December 2015, the European Commission published the Circular Economy Package, initiating one of the most significant changes in environmental and economic policy in recent years. The aim is to create a more circular economy, which is about preserving the value of products, materials, and resources within the economy for as long as possible and generating as little waste as possible. Intelligent product design, more recycling, and reuse should help to increasingly close the loop of product life cycles and achieve more effective value creation and use of all raw materials, products, and

waste. It outlines a comprehensive strategy to promote a circular economy within the European Union, focusing on resource efficiency, waste reduction, and sustainable consumption and production. Key aspects of the action plan include:

1. **Circular Economy Principles:** The plan promotes the adoption of circular economy principles, aiming to minimize waste generation, maximize resource use, and create a closed-loop system where materials are recycled and reused.
2. **Waste Reduction Targets:** The action plan sets specific targets to reduce landfilling and increase recycling rates for various types of waste, such as plastics, packaging, and electronics.
3. **Extended Producer Responsibility (EPR):** The plan emphasizes the role of producers in managing the entire lifecycle of their products, including responsible disposal and recycling.
4. **Product Design and Eco-design:** The plan encourages the design of products that are durable, repairable, and recyclable. Eco-design principles are promoted to reduce the environmental impact of products.
5. **Resource Efficiency:** The action plan aims to improve resource efficiency by promoting the efficient use of raw materials, water, and energy in various sectors.
6. **Eco-friendly Consumption:** The plan encourages consumers to make more sustainable choices by supporting products that have a lower environmental impact and longer lifespans.
7. **Innovation and Research:** The circular economy action plan promotes research and innovation in areas such as material science, recycling technologies, and business models that support circular practices.
8. **Circular Economy Financing:** The plan seeks to leverage public and private financing to support circular economy initiatives, such as recycling infrastructure and eco-innovation projects.
9. **International Cooperation:** The action plan acknowledges the global nature of resource challenges and aims to promote circular economy principles internationally through cooperation and dialogue.
10. **Monitoring and Reporting:** The plan includes mechanisms for monitoring progress, reporting, and regularly assessing the implementation of circular economy measures.

In summary, "Closing the Loop - An EU Action Plan for the Circular Economy" is an initiative launched by the European Commission in December 2015 to transition the EU towards a circular economy model. It focuses on waste reduction, sustainable production and consumption, resource efficiency, and collaboration between various stakeholders to create a more sustainable and resource-efficient future **(The European Commission 2015)**.

4.1.10 A sustainable bio-economy for Europe // November 2018

The Action Plan "A sustainable bio-economy for Europe" was published in November 2018 as a roadmap for the future of agriculture, economy, and industry. Bio-economy refers to the economic use of renewable biological resources from land and sea, such as plants, forests, fish, animals, and microorganisms for the production of food, materials and energy. This strategy aims to help the EU reduce carbon emissions and achieve a circular economy. The bio-economy will contribute to and strengthen the modernization of the EU's industrial sector by establishing new value chains and greener, more cost-efficient industrial processes while protecting biodiversity and the environment. The communication outlines the European Union's vision and strategy for developing a bio-economy that is environmentally sustainable, economically viable, and socially inclusive. Key points of the communication include:

1. **Definition of Bio-economy:** The document defines the bio-economy as an economy that uses renewable biological resources from land and sea to produce food, materials, and energy. It emphasizes the sustainable use of resources and the potential to replace fossilbased products with bio-based alternatives.
2. **Circular Approach:** The initiative promotes a circular economy approach by minimizing waste, promoting recycling, and enhancing the efficient use of resources within the bio-economy.
3. **Innovation and Research:** The communication emphasizes the importance of research, innovation, and technology development to drive the growth of the bio-economy. It encourages the development of new and sustainable bio-based products and processes.
4. **Sustainable Agriculture and Forestry:** The initiative highlights the significance of sustainable agricultural and forestry practices that contribute to the bio-economy while preserving biodiversity and ecosystem services.
5. **Bio-based Industries:** The communication supports the growth of bio-based industries, which use renewable resources to create products such as biofuels, bio-based materials, and chemicals.
6. **Bio-economy and Climate Change:** The strategy aligns with climate objectives by emphasizing the potential of the bio-economy to reduce greenhouse gas emissions through sustainable practices and the substitution of fossil-based resources.
7. **International Cooperation:** The initiative recognizes the global nature of the bio-economy and the importance of cooperation with international partners to address shared challenges.
8. **Cross-Sectoral Collaboration:** The communication underscores the need for collaboration among various sectors, including agriculture, industry, research, and environmental protection, to ensure the successful development of a sustainable bio-economy.
9. **Monitoring and Reporting:** The European Commission commits to monitoring progress, sharing best practices, and reporting on the implementation of the strategy.

In summary, "A Sustainable Bio-economy for Europe" is a strategic communication document published by the European Commission in November 2018. It outlines the EU's strategy for promoting a bio-economy that is sustainable, circular, and innovative, with an emphasis on responsible resource use, research, and cross-sectoral collaboration to achieve economic and environmental goals **(European Commission 2018)**.

4.1.11 Directive (EU) 2018/2001: Renewable Energy Directive II (REDII) // December 2018

RED II entered into force on 24 December 2018 and was transposed into national legislation on 30 June 2021, which meant that RED I expired. In contrast to RED I, the EU Commission has set a binding target of 32% renewable energy for the total electricity production of all EU member states by 2030. They are committed to achieving the 32% through the National Energy and Climate Plans (NECP). The achievement of this overall target is regulated for each member state in Art. 3 Para 1-9 of the Governance Regulation. Directive (EU) 2018/2001, also known as the Renewable Energy Directive II (REDII), was adopted by the European Union in December 2018. The directive is part of the EU's efforts to transition to a more sustainable and low-carbon energy system by promoting the use of renewable energy sources. Key aspects of the directive include:

1. **Binding Renewable Energy Targets:** REDII establishes a binding EU-wide target of achieving a 32% share of renewable energy in the final energy consumption by 2030.

2. **National Renewable Energy Targets:** Each member state is required to set its own specific renewable energy targets to contribute to the overall EU target.
3. **Guarantees of Origin:** The directive strengthens the system of guarantees of origin, which certifies the source of renewable energy, ensuring transparency and traceability in the energy market.
4. **Cross-Border Cooperation:** REDII encourages member states to cooperate on renewable energy projects, including joint support mechanisms, to optimize the use of renewable resources.
5. **Bioenergy Sustainability Criteria:** The directive introduces stricter sustainability criteria for the production and use of bioenergy, ensuring that it contributes to greenhouse gas emission reductions and avoids negative environmental impacts.
6. **Support Schemes and Auctions:** Member states are required to design support schemes and implement competitive auctions to ensure cost-efficient deployment of renewable energy projects.
7. **Renewables in Heating and Cooling:** REDII promotes the use of renewable energy sources in heating and cooling systems, aiming to increase their share in the sector.
8. **Electromobility and Renewable Fuels:** The directive encourages the use of renewable energy in transport through measures such as the deployment of electric vehicle charging infrastructure and the promotion of advanced biofuels.
9. **Reporting and Monitoring:** Member states must provide regular progress reports on their renewable energy targets and actions, promoting transparency and accountability.
10. **Simplification and Streamlining:** The directive aims to simplify administrative procedures and reduce regulatory barriers to facilitate the deployment of renewable energy projects.

In summary, Directive (EU) 2018/2001, the Renewable Energy Directive II (REDII), is a key instrument of the European Union's energy policy to increase the use of renewable energy sources. It sets binding targets, strengthens sustainability criteria, promotes cross-border cooperation, and encourages the integration of renewable energy in various sectors to contribute to the EU's climate and energy goals **(The European Parliament and Council of the European Union 2018b)**.

4.1.12 European Green Deal // December 2019

The European Commission presents the European Green Deal to achieve climate neutrality by 2050. The European Green Deal comprises a series of measures in the areas of financial market regulation, energy supply, transport, trade, industry and agriculture, and forestry. Initially, the European Union's CO_2 emissions were to be reduced by 40%t by 2030 compared to 1990 levels. Subsequently, the reduction was first tightened to 50% and finally to 55%. Particularly affected countries will be supported with a total of 100 billion euros in the conversion to an emission-free economy **(The European Commission 2019)**. The European Green Deal is to become a central component of the European Union's climate policy and addresses the anthropological climate crisis in its core issues. This was adopted on 11 December 2019 by the European Commission under the leadership of Ursula von der Leyen to reduce net greenhouse gas emissions in the European Union to zero by 2050 at the latest. This is expected to make Europe the first continent to achieve climate neutrality. The European Green Deal is a growth strategy to make the EU a prosperous and fair society with a competitive, modern, and resource-efficient economy, decoupling economic growth from unsustainable resource use **(The European Commission 2019)**. It identifies action strategies for the sectors with the highest greenhouse gas (GHG) emissions, including the transport sector, construction sector, agriculture, forestry, industry, and general energy production. In addition, promising systems such as the

circular economy, an emissions trading system at the EU and global level, and tax relief for the sustainable use of resources are discussed. A European Union procedural plan for the implementation of the Green Deal is shown in **figure 60**.

The European Green Deal is a comprehensive policy framework and strategy introduced by the European Commission in December 2019. It aims to transform the European Union into a more sustainable, climate-neutral, and environmentally friendly society and economy. Key points of the European Green Deal include:

1. **Climate Neutrality by 2050:** The central goal of the Green Deal is for the EU to achieve net-zero greenhouse gas emissions by 2050, meaning that the amount of GHG emissions released is balanced by the amount removed from the atmosphere.
2. **Ambitious Emission Reduction Targets:** The deal commits the EU to increase its emission reduction target for 2030 from the previous 40% to at least 55%, as compared to 1990 levels.
3. **Sustainable Energy Transition:** The Green Deal focuses on accelerating the transition to renewable energy sources, increasing energy efficiency, and supporting research and innovation in clean energy technologies.
4. **Circular Economy and Resource Efficiency:** The initiative aims to promote a circular economy by reducing waste, promoting recycling, and encouraging sustainable consumption and production.
5. **Biodiversity and Nature Protection:** The European Green Deal seeks to halt biodiversity loss, protect ecosystems, and restore degraded natural habitats to ensure the preservation of biodiversity.

Figure 60: The European Green Deal (Adapted from The European Commission 2019).

6. **Sustainable Agriculture and Food Systems:** The deal aims to promote sustainable farming practices, reduce the environmental impact of agriculture, and support the transition to more sustainable and healthy food systems.
7. **Clean Mobility:** The Green Deal emphasizes the development of clean and sustainable transportation systems, including the promotion of electric vehicles, public transportation, and the deployment of charging infrastructure.
8. **Renovation Wave:** The initiative aims to improve the energy efficiency of buildings across the EU, reducing their carbon footprint and energy consumption.
9. **Just Transition Mechanism:** The European Green Deal includes measures to ensure a fair transition for regions and industries heavily dependent on fossil fuels, helping them shift toward more sustainable alternatives.
10. **Financing and Investment:** The Green Deal seeks to mobilize public and private investments to support sustainable projects and initiatives across various sectors.
11. **International Leadership:** The European Green Deal envisions the EU as a global leader in climate action, promoting the green transition and cooperating with international partners to address climate challenges.

In summary, the European Green Deal is a comprehensive strategy introduced by the European Commission in December 2019 to achieve climate neutrality and promote sustainability across various sectors of the European Union's economy and society. It encompasses ambitious emission reduction targets, energy transition, circular economy principles, nature protection, clean mobility, and more, aiming to guide the EU toward a more sustainable and resilient future.

4.1.13 Circular Economy Action Plan – For a cleaner and more competitive Europe // March 2020

The Circular Economy Action Plan is a policy initiative launched by the European Commission in March 2020. It outlines a comprehensive strategy to promote a circular economy within the European Union, focusing on reducing resource consumption, minimizing waste, and fostering sustainable production and consumption. It is part of the achievement of the European Green Deal. Key aspects of the Circular Economy Action Plan include:

1. **Sustainable Products:** The plan aims to promote the design of products that are durable, repairable, and recyclable. It encourages extending the lifespan of products through better design and maintenance.
2. **Waste Reduction:** The initiative focuses on reducing waste generation and promoting resource efficiency by encouraging practices such as reusing, remanufacturing, and sharing economy models.
3. **Right to Repair:** The plan supports consumers' right to repair their products by ensuring access to spare parts, repair information, and service networks.
4. **Eco-design:** The action plan seeks to strengthen eco-design requirements for products, making them more energy-efficient, environmentally friendly, and circular by design.
5. **Digitalization:** The plan acknowledges the role of digital technologies in enabling a circular economy, such as through digital platforms for sharing and reusing products.
6. **Sustainable Packaging:** The initiative aims to reduce the environmental impact of packaging by promoting reusable and recyclable materials, and by combating single-use plastics.

7. **Textiles and Plastics:** The plan targets sectors with significant environmental impact, like textiles and plastics, promoting sustainable practices and reducing their negative effects.
8. **Biodegradable and Compostable Plastics:** The initiative emphasizes that biode-gradable and compostable plastics should be designed for specific waste management systems and not cause harm to the environment.
9. **Green Public Procurement:** The plan encourages public authorities to lead by example through green procurement practices that favour products with a lower environmental impact.
10. **Action on Food Waste:** The action plan addresses food waste by promoting measures to reduce it along the entire supply chain, including by redistributing surplus food.
11. **Reporting and Monitoring:** The plan establishes monitoring mechanisms to track the implementation of circular economy measures and assess their impact.

In summary, the Circular Economy Action Plan, launched by the European Commission in March 2020, is a comprehensive strategy to promote a circular economy within the European Union. It focuses on sustainable product design, waste reduction, right to repair, eco-design, digitalization, and other measures to reduce resource consumption and minimize environmental impact while enhancing economic competitiveness.

4.1.14 Regulation (EU) 2020/852: EU taxonomy for sustainable activities // June 2020

Regulation (EU) 2020/852, known as the EU Taxonomy Regulation, was adopted by the European Union in June 2020. This regulation establishes a framework to define and classify economic activities that can be considered environmentally sustainable. The EU Taxonomy aims to guide investors, businesses, and policymakers in identifying and promoting activities that contribute to the transition to a more sustainable economy. Key points of the EU Taxonomy Regulation include:

1. **Environmental Objectives:** The regulation sets out six environmental objectives that activities must substantially contribute in order to be considered environmentally sustainable. These objectives include climate change mitigation, climate change adaptation, sustainable use of water and marine resources, transition to a circular economy, pollution prevention and control, and protection and restoration of biodiversity and ecosystems.
2. **Technical Screening Criteria:** The regulation includes specific technical screening criteria that economic activities must meet in order to qualify as environmentally sustainable under each of the six objectives. These criteria are developed based on scientific and technical assessments.
3. **Disclosure Requirements:** The EU Taxonomy requires certain financial market participants, such as asset managers, to disclose the proportion of their portfolio invested in environmentally sustainable activities, using the taxonomy criteria.
4. **Green Bond Standard:** The regulation lays the foundation for a future EU Green Bond Standard that aligns with the EU Taxonomy, ensuring that bonds labeled as “green” are invested in environmentally sustainable projects.
5. **Extension to Other Objectives:** While the initial regulation focuses on climate change and other environmental objectives, it provides a framework for extending the taxonomy to other sustainability objectives in the future.
6. **Harmonization and Transparency:** The EU Taxonomy aims to harmonize definitions of environmentally sustainable activities across the EU and increase transparency in the financial sector by providing clear criteria for identifying green investments.

7. **Implementation Timetable:** The regulation outlines a phased approach for implementing the taxonomy criteria, with different economic sectors being included over time. It aims to provide flexibility for businesses to transition toward sustainability.

In summary, Regulation (EU) 2020/852, the EU Taxonomy Regulation, is a key instrument introduced by the European Union in June 2020 to establish a framework for defining and classifying environmentally sustainable economic activities. It sets environmental objectives, technical screening criteria, and disclosure requirements to guide investors and businesses in promoting sustainable practices and aligning financial activities with the transition to a more sustainable economy **(The European Parliament and Council of the European Union 2020)**.

4.1.15 2020/2077(INI) – New Circular Economy Action Plan // February 2021

The European Parliament resolution 2020/2077(INI) refers to the legislative initiative report on the New Circular Economy Action Plan, adopted in February 2021. This report outlines the European Parliament's position on the updated Circular Economy Action Plan introduced by the European Commission. The aim is path the way for Europe to grow into a sustainable economy, which will make it possible to achieve the targets of the European Green Deal. This is intended to reduce pressure on natural resources, create new jobs and take a more holistic view of the life cycle. In addition, the entire life cycle of products is to be considered in the future, with the circular economy being a central component of product design. In addition to these economic instruments, sustainable consumption by the population is also to be promoted. Overall, these measures are intended to ensure that the resources used in the EU economy are used for as long as possible, leading to an optimal and justified valorisation of resources. Key points of the resolution include:

1. **Welcome and Support:** The European Parliament welcomes the New Circular Economy Action Plan as an important step toward achieving sustainability goals and creating a more resilient and resource-efficient economy.
2. **Ambitious Goals:** The resolution emphasizes the need for ambitious targets and measures to promote the circular economy, reduce resource consumption, and minimize waste generation.
3. **Sustainable Product Design:** The European Parliament highlights the importance of extending the lifespan of products through eco-design, repairability, and durability, while minimizing negative environmental impacts.
4. **Right to Repair:** The resolution supports the right of consumers to repair their products, access spare parts, and receive repair information, thereby contributing to reducing electronic waste and fostering sustainable consumption.
5. **Eco-design:** The European Parliament calls for strengthening eco-design requirements to ensure that products are energy-efficient, environmentally friendly, and designed with circularity in mind.
6. **Plastics and Textiles:** The resolution emphasizes the need to address challenges related to plastics and textiles, including reducing micro plastic pollution, promoting sustainable alternatives, and encouraging the recycling of textiles.
7. **Green Public Procurement:** The European Parliament urges the implementation of green public procurement policies that prioritize sustainable and circular products and services.
8. **Regulatory Framework:** The resolution encourages the European Commission to establish a comprehensive regulatory framework that promotes circularity and addresses challenges related to hazardous substances, waste exports, and other relevant areas.
9. **International Cooperation:** The European Parliament stresses the im-

portance of promoting the circular economy globally and cooperating with international partners to address shared challenges.

10. **Reporting and Monitoring:** The resolution calls for improved reporting and monitoring mechanisms to track progress, assess the implementation of circular economy measures, and ensure transparency.

In summary, the European Parliament resolution 2020/2077(INI) regarding the New Circular Economy Action Plan reflects the Parliament's endorsement of the plan and provides specific recommendations and priorities for advancing the circular economy within the European Union. It emphasizes sustainable product design, the right to repair, eco-design, plastic and textile challenges, green public procurement, regulatory frameworks, international cooperation, and effective monitoring and reporting mechanisms.

4.1.16 Fit-for-55-Paket // July 2021

On 14 July 2021, the "Fit for 55 Package" was published. It contains more than ten cross-cutting legislative proposals that reshape existing climate, energy, and transport legislation. Commission presents a package of proposals to transform our economy to meet our 2030 climate targets. Negotiation and adoption of the legislative package by the European Parliament and the Member States to achieve our 2030 climate targets. The aim is to reduce net greenhouse gas emissions by 55% by 2030 compared to 1990 levels. The focus is on the following objectives: the breakdown of the 55% target, the reform of the European Emissions Trading Scheme, the introduction of a CO_2 limit compensation system, the adaptation of the EU Climate Protection Regulation, the adaptation of the Renewable Energy Directive and the Climate, Environment, and Energy Aid Guidelines. By the end of 2022, all EU member states must develop strategic plans that will guide the allocation of funds. Key aspects of the "Fit for 55" Package include:

1. **Strengthening the EU Emissions Trading System (ETS):** The package proposes to expand the ETS to include new sectors and tighten the emissions cap, thereby further incentivizing emissions reductions in industries.
2. **Carbon Border Adjustment Mechanism (CBAM):** The CBAM aims to prevent carbon leakage by imposing a carbon price on certain imported goods to ensure that foreign producers are subject to similar emissions costs as EU manufacturers.
3. **Renewable Energy and Energy Efficiency:** The package includes measures to increase the EU's renewable energy target to 40% of final energy consumption by 2030 and to improve energy efficiency, particularly in buildings.
4. **Transport and Mobility:** Proposals address the decarbonization of the transport sector through new emissions reduction targets for cars and vans, the expansion of electric vehicle charging infrastructure, and support for sustainable aviation and alternative fuels.
5. **Carbon Removal and Land Use:** The package focuses on enhancing carbon removal through afforestation, reforestation, and sustainable land use practices, as well as promoting agricultural practices that reduce emissions.
6. **Social Measures and Just Transition:** The "Fit for 55" Package includes provisions to ensure that the transition to a low-carbon economy is socially fair and that vulnerable groups are supported.
7. **Revision of the Effort Sharing Regulation:** The package revises the Effort Sharing Regulation to distribute emissions reduction targets among EU member states and ensure alignment with the 2030 climate target.
8. **Regulation of Methane Emissions:** The package introduces measures to reduce methane emissions from various sources, including the energy sector and agriculture.

9. **Strengthening Carbon Pricing:** The "Fit for 55" Package aims to enhance carbon pricing by expanding its coverage and aligning it with the increased emission reduction ambition.
10. **Sectoral Integration and Climate Action:** The package emphasizes the integration of various sectors, such as energy, transport, and industry, to ensure a holistic approach to achieving the EU's climate goals.

In summary, the "Fit for 55" Package is a comprehensive legislative and policy framework introduced by the European Commission in July 2021 to align the European Union's climate and energy policies with its ambitious emission reduction targets. The package addresses various sectors, including emissions trading, energy, transport, land use, and carbon pricing, while focusing on social fairness and a just transition to a more sustainable future **(The European Commission 2021)**.

4.1.17 REPowerEU

REPowerEU is a plan by the European Commission to rapidly reduce the European Union's dependence on Russian fossil fuels and accelerate the green transition. The plan was announced on May 18, 2022, in response to the Russian invasion of Ukraine.The REPowerEU plan includes several main pillars:

1. **Energy savings:** The plan aims to reduce the EU's energy consumption by 13% by 2030. This will be achieved through measures such as improving energy efficiency in buildings and industry, and promoting the use of public transportation.
2. **Diversification of energy supplies:** The plan aims to reduce the EU's reliance on Russian gas by two-thirds by 2030. This will be achieved by increasing imports of liquefied natural gas (LNG) from other countries, and by developing renewable energy sources such as solar and wind power.
3. **Accelerated deployment of renewable energy:** The plan aims to install 100 gigawatts of solar and wind power per year by 2025, and to make Europe climate neutral by 2050.
4. **Reduce gas consumption by 15% by 2030:** This will be achieved through measures such as energy efficiency improvements, switching to renewable heating and cooling, and using less gas for electricity generation.
5. **Diversify gas imports away from Russia:** This will be done by increasing imports from other countries, such as Norway, Algeria, and Qatar, and by developing renewable gas production, such as hydrogen and bio-methane.
6. **Accelerate the deployment of renewable energy:** This includes solar, wind, and offshore wind power, as well as renewable hydrogen production.
7. **Invest in energy efficiency:** This includes measures such as improving insulation in buildings, replacing old appliances, and using energy-efficient lighting.
8. **Support the development of new technologies:** This includes research and development into new renewable energy technologies, such as green hydrogen and carbon capture and storage.
9. **Strengthen the EU's energy security:** This includes measures to reduce Europe's reliance on imported fossil fuels and to ensure that the EU has a diversified and secure energy supply.
10. **Create jobs and boost economic growth:** The REPowerEU plan is expected to create millions of jobs and boost economic growth in the EU.
11. **Protect the environment:** The REPowerEU plan is in line with the EU's commitment to climate neutrality by 2050.

The REPowerEU plan is a major step forward in the EU's efforts to reduce its dependence on Russian fossil fuels and accelerate the green transition. It is an ambitious plan, but it is essential if the EU is to achieve its climate goals and ensure its energy security.

4.1.18 Further actions that have an impact on the biomass sector

The European Union (EU) has taken several important actions to promote biogas production and utilization as part of its efforts to advance sustainable energy, circular economy principles, and climate goals. Some of the most significant actions of the EU regarding biogas are discussed in chapter 1. In addition to the above efforts, other mechanisms incentivise biogas production as a renewable energy source and provide support mechanisms to promote its growth:

1. **Biofuels and Bioliquids Sustainability Criteria:** The EU established sustainability criteria for biofuels and bioliquids, including bio-methane derived from biogas. These criteria ensure that the production of bio-methane adheres to environmental and social standards, preventing adverse impacts on ecosystems and communities.
2. **Circular Economy Initiatives:** The EU's commitment to the circular economy aligns with biogas production's ability to transform organic waste into renewable energy and valuable byproducts. Biogas plants contribute to waste reduction and resource optimization.
3. **Funding Programs:** The EU supports biogas projects through funding programs like Horizon 2020, which provides financial assistance for research, innovation, and technological development in the field of energy, including biogas technologies.
4. **Bio-economy Strategy:** The EU's Bio-economy Strategy recognizes biogas production as a bio-based process that contributes to the sustainable use of biological resources. Biogas is an essential component of the circular bio-economy.
5. **Renewable Gas Initiative:** The European Commission launched the Renewable Gas Initiative to promote the use of renewable gases, including bio-methane from biogas, in the energy system. This initiative supports the integration of renewable gases into the energy mix.
6. **National and Regional Support:** Many member states have introduced national and regional incentives, grants, and policies to encourage biogas production. These actions contribute to the growth of biogas infrastructure and projects.
7. **Carbon Neutrality Goals:** The EU's commitment to achieving carbon neutrality by 2050 creates a favorable environment for biogas production, which can significantly reduce methane emissions and contribute to emissions reduction targets.
8. **Waste Management Objectives:** Biogas production addresses waste management challenges by utilizing organic waste as feedstock. The EU's waste management objectives align with the diversion of organic waste from landfills and waste-to-energy initiatives.
9. **Research and Innovation:** The EU emphasizes research and innovation to advance clean energy solutions, including biogas. Research projects and collaboration drive technological advancements in biogas production efficiency and environmental performance.

These actions collectively demonstrate the EU's commitment to promoting biogas production as a sustainable energy source, waste management solution, and contributor to its broader climate and environmental goals.

4.2 Evaluation of EU policy on biogas and bio-methane

This chapter draws explicit conclusions on the extent to which the respective EU policies are geared towards the production and use of biogas and bio-methane. In this context, the policy instruments mentioned in chapter 1 are discussed.

4.2.1 The Waste Framework Directive (Directive 2008/98/EC)

4.2.1.1 Explicit actions concerning biogas

The Waste Framework Directive primarily describes the collection, sorting, and treatment of waste and contains some directives for this purpose.

(1) *"It is important, in accordance with the waste hierarchy, and for the purpose of reduction of greenhouse gas emissions originating from waste disposal on landfills, to facilitate the separate collection and proper treatment of bio-waste in order to produce environmentally safe compost and other bio-waste based materials."* **(The European Parliament and Council of the European Union 2008)**.

This directive covers the production of biogas at 2 points. Firstly, separately collected bio-waste is a valuable secondary resource that can be used in pure form as a substrate for the production of biogas and bio-methane. Secondly, this process prevents the release of methane, which is harmful to the climate. Instead, it is collected and used as fuel. Additionally, the remaining fermentation residue can be ideally used as fertiliser.

(2) *"Animal by-products including processed products covered by Regulation (EC) No 1774/2002, except those which are destined for incineration, landfilling or use in a biogas or composting plant;"* **(The European Parliament and Council of the European Union 2008)**.

The handling of animal excreta is not covered in the WFP. Instead, reference is made to Regulation (EC) No 1774/2002, which specifically describes the requirements for a biogas plant using animal excrement as substrate **(The European Parliament and of the Council 2002)**.

(3) *"Bio-waste' means biodegradable garden and park waste, food and kitchen waste from households, restaurants, caterers and retail premises and comparable waste from food processing plants;"* **(The European Parliament and Council of the European Union 2008)**.

The collection of these secondary raw materials provides a good substrate basis for biogas plants in highly populated areas.

(4) *"Member States shall take measures, as appropriate, and in accordance with Articles 4 and 13, to encourage: (a) the separate collection of bio-waste with a view to the composting and digestion of bio-waste; (b) the treatment of bio-waste in a way that fulfills a high level of environmental protection; (c) the use of environmentally safe materials produced from bio-waste. The Commission shall carry out an assessment on the management of bio-waste with a view to submitting a proposal if appropriate. The assessment shall examine the opportunity of setting minimum requirements for bio-waste management and quality criteria for compost and digestate from bio-waste, in order to guarantee a high level of protection for human health and the environment."* **(The European Parliament and Council of the European Union 2008)**.

This guideline also addresses the optimal use of bio-waste as a substrate for preferably biogas plants.

(5) *"Waste management plans shall conform to the waste planning requirements laid down in Article 14 of Directive 94/62/EC and the strategy for the implementation of the reduction of biodegradable waste going to landfills, referred to in Article 5 of Directive 1999/31/EC."* **(The European Parliament and Council of the European Union 2008)**.

In future, bio-waste is to be better collected and separated in the EU countries. This material feed can underline the economic viability of biogas plants, especially in regions with a high population density. With these short transport routes, the waste heat, the electricity and the bio-methane can be used effectively.

4.2.1.2 Summary of actions concerning biogas

The Waste Framework Directive (WFD) and biogas production are intertwined due to the environmental and waste management goals they share. The WFD is a regulatory framework established by the European Union to guide the responsible management of waste, while biogas production, often derived from organic waste through anaerobic digestion, aligns with the WFD's principles and objectives. Here's how they intersect:

1. **Waste Prevention and Recovery:** The WFD promotes waste prevention, recovery, and recycling. Biogas production from organic waste, such as food waste and agricultural residues, contributes to waste recovery by diverting organic materials from landfills and incineration. Instead of treating these materials as waste, they are converted into valuable resources like biogas and nutrient-rich digestate.
2. **Renewable Energy Generation:** Biogas is a renewable energy source composed primarily of methane. It can be used for electricity generation, heat production, or as a fuel for vehicles. Biogas production aligns with the WFD's goal of sustainable energy production by utilizing organic waste to generate renewable energy.
3. **Reduction of Greenhouse Gas Emissions:** Biogas production through anaerobic digestion reduces methane emissions, as organic waste decomposes in an anaerobic environment. Since methane is a potent greenhouse gas, capturing it for energy use in biogas significantly mitigates its impact on climate change.
4. **Circular Economy:** Both the WFD and biogas production support the circular economy concept by turning waste into valuable resources. Biogas production exemplifies the circular approach by using organic waste as a feedstock to produce energy and nutrient-rich digestate, which can be used as a soil conditioner.
5. **Waste Management Innovation:** Biogas production is an innovative waste management approach that aligns with the WFD's goals. It offers an efficient and environmentally friendly method of treating organic waste, converting it into beneficial products while minimizing its environmental impact.
6. **Resource Efficiency:** Biogas production optimizes the use of organic waste, enhancing resource efficiency by extracting energy and valuable nutrients from materials that would otherwise be disposed of as waste.

In summary, the Waste Framework Directive and biogas production are interconnected as they share objectives related to responsible waste management, sustainable energy production, greenhouse gas reduction, and resource efficiency. Biogas production from organic waste is an effective way to achieve the WFD's goals by recovering value from waste materials and contributing to the circular economy.

4.2.2 Renewable Energy Directive I

4.2.2.1 Explicit actions concerning biogas

The Renewable Energy Directive serves primarily as a foundation for the European Renewable Energy Policy. It includes binding targets at EU level, National Climate Change Plans (NECP), support schemes, prohibition of deterioration, heating-cooling sector, transport sector. This directive was updated in 2018 by RED II. Despite this, RED I is a good basis to discuss the main points.

(1) *"The use of agricultural material such as manure, slurry, and other animal and organic waste for biogas production has, in view of the high greenhouse gas emission saving potential, significant environmental advantages in terms of heat and power production and its use as biofuel. Biogas installations can, as a result of their decentralised nature and the regional investment structure, contribute significantly to sustainable development in rural areas and offer farmers new income opportunities."* **(The European Parliament and of the Council 2009)**.

To comply with the renewable energy directive, agricultural residues such as animal excreta and organic waste are to be used to produce biogas. On the one hand, the emission of methane is reduced, as the waste is not just deposited in the environment, and on the other hand, valuable resources such as heat, electricity, and biofuels are obtained.

(2) *"'energy from renewable sources' means energy from renewable non-fossil sources, namely wind, solar, aerothermal, geothermal, hydrothermal and ocean energy, hydropower, biomass, landfill gas, sewage treatment plant gas and biogases;"* **(The European Parliament and of the Council 2009)**.

This phrase ensures that biogas is classified as a regenerative energy source.

(3) *"When designing their support systems, Member States may encourage the use of biofuels which give additional benefits, including the benefits of diversification offered by biofuels made from waste, residues, non-food cellulosic material, lignocellulosic material and algae, as well as nonirrigated plants grown in arid areas to fight desertification, by taking due account of the different costs of producing energy from traditional biofuels on the one hand and of those biofuels that give additional benefits on the other. Member States may encourage investment in research and development in relation to those and other renewable energy technologies that need time to become competitive."* **(The European Parliament and of the Council 2009)**.

The production of biogas and especially the purification into bio-methane also specifically addresses the production of bio-fuels with the help of processed waste. The efficiency of the fermentation of materials such as lignin, cellulose, and other plant raw materials is increased with progressive research.

In the following, the greenhouse saving potentials through the production of biogas from animal excreta and organic waste are listed. With 23 times the greenhouse gas potential, methane is even more harmful to the climate than carbon dioxide. By using resources such as animal excrement and organic waste, the methane produced can be captured and made usable. Thus, the construction of biogas plants with the use of waste leads to the reduction of climate-damaging gases and the development of an intact circular economy and bio-economy.

4.2.2.2 Summary of actions concerning biogas

The Renewable Energy Directive I (REDI) and biogas production are interconnected due to their shared objectives of promoting renewable energy sources and reducing greenhouse gas emissions. Biogas production, particularly through anaerobic digestion, aligns well with the goals and provisions of REDI. Here's how they intersect:

1. **Promotion of Renewable Energy Sources:** REDI's primary goal is to increase the share of renewable energy sources in the overall energy mix. Biogas production from organic waste, such as agricultural residues, food waste, and wastewater sludge, contributes to achieving this goal by generating renewable energy in the form of biogas.
2. **Diversification of Energy Sources:** Biogas production diversifies the renewable energy portfolio by offering an additional source of energy alongside wind, solar, and hydropower. This diversification enhances energy security and contributes to a more resilient energy system.
3. **Greenhouse Gas Emission Reduction:** Biogas production reduces greenhouse gas emissions, particularly methane, which is a potent contributor to global warming. Anaerobic digestion captures methane emissions that would otherwise be released into the atmosphere during organic waste decomposition, mitigating their impact on climate change.
4. **Waste Management and Circular Economy:** Biogas production aligns with REDI's objective of waste management and resource efficiency. Organic waste that would otherwise end up in landfills or incineration is utilized as a feedstock for biogas production, reducing waste volumes and contributing to the circular economy.
5. **Sustainability Criteria:** Biogas production can fulfill sustainability criteria outlined in REDI by ensuring that feedstocks are sourced responsibly, avoiding negative impacts on land use, and promoting sustainable practices in biogas production.
6. **Renewable Energy Targets:** The energy generated from biogas production contributes to meeting the renewable energy targets set by REDI, helping member states achieve their goals of increasing the share of renewables in their energy consumption.
7. **Incentives and Support:** REDI provides incentives and support mechanisms, such as feed-in tariffs and renewable energy certificates, that can encourage the development of biogas production facilities and their integration into the energy grid.
8. **Technological Innovation:** REDI's focus on advancing renewable technologies aligns with biogas production, which involves technological innovation in anaerobic digestion systems, biogas upgrading, and efficient utilization of the produced gas.
9. **Sustainable Bio-methane Production:** REDI encourages the use of sustainable biomass and biofuels, including bio-methane produced from biogas. This contributes to the reduction of fossil fuel dependence in sectors such as transportation.

In summary, the Renewable Energy Directive I and biogas production have several significant interferences, as biogas aligns with REDI's objectives of promoting renewable energy sources, reducing greenhouse gas emissions, enhancing waste management, and fostering sustainability. Biogas production offers a versatile and sustainable solution that contributes to the broader goals of transitioning to a cleaner and more renewable energy system.

4.2.3 Sustainable Development Goals

The sustainable development goals are a global non-profit project that was initiated by the United Nations in 2015. The aim is to make the earth a better place with 17 goals, where people, animals, living beings, and the environment can coexist peacefully.

4.2.3.1 Explicit actions concerning biogas

The SDGs consist of the 17 global goals, although no exact implementation is pre-

scribed. Therefore, there are only annual reports for these, in which the progress is presented. This means, however, that the document cannot be examined for the specific word "biogas" or there would be no hits. In the following, we will therefore look at what the SDGs have to do with biogas, both indirectly and in terms of content.

4.2.3.2 Summary of actions concerning biogas

The Sustainable Development Goals (SDGs) and biogas production are interconnected in various ways, as biogas production can contribute to several specific SDGs and their targets. The SDGs are a set of 17 global goals adopted by the United Nations to address a wide range of social, economic, and environmental challenges by 2030. Biogas production, particularly through anaerobic digestion, aligns with several of these goals:

1. **SDG 1:** No Poverty: Biogas production, especially in rural areas, can enhance energy access, improve livelihoods, and alleviate poverty by providing a source of energy for cooking, heating, and income-generating activities.
2. **SDG 2:** Zero Hunger: Biogas production can help address food security by converting agricultural residues into biogas while providing nutrient-rich digestate that can be used as a soil conditioner.
3. **SDG 6:** Clean Water and Sanitation: Biogas production can be applied to treat wastewater and sludge, reducing water pollution and promoting sustainable sanitation practices.
4. **SDG 7:** Affordable and Clean Energy: Biogas production contributes directly to SDG 7 by providing a source of affordable and clean energy. Biogas can be used for electricity generation, heating, cooking, and as a fuel for vehicles, offering a renewable alternative to fossil fuels.
5. **SDG 8:** Decent Work and Economic Growth: Biogas production contributes to economic growth by creating job opportunities in various stages, including feedstock collection, facility operation, and maintenance.
6. **SDG 9:** Industry, Innovation, and Infrastructure: Biogas production involves innovative technologies and infrastructure development, aligning with SDG 9's focus on promoting sustainable industrialization and enhancing technological progress.
7. **SDG 11:** Sustainable Cities and Communities: Biogas production can help create sustainable and resilient cities by addressing waste management challenges, reducing landfill use, and generating renewable energy within urban areas.
8. **SDG 12:** Responsible Consumption and Production: Biogas production supports responsible production and consumption by reducing waste generation through the conversion of organic waste into valuable resources like biogas and nutrient-rich digestate.
9. **SDG 13:** Climate Action: Biogas production significantly reduces greenhouse gas emissions, particularly methane, by capturing emissions from organic waste decomposition. This aligns with SDG 13's goal of combating climate change.
10. **SDG 15:** Life on Land: Biogas production promotes sustainable land use by diverting organic waste from landfills, reducing methane emissions, and generating renewable energy and soil-enhancing byproducts.
11. **SDG 17:** Partnerships for the Goals: The implementation of biogas projects often involves partnerships among governments, industries, communities, and NGOs, promoting cross-sectoral collaboration to achieve sustainable development objectives.

In summary, biogas production has significant interferences with multiple Sustainable Development Goals, particularly those related to clean energy, innovation, responsible consumption and production, climate action, sustainable cities, and partnerships.

In total, 11 of the 17 SDGs have an indirect or direct influence on the investigation and expansion of bio-energy. By addressing various social, economic, and environmental aspects, biogas production contributes to the broader agenda of achieving sustainable development and improving the well-being of people and the planet.

4.2.4 Paris Agreement

The Paris Agreement and biogas production are interconnected through their shared goals of addressing climate change and promoting sustainable development.

4.2.4.1 Explicit actions concerning biogas

Similar to the SDGs, the Paris Agreement is a very general document that sets targets but keeps implementation very general. It is also a very legal document, as it is an agreement between all signatory states to achieve the set targets. The search terms "biogas", "bio-energy" and "anaerobic digestion" could not be found in the document. Therefore, in the following paragraphs, the meaning of the Paris Agreement for biogas is discussed in general terms.

4.2.4.2 Summary of actions concerning biogas

The Paris Agreement, adopted in 2015 under the United Nations Framework Convention on Climate Change (UNFCCC), aims to limit global warming to well below 2 degrees Celsius above pre-industrial levels, with efforts to limit the increase to 1.5 degrees Celsius. Biogas production, particularly through anaerobic digestion, aligns with several aspects of the Paris Agreement:

1. **Mitigation of Greenhouse Gas Emissions:** The primary objective of the Paris Agreement is to mitigate greenhouse gas emissions to prevent dangerous levels of global warming. Biogas production contributes to this goal by capturing and utilizing methane emissions from organic waste, which would otherwise be released into the atmosphere. Since methane is a potent greenhouse gas, its reduction through biogas production directly aligns with the agreement's objectives.
2. **Renewable Energy Transition:** Biogas production provides a renewable energy source that can replace fossil fuels for electricity generation, heating, and transportation. By utilizing biogas, countries can reduce their reliance on carbon-intensive energy sources, contributing to the transition to a low-carbon energy system as envisioned by the Paris Agreement.
3. **Sustainable Development and Adaptation:** The Paris Agreement acknowledges the importance of sustainable development and adaptation to the impacts of climate change. Biogas production aligns with these aspects by promoting sustainable waste management practices, generating clean energy, and supporting local communities through job creation and energy access.
4. **Carbon Neutrality and Negative Emissions:** The Paris Agreement emphasizes the need to achieve carbon neutrality and explore negative emissions technologies. Biogas production can contribute to these goals by capturing methane emissions and using them for energy, thus preventing additional greenhouse gas emissions.
5. **Enhanced Nationally Determined Contributions (NDCs):** The Paris Agreement encourages countries to enhance their NDCs, which are national climate action plans. Biogas production can be included as a mitigation measure in NDCs to demonstrate efforts to reduce emissions from organic waste.
6. **Technology Transfer and Capacity Building:** The Paris Agreement promotes technology transfer and capacity building to support climate action in developing countries. Biogas production technology can be transferred to these countries to help address waste management challenges and promote

sustainable energy solutions.

7. **Cooperative Approaches:** The Paris Agreement encourages cooperation among countries to achieve their climate goals. Biogas production projects can involve international collaboration, technology sharing, and capacity-building efforts.

In summary, the Paris Agreement and biogas production are aligned in their efforts to mitigate climate change, promote sustainable development, and transition to a low-carbon energy system. Biogas production's ability to capture methane emissions, generate renewable energy, and support local communities makes it a valuable tool for achieving the goals outlined in the Paris Agreement.

4.2.5 Renewable Energy Directive II

The Renewable Energy Directive II (REDII) and biogas production are interconnected due to REDII's focus on promoting the use of renewable energy sources and biogas production's alignment with this objective.

4.2.5.1 Explicit actions concerning biogas

The RED II directive is very explicit and gives concrete trade recommendations and targets to be achieved by 2030. The term "biogas" appears 99 times in the document. This means that bioenergy is mentioned to a large extent in the directive as a renewable energy source. Not all mentioned terms will be discussed, but a short selection will be introduced.

(37) *"In order to ensure that the list of feedstock to produce advanced biofuels, other biofuels and biogas, as set out in an annex to this Directive, takes into account the principles of the waste hierarchy established in Directive 2008/98/EC, the Union sustainability criteria, and the need to ensure that that annex does not create additional demand for land while promoting the use of wastes and residues, the Commission, when regularly evaluating that annex, should consider the inclusion of additional feedstock that does not cause significant distortive effects on markets for (by-)products, wastes or residues."* **(The European Parliament and Council of the European Union 2018a)**.

(85) *"Advanced biofuels and other biofuels and biogas produced from feedstock listed in an annex to this Directive, renewable liquid and gaseous transport fuels of non-biological origin, and renewable electricity in the transport sector can contribute to low carbon emissions, stimulating the decarbonisation of the Union transport sector in a cost-effective manner, and improving, inter alia, energy diversification in the transport sector while promoting innovation, growth and jobs in the Union economy and reducing reliance on energy imports. An obligation on Member States to require fuel suppliers to ensure a minimum share of advanced biofuels and certain biogases, is intended to encourage continuous development of advanced fuels, including biofuels. It is important to ensure that that obligation also promotes improvements in the greenhouse gas performance of the fuels supplied to meet it. The Commission should assess the greenhouse gas performance, technical innovation and sustainability of those fuels."* **(The European Parliament and Council of the European Union 2018a)**.

(91) *"Feedstock which has low indirect land-use change impacts when used for biofuels, should be promoted for its contribution to the decarbonisation of the economy. Feedstock for advanced biofuels and biogas for transport, for which technology is more innovative and less mature and therefore needs a higher level of support, should, in particular, be included in an annex to this Directive. In order to ensure that it is updated in accordance with the latest technological developments while avoiding unintended negative effects, the Commission should review that annex in order to assess whether new feedstock should be added."*

(106) *"The minimum greenhouse gas emissions savings threshold for biofuels, bioliquids and biogas for transport produced in new*

installations should be increased in order to improve their overall greenhouse gas balance and to discourage further investments in installations with a low greenhouse gas emission savings performance. That increase provides investment safeguards for biofuels, bioliquids and biogas for transport production capacity."

(126) *"...specifying the methodology by which to determine the share of biofuel, and biogas for transport, resulting from biomass being processed with fossil fuels in a common process and the methodology by which to assess the greenhouse gas emissions savings from renewable liquid and gaseous transport fuels of non-biological origin and from recycled carbon fuels in order to ensure that credits from greenhouse gas emissions savings are given only once; amending by adding to, but not removing from, the lists of feedstock for the production of advanced biofuels and other biofuels and biogas; and supplementing or amending the rules for calculating the greenhouse gas impact of bio-fuels, bioliquids and their fossil fuel comparators."*

4.2.5.2 Summary of actions concerning biogas

REDII sets out rules and targets for increasing the share of renewable energy in the European Union's energy mix by 2030. Biogas production, especially through anaerobic digestion, can contribute to several aspects of REDII:

1. **Renewable Energy Targets:** REDII establishes binding renewable energy targets for EU member states. Biogas production, as a form of renewable energy derived from organic waste, can contribute to meeting these targets and increasing the overall share of renewables in the energy mix.
2. **Bioenergy and Biomethane:** REDII recognizes bioenergy, including bio-methane derived from biogas, as a renewable energy source that can contribute to the EU's energy goals. Biogas production aligns with this recognition and supports the sustainable use of biomass resources.
3. **Support Mechanisms:** REDII provides support mechanisms such as feed-in tariffs, premium payments, and guarantees of origin to incentivize the production and use of renewable energy. These mechanisms can benefit biogas projects and encourage the growth of the biogas sector.
4. **Transport Sector:** REDII emphasizes the use of renewable energy in the transport sector. Biomethane produced from biogas can be used as a renewable fuel for vehicles, contributing to the reduction of greenhouse gas emissions in the transportation sector.
5. **Sustainability Criteria:** REDII sets sustainability criteria for the use of biomass in bioenergy production. Biogas production can meet these criteria by ensuring that feedstocks are sourced sustainably and do not contribute to deforestation or other negative environmental impacts.
6. **Decarbonization:** REDII aims to decarbonize the energy sector and reduce green-house gas emissions. Biogas production contributes to this objective by capturing methane emissions from organic waste and converting them into a useful energy source.
7. **Waste Management:** REDII promotes waste-to-energy solutions that can help address waste management challenges. Biogas production offers an environmentally friendly method of managing organic waste, transforming it into energy and valuable by products.
8. **Circular Economy:** Both REDII and biogas production support the principles of the circular economy by converting waste materials into valuable resources. Biogas production enhances circularity by repurposing organic waste and reducing waste disposal.
9. **Local Energy Production:** Biogas production facilities can be located close to sources of organic waste, promoting local energy production and reduc-

ing the need for long-distance transportation of waste materials.

In summary, the Renewable Energy Directive II and biogas production have several significant interferences, as biogas aligns with REDII's objectives of increasing the use of renewable energy sources, reducing greenhouse gas emissions, promoting sustainable biomass use, and contributing to the transition to a low-carbon energy system. Biogas production offers a versatile and sustainable solution that complements the broader goals outlined in REDII.

4.2.6 European Green Deal

The European Green Deal and biogas production are interconnected through their shared objectives of advancing sustainability, mitigating climate change, and transitioning to a more environmentally friendly and resource-efficient economy.

4.2.6.1 Explicit actions concerning biogas

The European Green Deal does not explicitly mention biogas, bioenergy or anaerobic digestion. However, the term bio-economy is used, which includes the bio-based industry and thus also the production of biogas. Furthermore, the circular economy, which includes anaerobic digestion, is also mentioned in the document in many ways. In the following chapter, all other aspects that influence biogas, both directly and indirectly, are highlighted.

4.2.6.2 Summary of actions concerning biogas

The European Green Deal is the European Union's comprehensive plan to make the EU's economy sustainable and achieve climate neutrality by 2050. Biogas production, especially through anaerobic digestion, aligns with several aspects of the European Green Deal:

1. **Climate Neutrality:** The European Green Deal aims to achieve climate neutrality by 2050. Biogas production contributes to this goal by capturing and utilizing methane emissions from organic waste, which is a potent greenhouse gas, thus preventing its release into the atmosphere.
2. **Renewable Energy:** The European Green Deal emphasizes the expansion of renewable energy sources. Biogas production provides a renewable energy source that can help diversify the energy mix and reduce reliance on fossil fuels.
3. **Circular Economy:** Both the European Green Deal and biogas production support the circular economy by utilizing organic waste as a resource to generate energy and valuable byproducts. Biogas production contributes to waste reduction, resource efficiency, and the repurposing of organic materials.
4. **Waste Management:** The European Green Deal addresses waste management challenges as part of its sustainability agenda. Biogas production offers an environmentally friendly solution to organic waste management, reducing landfilling and promoting a more sustainable approach.
5. **Sustainable Agriculture:** Biogas production can be linked to sustainable agriculture by utilizing agricultural residues and byproducts as feedstock. This supports efficient resource utilization and contributes to agricultural sustainability.
6. **Local Energy Generation:** Biogas production facilities can be established in local communities, promoting decentralized energy generation and reducing energy transportation losses.
7. **Innovation and Research:** The European Green Deal encourages innovation and research to drive sustainable development. Biogas production involves innovative technologies and practices that align with the spirit of innovation promoted by the Green Deal.

8. **Emissions Reduction:** Biogas production contributes to reducing greenhouse gas emissions from waste and energy sectors. By capturing methane emissions and using them for energy, biogas helps meet emissions reduction targets outlined in the European Green Deal.
9. **Rural Development:** Biogas production projects can stimulate rural development by creating jobs, supporting local economies, and providing new opportunities for farmers and communities.
10. **Bioeconomy:** The European Green Deal promotes a sustainable and circular bioeconomy. Biogas production is a bio-based process that contributes to the utilization of organic materials in energy production and waste management.

In summary, the European Green Deal and biogas production have several significant interferences, as biogas aligns with the deal's objectives of climate neutrality, renewable energy expansion, circular economy promotion, waste management, and sustainable agriculture. Biogas production offers a practical and sustainable solution that supports the broader goals outlined in the European Green Deal.

4.2.7 EU Action Plan for Circular Economy

"Closing the Loop - An EU Action Plan for the Circular Economy" and biogas production are interconnected through their shared objectives of promoting resource efficiency, waste reduction, sustainable energy generation, and the transition to a circular economy.

4.2.7.1 Explicit actions concerning biogas

The EU action plan for circular economy does not explicitly mention biogas, bioenergy or anaerobic digestion. However, the term bioeconomy is used, which includes the bio-based industry and thus also the production of biogas. Furthermore, the circular economy, which includes anaerobic digestion, is also mentioned in the document in many ways. In the following chapter, all other aspects that influence biogas, both directly and indirectly, are highlighted.

4.2.7.2 Summary of actions concerning biogas

Several interferences between the action plan and biogas can be identified:

1. **Waste Valorization:** The action plan aims to transform waste into valuable resources. Biogas production aligns with this objective by converting organic waste into renewable energy (biogas) and nutrient-rich digestate, thus contributing to waste valorization and efficient resource use.
2. **Circular Economy:** Both the action plan and biogas production support the transition to a circular economy. Biogas production exemplifies circular practices by repurposing organic waste, reducing waste sent to landfills, and generating renewable energy and usable byproducts.
3. **Waste Reduction:** The action plan emphasizes reducing waste generation. Biogas production contributes to waste reduction by diverting organic waste from disposal sites and utilizing it as a resource for energy and other products.
4. **Emissions Reduction:** Biogas production helps mitigate greenhouse gas emissions by capturing methane from organic waste decomposition and using it as a renewable energy source. This aligns with the action plan's goal of reducing the environmental impact of waste management.
5. **Renewable Energy Generation:** Biogas is a renewable energy source that aligns with the action plan's objective of promoting sustainable energy production. It diversifies the energy mix and reduces reliance on fossil fuels.

6. **Waste Management:** Both the action plan and biogas production address waste management challenges by promoting sustainable alternatives to traditional waste disposal methods, such as landfilling. Biogas production offers an eco-friendly solution for organic waste management.
7. **Resource Efficiency:** The action plan emphasizes efficient resource use. Biogas production optimizes the use of organic waste materials, converting them into valuable energy and by-products, contributing to resource efficiency.
8. **Bioeconomy Promotion:** Biogas production is a part of the bio-economy, utilizing organic materials for energy and value creation. The action plan's focus on the bioeconomy aligns with the circular bioenergy approach of biogas production.
9. **Local Economic Development:** Biogas production facilities can create local jobs and stimulate economic activity. This corresponds with the action plan's goal of fostering sustainable economic growth at the local level.
10. **Innovation and Collaboration:** Both the action plan and biogas production require innovation, technology adoption, and collaboration among stakeholders. Biogas projects often involve multidisciplinary cooperation among waste management, energy, and agriculture sectors.

In summary, "Closing the Loop - An EU Action Plan for the Circular Economy" and biogas production share several significant interferences, as they both contribute to the circular economy's principles of waste reduction, resource efficiency, sustainable energy production, and responsible waste management. Biogas aligns well with the action plan's goals and exemplifies a practical solution to achieve circularity and sustainability within the EU.

4.2.8 Farm to Fork

The Farm to Fork Strategy and biogas production are interconnected through their shared objectives of promoting sustainable agriculture, reducing greenhouse gas emissions, improving waste management, and enhancing the circular economy.

4.2.8.1 Explicit actions concerning biogas

The Farm to Folk Strategy mentions biogas twice. It is an essential component for closing nutrient cycles and producing renewable energy.

"The circular bio-based economy is still a largely untapped potential for farmers and their cooperatives. For example, advanced bio-refineries that produce bio-fertilisers, protein feed, bioenergy, and bio-chemicals offer opportunities for the transition to a climate-neutral European economy and the creation of new jobs in primary production. Farmers should grasp opportunities to reduce methane emissions from livestock by developing the production of renewable energy and investing in anaerobic digesters for biogas production from agriculture waste and residues, such as manure. Farms also have the potential to produce biogas from other sources of waste and residues, such as from the food and beverage industry, sewage, wastewater and municipal waste. Farm houses and barns are often perfect for placing solar panels and such investments should be prioritised in the future CAP Strategic Plans12. The Commission will take action to speed-up market adoption of these and other energy efficiency solutions in the agriculture and food sectors as long as these investments are carried out in a sustainable manner and without compromising food security or biodiversity, under the clean energy initiatives and programmes".

4.2.8.2 Summary of actions concerning biogas

Several interferences between the Farm to Fork Strategy and biogas can be identified:

1. **Sustainable Agriculture:** The Farm to Fork Strategy aims to promote sustain-

able and environmentally friendly agricultural practices. Biogas production can support these objectives by utilizing agricultural residues and byproducts as feedstock, reducing waste and enhancing resource efficiency.

2. **Emissions Reduction:** Biogas production directly aligns with the Farm to Fork Strategy's goal of reducing greenhouse gas emissions. By capturing methane emissions from organic waste and utilizing them for energy, biogas production prevents the release of potent greenhouse gases into the atmosphere.
3. **Circular Economy:** Both the Farm to Fork Strategy and biogas production support the principles of the circular economy. Biogas production repurposes organic waste into valuable energy (biogas) and nutrient-rich byproducts (digestate), contributing to waste reduction and resource optimization.
4. **Waste Reduction and Valorization:** The Farm to Fork Strategy promotes reducing food and organic waste. Biogas production addresses this by converting organic waste, including food waste and agricultural residues, into biogas and digestate, contributing to efficient waste management and energy generation.
5. **Nutrient Cycling:** Biogas production generates digestate, a nutrient-rich residue that can be used as a natural fertilizer. This aligns with the Farm to Fork Strategy's objective of optimizing nutrient cycling in agriculture and reducing the reliance on synthetic fertilizers.
6. **Renewable Energy Generation:** Biogas production contributes to the generation of renewable energy, supporting the Farm to Fork Strategy's aim of promoting cleaner and more sustainable energy sources within the food supply chain.
7. **Local Energy Production:** Biogas production facilities can be established locally, promoting decentralized energy generation and supporting rural development, which resonates with the Farm to Fork Strategy's focus on strengthening rural areas.
8. **Collaboration and Multi-Sectoral Approach:** Both the Farm to Fork Strategy and biogas production require collaboration among different stakeholders, including farmers, industry, policymakers, and consumers. They highlight the need for coordinated efforts to achieve sustainable and integrated solutions.
9. **Regenerative Practices:** The Farm to Fork Strategy emphasizes regenerative agricultural practices that enhance soil health and biodiversity. Biogas production can complement these practices by recycling organic materials and contributing to soil enrichment through digestate application.

In summary, the Farm to Fork Strategy and biogas production share several important interferences, as both aim to promote sustainable agriculture, reduce emissions, enhance waste management, and contribute to a more circular and resource-efficient food system. Biogas production aligns well with the overarching goals of the Farm to Fork Strategy by addressing environmental, agricultural, and energy-related challenges.

4.2.9 EU Taxonomy for Sustainable Activities

Regulation (EU) 2020/852 on the EU taxonomy for sustainable activities and biogas production are interconnected through their relevance to sustainable development, environmental protection, and the transition to a low-carbon economy.

4.2.9.1 Explicit actions concerning biogas

The EU Taxonomy for Sustainable Activities does not explicitly mention biogas, bioenergy or anaerobic digestion. However, the term circular economy and waste management are used, which also includes the production of biogas. Furthermore, the circular

economy, which includes anaerobic digestion, is mentioned in many places in the document. In the following chapter, all other aspects that have both a direct and indirect influence on biogas are highlighted.

4.2.9.2 Summary od actions concerning biogas

The EU taxonomy sets out criteria for determining whether an economic activity qualifies as environmentally sustainable. Biogas production, particularly through anaerobic digestion, can have several intersections with the EU taxonomy:

1. **Environmental Objectives:** The EU taxonomy establishes six environmental objectives that activities must contribute to in order to be considered environmentally sustainable. Biogas production aligns with these objectives, including climate change mitigation, circular economy transition, and pollution prevention and control.
2. **Climate Mitigation:** Biogas production reduces greenhouse gas emissions by capturing methane from organic waste decomposition and utilizing it as a renewable energy source. This directly contributes to the climate change mitigation objective of the EU taxonomy.
3. **Circular Economy:** Biogas production is an example of circular economy practices, as it transforms organic waste into valuable resources such as renewable energy (biogas) and nutrient-rich digestate. This aligns with the circular economy transition objective of the EU taxonomy.
4. **Pollution Prevention:** Biogas production helps prevent pollution by diverting organic waste from landfills, reducing methane emissions, and promoting sustainable waste management practices.
5. **Technical Screening Criteria:** The EU taxonomy includes specific technical screening criteria that economic activities must meet to be considered environmentally sustainable. Biogas production projects need to fulfill these criteria, such as demonstrating emission reductions and adhering to waste management standards.
6. **Disclosure and Transparency:** The EU taxonomy requires financial market participants to disclose the proportion of their portfolio invested in environmentally sustainable activities, using the taxonomy criteria. Biogas production projects that meet the criteria can be considered sustainable investments.
7. **Alignment with Climate Goals:** Biogas production contributes to the achievement of the EU's climate targets and commitments under the Paris Agreement. It supports the transition to renewable energy sources and reductions in greenhouse gas emissions.
8. **Renewable Energy:** Biogas production is a renewable energy source, and its inclusion in the EU taxonomy can encourage investments in sustainable energy projects.
9. **Innovation and Research:** Biogas production involves innovative technologies and practices. This aligns with the EU taxonomy's focus on promoting innovation for sustainability.

In summary, Regulation (EU) 2020/852 on the EU taxonomy for sustainable activities and biogas production have significant interferences, as biogas aligns with the taxonomy's environmental objectives, technical screening criteria, and principles of sustainability. Biogas production contributes to climate change mitigation, circular economy promotion, pollution prevention, and renewable energy generation, making it a relevant candidate for inclusion in the EU taxonomy for sustainable activities.

4.2.10 Fit for 55 Package

The "Fit for 55" Package and biogas production are interconnected through their shared objectives of advancing sustainabili-

ty, reducing greenhouse gas emissions, and promoting renewable energy sources.

4.2.10.1 Explicit actions concerning biogas

The Fit for 55 Packagefor Sustainable Activities does not explicitly mention biogas, bioenergy or anaerobic digestion. However, the term bio-methane, methane for the transport sector and biofuels are used, which includes the production of biogas. Furthermore, the circular economy, which includes anaerobic digestion, is mentioned in many places in the document. In the following chapter, all other aspects that have both a direct and indirect influence on biogas are highlighted.

4.2.10.2 Summary of actions concerning biogas

The "Fit for 55" Package consists of various legislative proposals aimed at aligning the European Union's policies with more ambitious climate targets. Biogas production, particularly through anaerobic digestion, can have several intersections with the "Fit for 55" Package:

1. **Climate Goals:** The "Fit for 55" Package aims to further the EU's climate goals, including a more ambitious target to reduce net greenhouse gas emissions by at least 55% by 2030 compared to 1990 levels. Biogas production contributes directly to this goal by reducing methane emissions and promoting renewable energy generation.
2. **Renewable Energy Transition:** Biogas production aligns with the package's emphasis on transitioning to renewable energy sources. It offers a renewable energy solution derived from organic waste, contributing to the diversification of the energy mix and reducing dependence on fossil fuels.
3. **Emissions Reduction:** Biogas production captures methane emissions from organic waste, which is a potent greenhouse gas, and uses it as a renewable energy source. This directly supports the "Fit for 55" Package's objectives of reducing methane emissions and overall greenhouse gas emissions.
4. **Bioenergy and Bio-methane:** The "Fit for 55" Package recognizes the importance of sustainable bioenergy and bio-methane in achieving climate targets. Biogas production provides a source of bio-methane, a renewable fuel that can be used in various sectors, including transportation.
5. **Waste Management:** Biogas production aligns with the waste management goals of the "Fit for 55" Package by utilizing organic waste as a resource and reducing the need for landfilling and incineration.
6. **Sustainable Agriculture:** Biogas production utilizes agricultural residues and by-products as feedstock, supporting sustainable agricultural practices and resource utilization.
7. **Local Energy Generation:** Biogas production can promote local energy generation, contributing to energy security and reducing transmission losses.
8. **Cross-Sectoral Integration:** The "Fit for 55" Package emphasizes cross-sectoral integration to achieve climate goals. Biogas production involves multiple sectors, including waste management, energy, and agriculture, making it a versatile solution that aligns with this approach.
9. **Innovation and Research:** Biogas production involves innovative technologies and practices, which align with the "Fit for 55" Package's focus on innovation and research for sustainable development.

In summary, the "Fit for 55" Package and biogas production have significant interferences, as biogas aligns with the package's objectives of emissions reduction, renewable energy transition, waste management, and sustainable development. Biogas pro-

duction offers a practical and sustainable solution that complements the broader goals outlined in the "Fit for 55" Package.

4.2.11 REPower EU

4.2.11.1 Explicit actions concerning biogas

Boosting sustainable biomethane production to 35 bcm by 2030 is a cost-efficient path to achieve our ambition to reduce imports of natural gas from Russia. To increase the capacity of biogas production in the EU and promote its conversion into bio-methane, the estimated investment needs amount to EUR 37 billon euro over the period.

As outlined in the Biomethane Action Plan in the accompanying staff working document, the Commission proposes to address the main barriers to increased sustainable bio-methane production and use and facilitation of its integration into the EU internal gas market by:

- Establishing an industrial biogas and biomethane partnership to stimulate the renewable gases value chain.
- Taking additional measures to encourage biogas producers to create energy communities.
- Providing incentives for biogas upgrading into biomethane;
- Promoting the adaptation and adjustment of existing and the deployment of new infrastructure for the transport of more bio-methane through the EU gas grid.
- Addressing gaps in research, development and innovation
- Facilitating access to finance, and mobilise EU funding under CEF, Cohesion Policy, RRF and the Common Agricultural Policy.

"Energy efficiency, fuel substitution, electrification, and an enhanced uptake of renewable hydrogen, biogas and bio-methane by industry could save up to 35 bcm of natural gas by 2030 on top of what is foreseen under the Fit for 55 proposals. Production of non-metallic minerals, cement, glass and ceramics, production of chemicals and refineries provide the biggest opportunities for reducing fossil gas demand – almost 22 bcm."

"The regional assessment of additional gas infrastructure needs for REPowerEU shows that it will be possible to fully compensate the equivalent of Russian gas imports by a combination of demand reduction, a ramp up of domestic production of biogas/bio-methane and hydrogen, and limited additions of gas infrastructure. The most important needs are linked to meet demand in Central and Eastern Europe, and in the northern part of Germany, as well as the reinforcement of the Southern gas corridor. This limited additional infrastructure, as described in annex 3, should solve the needs for the forthcoming decade, without leading to a lock-in of fossil fuels and stranded assets that inhibit the long-term transition to a climate-neutral economy."

The assessment shows that it will be possible to fully compensate the end to Russian gas imports by a combination of demand reductions as envisaged by the Commission's fit for 55 package, a ramp up of domestic production of biogas and fossil-free hydrogen in particular, and rather limited additions of gas infrastructure beyond what is already included in the current 5th PCI list. Mitigating the few remaining bottlenecks will also increase the European gas system's resilience and flexibility.

4.2.11.2 Summary of actions concerning biogas

The REPower EU plan has sevearl implications for biogas production.

1. **Increased demand for biogas:** The plan aims to increase the use of biogas in the EU, as a way to reduce dependence on Russian gas. This will create new demand for biogas production.
2. **Support for biogas projects:** The

plan includes financial support for biogas projects, which will help to make them more viable.

3. **Regulation of biogas production:** The plan includes new regulations for biogas production, which will aim to ensure sustainability of biogas plants.
4. **Research and development:** The plan includes funding for research and development into biogas production, which will help to improve the efficiency and sustainability of the process.

The REPowerEU plan is a major opportunity for the biogas industry. It is likely to lead to increased investment in biogas production and the development of new biogas technologies. This will help to make biogas a more competitive and sustainable energy source.

4.2.11.3 Current crisis influencing the policy for the biogas sector

European dependence on gas imports from Russia combined with Russia's war against Ukraine led to an energy price crisis. This war is prompting a rethink of Europe's resource and energy policy, especially with politically unstable regions and states with totalitarian structures. For this purpose, the EU Commission presented a communication on remedial measures on 8 March 2022. These include accelerating the expansion of renewable energies by speeding up planning and approval procedures. Originally, in the Green Deal, the European Commission had set the target for bio-methane or biogas production at 17 billion m^3 by 2030. For the occasion, the target was raised to 35 billion m3. From today's level, this means a tenfold increase in current production in the EU **(The European Commission 2022)**. In detail, independence from Russian fossil fuels will be achieved by 2030 as follows:

- Diversifying gas supplies
- Larger volumes of bio-methane and renewable hydrogen production and imports
- Reducing faster the use of fossil fuels in our homes, buildings, industry, and power system
- Boosting energy efficiency
- Increasing renewables and electrification
- Addressing infrastructure bottlenecks

4.2.12 Summary of actions the EU have done to promote biogas

The European Union (EU) has taken several actions to promote and support biogas production as part of its efforts to advance sustainability, reduce greenhouse gas emissions, and transition to a more renewable energy system. Some of the key actions taken by the EU to promote biogas production include:

1. **Legislation and Directives:** The EU has established legislative frameworks and directives that encourage the use of renewable energy sources, including biogas. Directives such as the Renewable Energy Directive I (2009/28/EC) and Renewable Energy Directive II (2018/2001/EU) set targets for increasing the share of renewables in the energy mix and provide incentives for biogas production.
2. **Support Mechanisms:** The EU provides financial support and incentives for biogas production through mechanisms like feed-in tariffs, premium payments, and renewable energy certificates. These mechanisms help make biogas projects economically viable and attractive to investors.
3. **Sustainability Criteria:** The EU has established sustainability criteria for biofuels and bioliquids, including bio-methane produced from biogas. These criteria ensure that biogas production follows environmentally and socially responsible practices.
4. **Research and Innovation:** The EU supports research, innovation, and technological development in the field of biogas production. Funding programs such as

Horizon 2020 provide financial support for projects that advance biogas technologies and improve efficiency.

5. **EU Taxonomy:** The EU taxonomy for sustainable activities includes criteria for determining whether an economic activity, including biogas production, is environmentally sustainable. This provides clarity for investors and promotes sustainable biogas projects.
6. **Circular Economy Initiatives:** The EU's circular economy initiatives encourage the utilization of organic waste as a resource, aligning with biogas production's ability to convert waste into valuable energy and by-products.
7. **Decarbonization Goals:** The EU's commitments to decarbonization and reducing greenhouse gas emissions create a favorable environment for biogas production, which contributes to emissions reduction through methane capture.
8. **Policy Integration:** Biogas production intersects with various EU policies, including waste management, agriculture, and energy. The integration of these policies supports the development of biogas projects that address multiple sustainability objectives.
9. **Capacity Building:** The EU supports capacity building and knowledge sharing among member states to promote the development of biogas infrastructure and technologies.
10. **International Cooperation:** The EU collaborates with international organizations and partners to promote sustainable biogas production globally, sharing best practices and supporting capacity building in developing countries.
11. **Renewable Gas Initiative:** The European Commission launched the Renewable Gas Initiative to promote the use of renewable gases, including bio-methane from biogas, in the energy system.

These actions collectively create an enabling environment for biogas production, encouraging its growth as a sustainable energy source and waste management solution within the EU.

4.3 Biogas Landscape in Europe

This chapter compares the biogas and bio-methane sectors of European countries. The 27 EU countries are discussed, as well as the United Kingdom, Norway, Switzerland, Ukraine, and Iceland as important biogas and bio-methane producers. In 2020, 191 TWh of biogas and bio-methane were produced in the entire European Union. This corresponds to about 4.6% of gas consumption in the EU. **Figure 61** shows the development of combined biogas and bio-methane production from 2011 to 2020.

While the development of biogas production is stagnating because it is difficult to generate usable electricity and heat. Particularly the interest in the production of biomethane has risen sharply in the last ten years. In contrast, biomethane production is growing strongly due to high demand, storage capacity, and rising prices for natural gas. In 2019, 26 TWh of bio-methane were produced, and in the following year already 32 TWh, which means an increase of 23%. A total of 161 new methane plants came on stream during this period. The United Kingdom saw the strongest biomethane growth in 2020, with an increase of 1,689 GWh. This is followed by Denmark, with an increase of 1,347 GWh, France, with an increase of 972 GWh, Italy, with an increase of 805 GWh and the Netherlands, with an increase of 698 GWh. In terms of absolute numbers, Germany leads as a methane producer with 11,200 GWh, followed by the United Kingdom, with a production of 6,944 GWh, and Denmark, with a production of 4,041 GWh. The amount of biogas and bio-methane production varies greatly between European countries due to

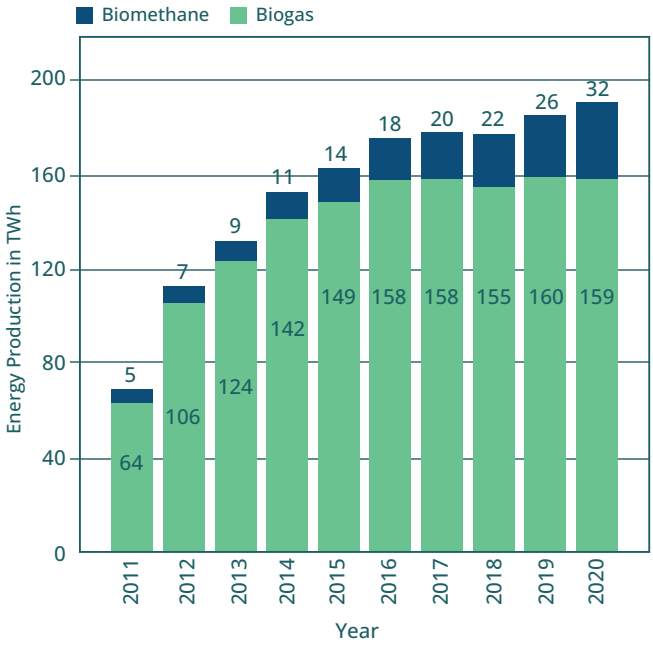


Figure 61: Trends and developments in biogas and bio-methane production in Europe (Adapted from the European Biogas Association 2021).

the respective national governments. Germany, for example, has a large number of biogas plants due to the introduction of the Renewable Energy Sources Act and the feed-in tariff, while countries like Sweden have more bio-methane plants due to the promotion of bio-methane as a fuel. In general, however, the statistics show that the production of bio-methane is on an upward trend.

Figure 62 shows the countries that produce the most biogas and biomethane. Germany produces the most biogas and biomethane with 82.428 GWh. As mentioned above, this is due to the strong promotion of biogas production with the EEG of the German government. Germany is followed by the United Kingdom and Italy, which produce about one-third of what Germany produces.

In the following, the countries with the highest biogas and biomethane production are presented, which are listed in **Figure 62**. In the further explanations, exact figures are discussed, such as the number of biogas and bio-methane plants, trends and stagnation, substrates used, implementation of EU regulations, national ideas, and support levers of the individual countries as well as regional differences.

The largest share of biogas plants, 63%, is found in the agricultural sector. In comparison, landfills account for the second-largest share, 15%. Among the biomethane plants, the largest share is also found in the agricultural sector with a share of 53%, whereas organic municipal waste is the second-largest source of biomethane production with 11% **(European Biogas Association 2021)**.

Developments to promote biomethane

A biomethane register issues certificates to biomethane producers that prove to the gas consumer that renewable gas has been fed in. This fulfills the criteria of RED II. New regulation for the injection of bio-methane into the gas grid is to replace the current subsidies for biogas and bio-methane. Currently, the production of electricity and bio-methane is subsidized for at least 20 years until 2032. Furthermore, a tendering system for biomethane plants was introduced in 2020. Denmark, Sweden, and Estonia are the only countries that produce more biomethane than biogas **(European Biogas Association 2021)**.

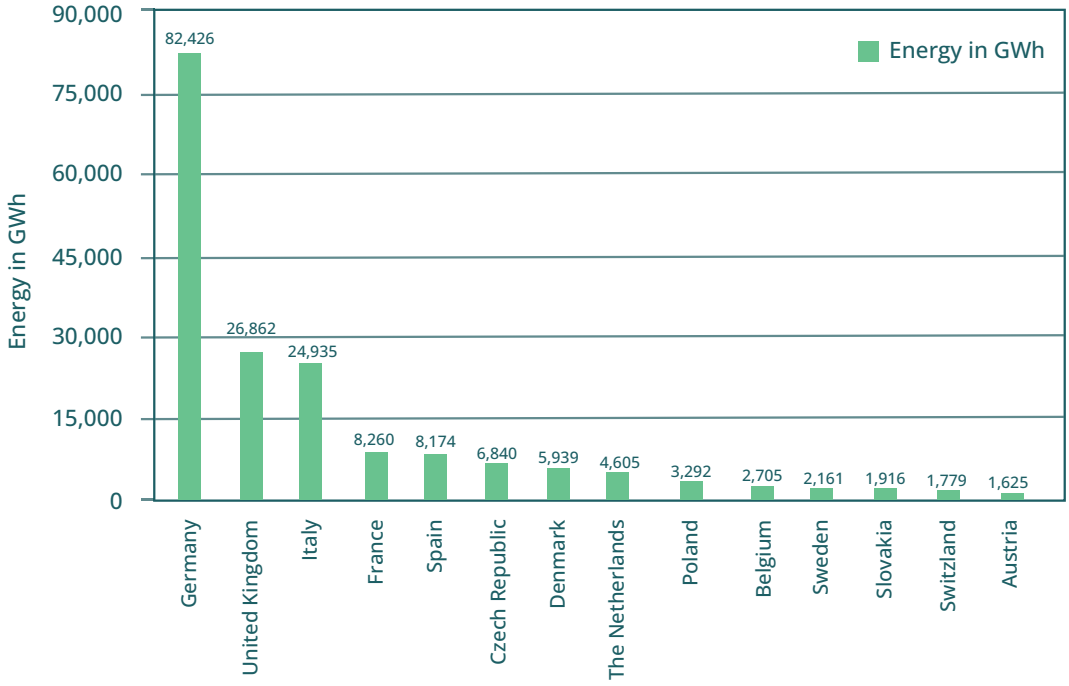


Figure 62: Top Biogas producers in Europe given in GWh (Adapted from the European Biogas Association 2021).

4.3.1 Germany

Germany has been a frontrunner in promoting biogas production and utilization through a combination of policies, incentives, and support mechanisms. Some key actions and measures taken by Germany to promote biogas include:

1. **Feed-in Tariffs and Renewable Energy Act (EEG):** Germany's EEG introduced feed-in tariffs for renewable energy sources, including biogas. These tariffs guarantee fixed payments to biogas producers for the electricity they generate, creating financial incentives for biogas projects.
2. **Biogas Act (BioGasV):** The Biogas Act sets regulations and standards for the production, storage, and distribution of biogas. It establishes feed-in tariffs, quality requirements for feedstocks, and technical specifications for biogas facilities.
3. **Support for Small-Scale Biogas Plants:** Germany supports small-scale biogas plants through simplified administrative procedures and more favorable feed-in tariffs. This encourages decentralized energy generation and promotes rural development.
4. **Renewable Heat Incentives:** Germany provides incentives for biogas utilization in heat applications. Biogas can be used for heating buildings, industrial processes, and district heating systems, contributing to energy efficiency.
5. **Biogas Upgrading and Injection:** Germany promotes the upgrading of biogas to biomethane quality for injection into the natural gas grid. Producers receive premium payments for injecting biomethane, expanding the utilization options for biogas.
6. **Anaerobic Digestion Research:** Germany invests in research and development of anaerobic digestion technologies to improve the efficiency and performance of biogas production processes.
7. **Agricultural Support:** Germany supports the use of agricultural residues and organic waste as feedstock for biogas production. This aligns with

sustainable agricultural practices and reduces waste.

8. **Biogas Associations and Networks:** Various biogas associations and networks in Germany provide information, advocacy, and knowledge exchange for biogas producers and stakeholders.
9. **Energy Efficiency and Emission Standards:** Germany enforces energy efficiency and emission standards for biogas facilities to ensure their environmental and operational performance.
10. **Regional Initiatives:** Some German states offer additional incentives, grants, and programs to promote biogas production, depending on their energy and climate policies.
11. **Research and Innovation:** Germany supports research projects focused on biogas technologies, efficiency improvements, and the integration of biogas into the energy system.
12. **Economic Benefits:** Biogas projects contribute to economic growth by creating jobs in construction, operation, and maintenance.

Germany's comprehensive approach to promoting biogas encompasses various aspects, from policy frameworks and financial incentives to technological advancements and research initiatives. These measures have contributed to Germany's significant role in biogas production and its integration into the country's energy transition and sustainable development goals.

4.3.2 The United Kingdom

The United Kingdom (UK) has implemented several measures to promote biogas production and utilization as part of its efforts to increase renewable energy generation, reduce greenhouse gas emissions, and address waste management challenges. Some key actions and initiatives taken by the UK to promote biogas include:

1. **Renewable Heat Incentive (RHI):** The UK's RHI provides financial incentives to support the generation of renewable heat, including biogas utilization for heating applications. This scheme encourages the use of biogas in various sectors, such as agriculture, industry, and residential buildings.
2. **Feed-in Tariffs (FiTs):** The UK previously offered FiTs for small-scale renewable energy projects, including biogas production. These tariffs provided guaranteed payments to biogas producers for the electricity they generated and exported to the grid.
3. **Renewable Transport Fuel Obligation (RTFO):** The RTFO requires fuel suppliers to include a certain percentage of renewable fuels in their transportation fuel mix, including bio-methane produced from biogas. This promotes the use of biogas as a clean transportation fuel.
4. **Anaerobic Digestion Strategy and Action Plan:** The UK government released an Anaerobic Digestion Strategy and Action Plan to support the growth of the anaerobic digestion sector, including biogas production. The plan includes targets, research funding, and policy support.
5. **Waste-to-Energy:** The UK encourages the use of organic waste as feedstock for biogas production, supporting waste-to-energy initiatives and diverting waste from landfills.
6. **Research and Development:** The UK invests in research and innovation related to biogas production technologies, efficiency improvements, and waste utilization.
7. **Gas Grid Injection:** The UK promotes the injection of bio-methane produced from biogas into the natural gas grid. Biomethane can be used for heating, cooking, and transportation, offering an additional revenue stream for biogas producers.
8. **Biofertilizer Production:** Biogas production generates digestate, which can be used as a nutrient-rich biofertilizer in agriculture, reducing the need for synthetic fertilizers.

9. **Regional Initiatives:** Different regions within the UK may offer their own incentives, grants, and support for biogas projects, aligned with their local energy and waste management strategies.
10. **Knowledge Sharing and Networking:** The UK hosts conferences, workshops, and industry events to facilitate knowledge exchange and networking opportunities for biogas stakeholders.
11. **Policy Support:** The UK's broader energy and climate policies, including its commitment to net-zero emissions by 2050, provide a favorable environment for renewable energy sources like biogas.
12. **Circular Economy Approach:** Biogas production supports the UK's circular economy goals by repurposing organic waste and contributing to waste reduction and resource efficiency.

These actions collectively demonstrate the UK's commitment to promoting biogas production as a renewable energy source, waste management solution, and contributor to its climate and sustainability objectives.

4.3.3 Italy

Italy has taken various measures to promote biogas production and utilization as part of its efforts to increase renewable energy generation, reduce greenhouse gas emissions, and advance sustainable waste management. Some key actions and initiatives taken by Italy to promote biogas include:

1. **Feed-in Tariffs (Conto Energia):** Italy has provided feed-in tariffs through the Conto Energia schemes to incentivize the production of renewable energy, including biogas. These tariffs guarantee fixed payments for the electricity generated from biogas projects, providing financial incentives for investment.
2. **National Strategic Framework for Biogas:** Italy developed a National Strategic Framework for Biogas to support the growth of the biogas sector. The framework includes targets, regulatory measures, and support mechanisms to encourage biogas production.
3. **Renewable Energy Certificates:** Italy has implemented a renewable energy certificate system that allows biogas producers to trade certificates representing the renewable attributes of their energy production.
4. **Waste-to-Energy Initiatives:** Italy encourages the utilization of organic waste for biogas production, contributing to waste-to-energy initiatives and reducing the environmental impact of waste disposal.
5. **Regional Incentives:** Different regions within Italy may offer specific incentives, grants, and support for biogas projects based on their local energy and waste management policies.
6. **Agricultural Residues Utilization:** Italy promotes the use of agricultural residues and byproducts as feedstock for biogas production, aligning with sustainable agricultural practices and waste reduction goals.
7. **Biogas Upgrading:** Italy supports the upgrading of biogas to biomethane quality for injection into the natural gas grid. This enhances the utilization of biogas beyond electricity generation.
8. **Research and Development:** Italy invests in research and innovation related to biogas production technologies, process efficiency improvements, and environmental performance.
9. **Decentralized Energy Generation:** Italy's energy policies encourage decentralized energy generation, which aligns with biogas production's potential for distributed energy supply.
10. **Circular Economy:** Biogas production supports Italy's circular economy goals by converting organic waste into valuable energy and biofertilizers, contributing to waste reduction and resource efficiency.
11. **Climate and Energy Policies:** Italy's broader climate and energy policies,

including its commitment to renewable energy and emissions reduction targets, create a conducive environment for biogas projects.

12. **Collaboration and Networking:** Italy hosts events, conferences, and industry gatherings to facilitate knowledge exchange, collaboration, and networking among biogas stakeholders.
13. **Green Public Procurement:** Italy encourages the use of renewable energy sources, including biogas, in public procurement for energy consumption.

These actions collectively highlight Italy's efforts to promote biogas production as a sustainable energy source, waste management solution, and contributor to its climate and sustainability objectives.

4.3.4 France

France has implemented various measures to promote biogas production and utilization as part of its efforts to increase renewable energy generation, reduce greenhouse gas emissions, and improve waste management practices. Some key actions and initiatives taken by France to promote biogas include:

1. **Feed-in Tariffs and Premiums:** France has provided feed-in tariffs and premium payments for biogas producers as part of its support for renewable energy. These incentives guarantee payments for the electricity generated from biogas projects and contribute to the financial viability of biogas installations.
2. **Biogas Support Scheme (CS2G):** France's CS2G scheme provides financial support for the injection of biogas into the natural gas grid. Producers receive a premium for bio-methane injection, incentivizing the production of high-quality biogas.
3. **Waste-to-Energy Initiatives:** France encourages the use of organic waste for biogas production, supporting waste-to-energy projects and reducing the environmental impact of waste disposal.
4. **Circular Economy:** Biogas production aligns with France's circular economy goals by repurposing organic waste and creating valuable energy and biofertilizers.
5. **Anaerobic Digestion Development Plan:** France has developed an Anaerobic Digestion Development Plan to support the growth of the biogas sector. The plan includes targets, regulatory measures, and financial incentives for biogas projects.
6. **Renewable Heat Incentives:** France offers incentives for biogas utilization in heating applications, supporting the use of biogas for space heating, industrial processes, and district heating systems.
7. **Research and Innovation:** France invests in research and development related to biogas production technologies, efficiency improvements, and waste utilization.
8. **Agricultural Residues Utilization:** France promotes the use of agricultural residues and byproducts as feedstock for biogas production, aligning with sustainable agricultural practices and waste reduction goals.
9. **Decentralized Energy Generation:** France's energy policies encourage decentralized energy generation, which aligns with biogas production's potential for distributed energy supply.
10. **Biofertilizer Production:** Biogas production generates digestate, which can be used as a nutrient-rich biofertilizer in agriculture, reducing the need for synthetic fertilizers.
11. **Regulatory Framework:** France has established a regulatory framework for biogas production, ensuring compliance with environmental, safety, and quality standards.
12. **Regional Initiatives:** Different regions within France may offer specific incentives, grants, and support for biogas projects based on their local energy and waste management policies.
13. **Knowledge Sharing and Networking:** France hosts conferences, seminars, and industry events to facilitate knowl-

edge exchange, collaboration, and networking among biogas stakeholders.

14. **Climate and Energy Goals:** France's broader climate and energy policies, including its commitment to renewable energy targets and emissions reduction, create a supportive environment for biogas projects.

These actions collectively reflect France's commitment to promoting biogas production as a renewable energy source, waste management solution, and contributor to its climate and sustainability objectives.

4.3.5 Spain

Spain has taken various measures to promote biogas production and utilization as part of its efforts to increase renewable energy generation, reduce greenhouse gas emissions, and advance sustainable waste management. Some key actions and initiatives taken by Spain to promote biogas include:

1. **Renewable Energy Support:** Spain provides support mechanisms such as feed-in tariffs and premiums for biogas producers as part of its commitment to renewable energy. These incentives guarantee payments for the electricity generated from biogas projects, enhancing the financial viability of biogas installations.
2. **Circular Economy Strategy:** Spain's Circular Economy Strategy emphasizes the use of organic waste as a resource. Biogas production aligns with this strategy by repurposing organic waste and contributing to waste reduction and resource efficiency.
3. **Waste-to-Energy Initiatives:** Spain encourages the utilization of organic waste for biogas production, contributing to waste-to-energy projects and reducing the environmental impact of waste disposal.
4. **Renewable Heat Incentives:** Spain provides incentives for biogas utilization in heating applications, supporting the use of biogas for space heating, industrial processes, and district heating systems.
5. **Research and Innovation:** Spain invests in research and development related to biogas production technologies, process improvements, and environmental performance.
6. **Regional Incentives:** Different regions within Spain may offer specific incentives, grants, and support for biogas projects based on their local energy and waste management policies.
7. **Decentralized Energy Generation:** Spain's energy policies encourage decentralized energy generation, which aligns with biogas production's potential for distributed energy supply.
8. **Biofertilizer Production:** Biogas production generates digestate, which can be used as a nutrient-rich biofertilizer in agriculture, reducing the need for synthetic fertilizers.
9. **Climate and Energy Goals:** Spain's broader climate and energy policies, including its commitment to renewable energy targets and emissions reduction, create a conducive environment for biogas projects.
10. **Knowledge Sharing and Networking:** Spain hosts conferences, seminars, and industry events to facilitate knowledge exchange, collaboration, and networking among biogas stakeholders.
11. **Regulatory Framework:** Spain has established a regulatory framework for biogas production, ensuring compliance with environmental, safety, and quality standards.
12. **Green Public Procurement:** Spain encourages the use of renewable energy sources, including biogas, in public procurement for energy consumption.
13. **Bioeconomy Development:** Biogas production contributes to Spain's bioeconomy goals by utilizing organic waste as a feedstock for energy and bio-based products.

These actions collectively demonstrate Spain's commitment to promoting biogas production as a renewable energy source, waste management solution, and contributor to its climate and sustainability objectives.

4.3.6 The Czech Republic

The Czech Republic has taken various measures to promote biogas production and utilization as part of its efforts to increase renewable energy generation, reduce greenhouse gas emissions, and enhance sustainable waste management. Some key actions and initiatives taken by the Czech Republic to promote biogas include:

1. **Feed-in Tariffs and Support Schemes:** The Czech Republic has implemented feed-in tariffs and support schemes to incentivize the production of renewable energy, including biogas. These mechanisms provide financial incentives for biogas producers and guarantee payments for the electricity generated.
2. **Renewable Energy Act:** The Czech Republic's Renewable Energy Act outlines the regulatory framework for renewable energy production, including biogas. It sets rules for grid connection, payments, and quality standards.
3. **Research and Innovation:** The Czech Republic invests in research and development related to biogas production technologies, process efficiency improvements, and waste utilization.
4. **Waste-to-Energy Initiatives:** The utilization of organic waste for biogas production aligns with the Czech Republic's waste-to-energy initiatives and waste management goals.
5. **Renewable Heat Incentives:** The Czech Republic offers incentives for biogas utilization in heating applications, supporting the use of biogas for space heating, industrial processes, and district heating systems.
6. **Decentralized Energy Generation:** The Czech Republic's energy policies encourage decentralized energy generation, which aligns with biogas production's potential for distributed energy supply.
7. **Regional Initiatives:** Different regions within the Czech Republic may offer specific incentives, grants, and support for biogas projects based on their local energy and waste management policies.
8. **Climate and Energy Goals:** The Czech Republic's broader climate and energy policies, including its commitment to renewable energy targets and emissions reduction, create a conducive environment for biogas projects.
9. **Circular Economy:** Biogas production contributes to the circular economy by converting organic waste into valuable energy and biofertilizers.
10. **Knowledge Sharing and Networking**: The Czech Republic hosts conferences, seminars, and industry events to facilitate knowledge exchange, collaboration, and networking among biogas stakeholders.
11. **Regulatory Framework:** The Czech Republic has established regulations and quality standards for biogas production and utilization, ensuring compliance with environmental, safety, and technical requirements.
12. **Green Public Procurement:** The Czech Republic encourages the use of renewable energy sources, including biogas, in public procurement for energy consumption.
13. **Agricultural Residues Utilization:** The utilization of agricultural residues and byproducts as feedstock for biogas production aligns with sustainable agricultural practices and waste reduction goals.

These actions collectively reflect the Czech Republic's commitment to promoting biogas production as a renewable energy source, waste management solution, and contributor to its climate and sustainability objectives.

4.3.7 Denmark

Denmark has taken various measures to promote biogas production and utilization as part of its efforts to increase renewable energy generation, reduce greenhouse gas emissions, and advance sustainable waste management. Some key actions and initiatives taken by Denmark to promote biogas include:

1. **Feed-in Tariffs and Premiums:** Denmark offers feed-in tariffs and premium payments to biogas producers, providing financial incentives for the production of renewable energy from biogas.
2. **Renewable Energy Support:** Denmark's support mechanisms for renewable energy include grants, subsidies, and financial support for biogas projects, enhancing their economic viability.
3. **Renewable Energy Expansion Plans:** Denmark has set ambitious goals for renewable energy expansion, including biogas. These goals provide a framework for increased biogas production and utilization.
4. **Biogas Upgrading and Injection:** Denmark promotes the upgrading of biogas to biomethane quality for injection into the natural gas grid. This enhances the utilization of biogas beyond electricity generation.
5. **Waste-to-Energy Initiatives:** Denmark encourages the use of organic waste for biogas production, contributing to waste-to-energy projects and reducing the environmental impact of waste disposal.
6. **Green Public Procurement:** Denmark encourages the use of renewable energy sources, including biogas, in public procurement for energy consumption.
7. **Climate and Energy Goals:** Denmark's broader climate and energy policies, including its commitment to renewable energy targets and emissions reduction, create a supportive environment for biogas projects.
8. **Circular Economy Approach:** Denmark's circular economy initiatives align with biogas production, as it repurposes organic waste and contributes to waste reduction and resource efficiency.
9. **Research and Innovation:** Denmark invests in research and development related to biogas production technologies, efficiency improvements, and environmental performance.
10. **Agricultural Residues Utilization:** Denmark promotes the use of agricultural residues and byproducts as feedstock for biogas production, aligning with sustainable agricultural practices and waste reduction goals.
11. **Decentralized Energy Generation:** Denmark's energy policies encourage decentralized energy generation, which aligns with biogas production's potential for distributed energy supply.
12. **Knowledge Sharing and Networking:** Denmark hosts conferences, seminars, and industry events to facilitate knowledge exchange, collaboration, and networking among biogas stakeholders.
13. **Regional Initiatives:** Different regions within Denmark may offer specific incentives, grants, and support for biogas projects based on their local energy and waste management policies.
14. **Regulatory Framework:** Denmark has established regulations and quality standards for biogas production and utilization, ensuring compliance with environmental, safety, and technical requirements.

These actions collectively demonstrate Denmark's commitment to promoting biogas production as a renewable energy source, waste management solution, and contributor to its climate and sustainability objectives.

4.3.8 The Netherlands

The Netherlands has implemented several measures to promote biogas production and utilization as part of its efforts to increase renewable energy generation, reduce greenhouse gas emissions, and enhance sustainable waste management. Some key actions

and initiatives taken by the Netherlands to promote biogas include:

1. **Feed-in Tariffs and Subsidies:** The Netherlands provides feed-in tariffs and subsidies for biogas producers, creating financial incentives for the production of renewable energy from biogas.
2. **SDE+ Scheme:** The Stimulating Sustainable Energy Production (SDE+) scheme offers financial support for various renewable energy projects, including biogas. This scheme encourages the development of cost-effective and sustainable energy projects.
3. **Biogas Upgrading and Injection:** The Netherlands promotes the upgrading of biogas to bio-methane quality for injection into the natural gas grid. This enhances the utilization of biogas beyond electricity generation.
4. **Waste-to-Energy Initiatives:** The Netherlands encourages the use of organic waste for biogas production, contributing to waste-to-energy projects and reducing the environmental impact of waste disposal.
5. **Green Public Procurement:** The Netherlands encourages the use of renewable energy sources, including biogas, in public procurement for energy consumption.
6. **Climate and Energy Goals:** The Netherlands' broader climate and energy policies, including its commitment to renewable energy targets and emissions reduction, create a supportive environment for biogas projects.
7. **Circular Economy Approach:** Biogas production aligns with the Netherlands' circular economy initiatives by repurposing organic waste and contributing to waste reduction and resource efficiency.
8. **Research and Innovation:** The Netherlands invests in research and development related to biogas production technologies, efficiency improvements, and environmental performance.
9. **Decentralized Energy Generation:** The Netherlands' energy policies encourage decentralized energy generation, which aligns with biogas production's potential for distributed energy supply.
10. **Knowledge Sharing and Networking:** The Netherlands hosts conferences, seminars, and industry events to facilitate knowledge exchange, collaboration, and networking among biogas stakeholders.
11. **Regional Initiatives:** Different regions within the Netherlands may offer specific incentives, grants, and support for biogas projects based on their local energy and waste management policies.
12. **Regulatory Framework:** The Netherlands has established regulations and quality standards for biogas production and utilization, ensuring compliance with environmental, safety, and technical requirements.

These actions collectively demonstrate the Netherlands' commitment to promoting biogas production as a renewable energy source, waste management solution, and contributor to its climate and sustainability objectives.

4.3.9 Poland

Poland has taken various measures to promote biogas production and utilization as part of its efforts to increase renewable energy generation, reduce greenhouse gas emissions, and enhance sustainable waste management. Some key actions and initiatives taken by Poland to promote biogas include:

1. **Feed-in Tariffs and Subsidies:** Poland provides feed-in tariffs and subsidies for biogas producers, offering financial incentives for the production of renewable energy from biogas.
2. **Renewable Energy Support:** Poland's support mechanisms for renewable energy include grants, subsidies, and financial incentives for biogas projects, enhancing their economic viability.

3. **Green Certificates:** Poland has implemented a green certificate system that allows biogas producers to trade certificates representing the renewable attributes of their energy production.
4. **Waste-to-Energy Initiatives:** Poland encourages the use of organic waste for biogas production, contributing to waste-to-energy projects and reducing the environmental impact of waste disposal.
5. **Circular Economy Initiatives:** Biogas production aligns with Poland's circular economy initiatives by repurposing organic waste and contributing to waste reduction and resource efficiency.
6. **Climate and Energy Goals:** Poland's broader climate and energy policies, including its commitment to renewable energy targets and emissions reduction, create a supportive environment for biogas projects.
7. **Renewable Heat Incentives:** Poland provides incentives for biogas utilization in heating applications, supporting the use of biogas for space heating, industrial processes, and district heating systems.
8. **Research and Innovation:** Poland invests in research and development related to biogas production technologies, efficiency improvements, and environmental performance.
9. **Decentralized Energy Generation:** Poland's energy policies encourage decentralized energy generation, which aligns with biogas production's potential for distributed energy supply.
10. **Knowledge Sharing and Networking:** Poland hosts conferences, seminars, and industry events to facilitate knowledge exchange, collaboration, and networking among biogas stakeholders.
11. **Regional Initiatives:** Different regions within Poland may offer specific incentives, grants, and support for biogas projects based on their local energy and waste management policies.
12. **Regulatory Framework:** Poland has established regulations and quality standards for biogas production and utilization, ensuring compliance with environmental, safety, and technical requirements.
13. **Biofertilizer Production:** Biogas production generates digestate, which can be used as a nutrient-rich biofertilizer in agriculture, reducing the need for synthetic fertilizers.

These actions collectively demonstrate Poland's commitment to promoting biogas production as a renewable energy source, waste management solution, and contributor to its climate and sustainability objectives.

4.3.10 Belgium

Belgium has implemented various measures to promote biogas production and utilization as part of its efforts to increase renewable energy generation, reduce greenhouse gas emissions, and enhance sustainable waste management. Some key actions and initiatives taken by Belgium to promote biogas include:

1. **Renewable Energy Support Mechanisms:** Belgium offers feed-in tariffs, subsidies, and renewable energy certificates to incentivize biogas producers, providing financial support for the production of renewable energy from biogas.
2. **Green Certificates:** Belgium's green certificate system allows biogas producers to trade certificates representing the renewable attributes of their energy production.
3. **Waste-to-Energy Initiatives:** Belgium encourages the use of organic waste for biogas production, contributing to waste-to-energy projects and reducing the environmental impact of waste disposal.
4. **Climate and Energy Goals:** Belgium's broader climate and energy policies, including its commitment to renewable energy targets and emissions reduction, create a supportive environ-

ment for biogas projects.

5. **Renewable Heat Incentives:** Belgium provides incentives for biogas utilization in heating applications, supporting the use of biogas for space heating, industrial processes, and district heating systems.
6. **Circular Economy Initiatives:** Biogas production aligns with Belgium's circular economy initiatives by repurposing organic waste and contributing to waste reduction and resource efficiency.
7. **Research and Innovation:** Belgium invests in research and development related to biogas production technologies, efficiency improvements, and environmental performance.
8. **Decentralized Energy Generation:** Belgium's energy policies encourage decentralized energy generation, which aligns with biogas production's potential for distributed energy supply.
9. **Knowledge Sharing and Networking:** Belgium hosts conferences, seminars, and industry events to facilitate knowledge exchange, collaboration, and networking among biogas stakeholders.
10. **Regional Initiatives:** Different regions within Belgium may offer specific incentives, grants, and support for biogas projects based on their local energy and waste management policies.
11. **Regulatory Framework:** Belgium has established regulations and quality standards for biogas production and utilization, ensuring compliance with environmental, safety, and technical requirements.
12. **Biofertilizer Production:** Biogas production generates digestate, which can be used as a nutrient-rich biofertilizer in agriculture, reducing the need for synthetic fertilizers.

These actions collectively demonstrate Belgium's commitment to promoting biogas production as a renewable energy source, waste management solution, and contributor to its climate and sustainability objectives.

4.3.11 Sweden

Sweden has implemented various measures to promote biogas production and utilization as part of its efforts to increase renewable energy generation, reduce greenhouse gas emissions, and advance sustainable waste management. Some key actions and initiatives taken by Sweden to promote biogas include:

1. **Biogas in Transport:** Sweden has been a pioneer in promoting biogas as a transportation fuel. It offers incentives for the use of biogas in vehicles, including tax exemptions and reduced fuel taxes.
2. **Renewable Energy Support:** Sweden provides feed-in tariffs, subsidies, and grants for biogas producers to encourage the production of renewable energy from biogas.
3. **Waste-to-Energy Initiatives:** Sweden encourages the utilization of organic waste for biogas production, contributing to waste-to-energy projects and reducing the environmental impact of waste disposal.
4. **Biogas Upgrading and Injection:** Sweden promotes the upgrading of biogas to biomethane quality for injection into the natural gas grid. This enhances the utilization of biogas beyond electricity generation.
5. **Circular Economy Initiatives:** Biogas production aligns with Sweden's circular economy initiatives by repurposing organic waste and contributing to waste reduction and resource efficiency.
6. **Climate and Energy Goals:** Sweden's broader climate and energy policies, including its commitment to renewable energy targets and emissions reduction, create a supportive environment for biogas projects.
7. **Renewable Heat Incentives:** Sweden provides incentives for biogas utilization in heating applications, supporting the use of biogas for space heat-

ing, industrial processes, and district heating systems.

8. **Research and Innovation:** Sweden invests in research and development related to biogas production technologies, efficiency improvements, and environmental performance.
9. **Decentralized Energy Generation:** Sweden's energy policies encourage decentralized energy generation, which aligns with biogas production's potential for distributed energy supply.
10. **Knowledge Sharing and Networking:** Sweden hosts conferences, seminars, and industry events to facilitate knowledge exchange, collaboration, and networking among biogas stakeholders.
11. **Regional Initiatives:** Different regions within Sweden may offer specific incentives, grants, and support for biogas projects based on their local energy and waste management policies.
12. **Regulatory Framework:** Sweden has established regulations and quality standards for biogas production and utilization, ensuring compliance with environmental, safety, and technical requirements.
13. **Biofertilizer Production:** Biogas production generates digestate, which can be used as a nutrient-rich biofertilizer in agriculture, reducing the need for synthetic fertilizers.

These actions collectively demonstrate Sweden's commitment to promoting biogas production as a renewable energy source, waste management solution, and contributor to its climate and sustainability objectives.

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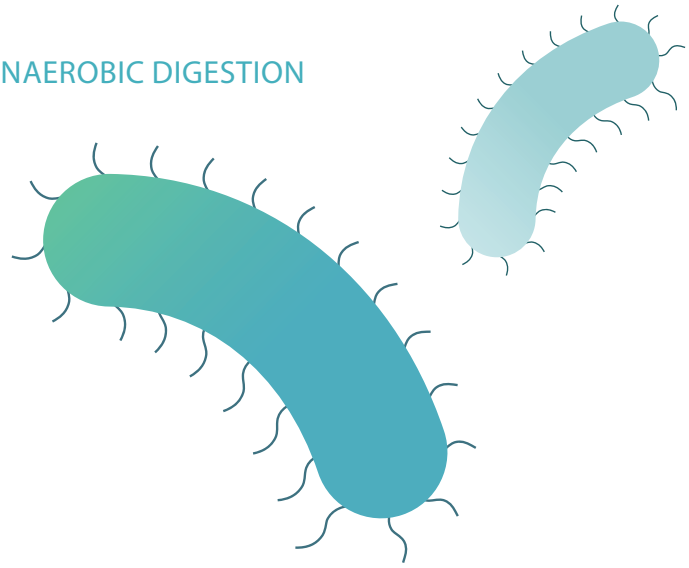

05

Microorganisms involved in anaerobic digestion

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Abstract:

Microbial ecosystems, generally referred to as microbiomes, have become a topic of interest in recent years due to their applications in a variety of fields such as agriculture, biotechnology, and particularly human medicine. Microbiome research is often necessary for understanding and improving the processes of larger biological systems **(Berg *et al.*, 2020)**. One such system that deeply relies on the microbiome is the production of biogas through Anaerobic Digestion (AD). Microorganisms, present in high-abundances, are the true workhorses (or heroes!) of the process being responsible for all the conversions taking place inside a digester. A surprising fact is that only one gram of digestate on average contains as many bacteria and archaea as there are humans inhabiting planet earth.

AD is a multi-step process in which organic materials are converted through fermentation and anaerobic respiration into methane and carbon dioxide as main end products. In this degradation process multiple groups of microorganisms from all three domains of life are

involved: archaea, bacteria and eukarya (see **figure 63**). In a digester, bacteria are responsible for hydrolyzing and fermenting carbohydrates, proteins and lipids yielding hydrogen, carbon dioxide and acetic acid as main end products. The methanogenic archaea, one of the oldest life forms on earth, are afterwards responsible for producing methane out of hydro-gen and carbon dioxide as well as converting acetic acid into methane and carbon dioxide. Eukaryotes, such as anaerobic fungi and other eukaryotes informally classified as protozoa, are higher organisms which have potential importance in the anaerobic digestion process, but are currently an overlooked group of microorganisms.

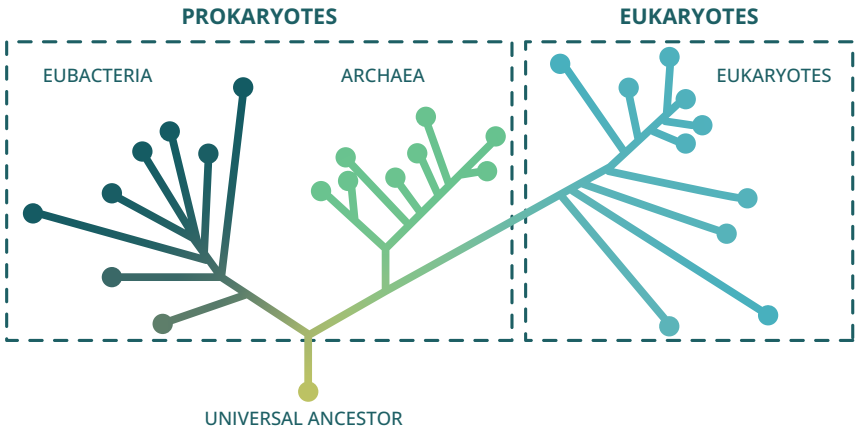
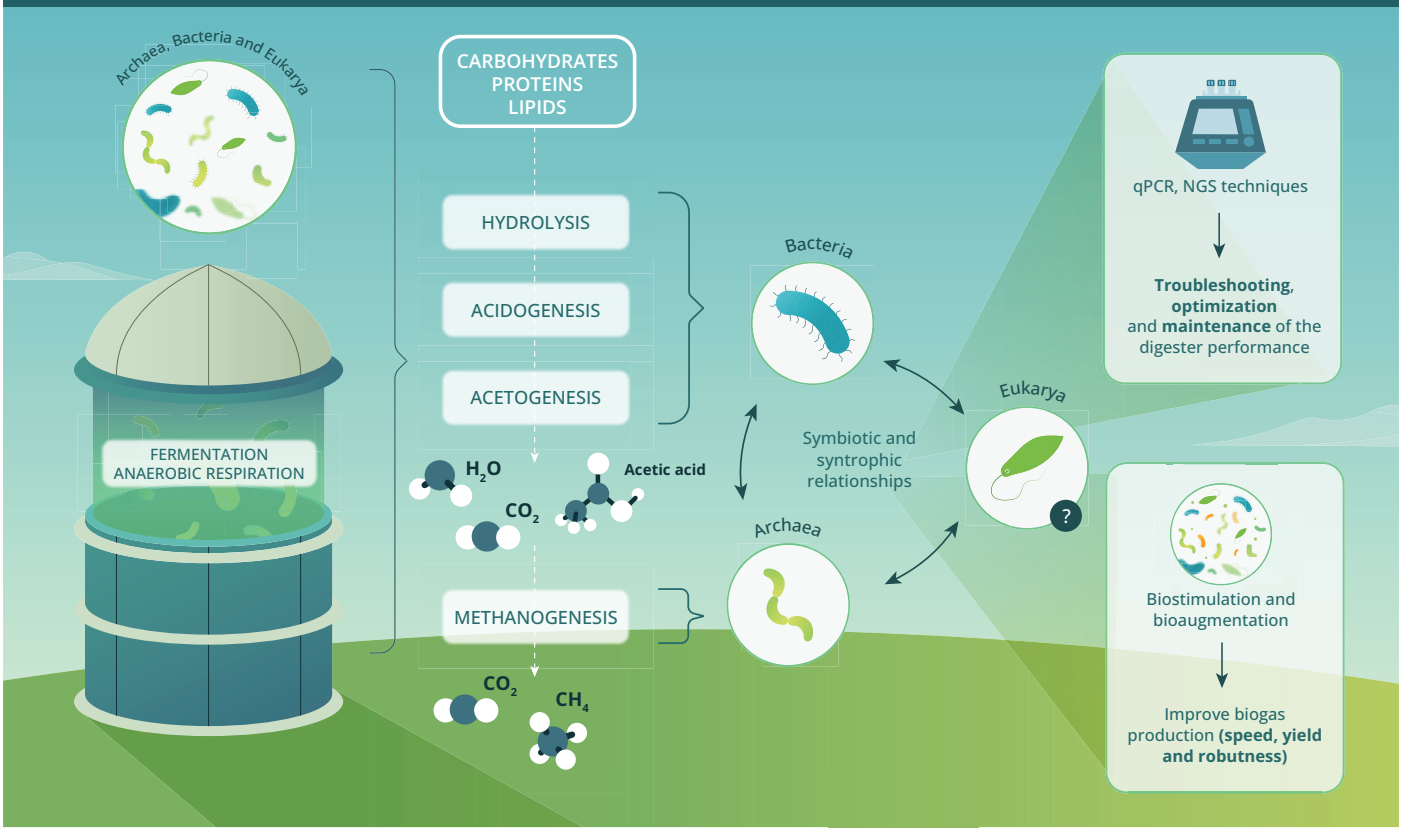


Figure 63: The three domains of life: archaea, bacteria and eukarya (Source: The Three Domains).

AD is a robust and elegant process. As in the anaerobic digester environment energy yields are low, microorganisms are collaborating through symbiotic and syntrophic relationships that are based on the principles of thermodynamics. The waste product of one group of microorganisms, intermediate products will not accumulate. Furthermore, the gaseous end-products of the process cannot accumulate as well as they are bubbling out of the reactor. Hence, in any successful anaerobic digestion process, the degradation process will automatically continue until all degradable substrates are converted.

This chapter firstly describes the various steps and conditions of AD processes (*paragraph 5.2*). Along the way important concepts such as symbiosis, syntrophy and direct interspecies electron transfer are explained in detail. The following paragraphs go in depth on the role of microorganisms from the three different domains of life that are involved in AD (*paragraph 5.3* on bacteria, *5.4* on archaea and *5.5* on eukaryotes). Throughout these paragraphs specific techniques such as culture-independent analysis and bioaugmentation are discussed.

Anaerobic Digestion is a complex biological process in which microorganisms from multiple domains of life work closely together to produce green methane out of residual biomass substrates.



5.1 Anaerobic Digestion

AD occurs through a series of biochemical reaction steps which are performed or mediated by a variety of microbes (Nie *et al.*, 2021). The microbial interactions and relationships within each of these steps are critical for the productivity and stability of AD. It is thought that a greater number of trophic relationships brings more stability in an ecosystem such as AD that is subjected to disturbances (Johnson *et al.*, 1996; Rooney & McCann, 2012).

Steps in AD

The microbial processes of AD can be described by the sequential steps of hydrolysis, acidogenesis, acetogenesis, and methanogenesis (**figure 64**). Each of these steps is accomplished by a group of microorganisms, and it is critical to maintain a balanced reaction rate among the steps or guilds to ensure rapid and stable digestion.

Bacteria are solely responsible for the first 3 sequential degradation steps where organic matter is broken down by specialized bacteria into smaller compounds (Kouzuma et al., 2015, Carballa et al., 2015).

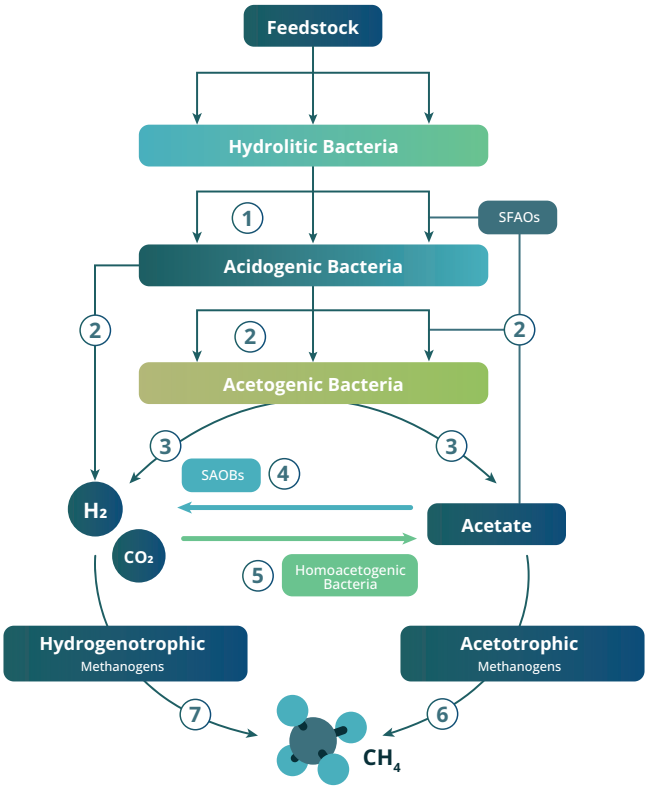


Figure 64: Flow chart showing the main stages and pathways involved in the anaerobic digestion of organic waste (compiled by Bioclear earth b.v.)

Hydrolysis involves the breakdown of polymeric substrates, such as polysaccharides into sugars, lipids into glycerol and long chain fatty acids and proteins into amino acids as end products. This is done by using extracellular enzymes produced by anaerobic hydrolytic bacteria. These enzymes generally include amylases, cellulases, lipases, pectinases, and proteases. Hydrolysis is the limiting step when digesting complex plant materials such as agricultural residues, manure and green wastes and long residence times are required. The extend of hydrolysis is limiting the final biogas yield. The speed of hydrolysis determines the biogas production rate and also determines the final biogas yield in combination with the Solid Residence Time in the system.

The next step in AD is acidogenesis, in which bacteria use the products of hydrolytic bacteria as electron acceptors to generate fermentation products such as formic acid, acetic acid, propionic acid, butyric acid, pentatonic acid, alcohols, CO_2, and H_2 **(Nguyen *et al.*, 2018)**. From the fermentation products, only acetate, formate, H_2, and CO_2, resulted from acidogenesis, can be directly utilized by methanogens for biogas production **(Lim *et al.*, 2020)**.

The third stage in AD is acetogenesis, where fermented products are used by acetogenic bacteria and transformed into acetate, CO_2 and H_2, especially fatty acids and alcohols. These bacteria are called acetogens, non-syntrophic acetogens and are a obligatory source of H_2 **(Hassan *et al.*, 2022)**. Furthermore two special groups of bacteria need to be mentioned here. Homoacetogenic bacteria can directly produce acetate out of CO_2 and H_2 and Syntrophic Acetate Oxidizing bacteria can convert acetate into CO_2 and H_2, yielding energy in combination with a hydrogenotrophic methanation step. These two additional pathways can offer flexibility in methane producing routes, thereby providing extra stability and robustness to the digestion process.

In the final step of AD, known as methanogenesis, specialized archaea produce methane as an end product **(De Vrieze & Verstraete, 2016)**. In AD methanogenesis is the rate limiting step for substrates such as sugars, glycerol and short chain fatty acids that are easily degradable and thus there is much interest in optimizing this particular metabolic conversion. High VFA concentrations are harmful to methanogens and other microbes in AD and inhibit their growth and function which in turn limits community stability and methane production **(Pan *et al.*, 2021)**. The substrate loading rate of a successful AD process is chosen in such a way that VFA's do not accumulate either directly (acetate) or indirectly (propionate, butyrate through elevated levels of hydrogen).

Methane can be produced via three different pathways, acetoclastic, hydrogenotrophic and methylotrophic, that differ in many aspects of their biochemistry and physiology.

Acetate is directly dismutated or fermented to methane and carbon dioxide by acetoclastic methanogens. Only two genera of methanogenic archaea, *Methanosarcina* and *Methanothrix* (former *Methanosaeta*), are able to grow with acetate as sole energy and carbon source. Considering that acetate is the main end product of the biomass degradation by bacteria, considering the low diversity of acetoclastic methanogens, further considering their high sensitivity towards their environment and their slow growth rate, acetoclastic methanation is often considered as the most vulnerable link in any digestion process. Syntrophic Acetate Oxidizing Bacteria in combination with hydrogenotrophic methanogens may have a stabilizing effect by providing an alternative pathway of converting acetate into methane.

The largest phylogenetic diversity is found within the hydrogenotrophic methanogens. They utilize hydrogen as an electron donor for the reduction of carbon dioxide to methane in six steps via the reductive acetyl-CoA or Wood–Ljungdahl pathway **(Berghuis *et al.*, 2019)**. Hydrogenotrophic methanogens keep the hydrogen levels in the digester and in the biogas low. This is critical for the success of the whole AD process. In the case hydrogen accumulates in the system, the fermentation steps in which hydrogen is produced are affected. Typically the conversion of butyrate and propionate to acetate will be thermodynamically blocked at elevated hydrogen partial pressures. This leads to accumulation of these VFA's constraining the growth of methanogenic archaea **(Braga Nan *et al.*, 2020)**.

Methylotrophic methanogens exhibit the ability to thrive on methylated compounds like methanol or methylamines by dismutation. Typically, this reaction involves four C1 compounds: one in which the methyl group is oxidized to CO_2, while the remaining three methyl groups are reduced to methane by the methyl-coenzyme M reductase **(Berghuis *et al.*, 2019)**. This pathway contributes to a very small amount of methane accumulation comparing with the hydrogenotrophic and acetoclastic methanogenic pathways **(Conrad, 2005)**.

A more detailed and in-depth description of the main microorganisms involved in the different stages within anaerobic digestion is described in *chapter 5.3* on bacteria (Hydrolysis, Acidogenesis and Acetogenesis) and *chapter 5.4* on archaea (Methanogenesis).

Physicochemical conditions

The physicochemical conditions within a digester create a unique and often harsh environment, posing challenges for bacteria to thrive. The microbial taxa involved in each degradation phase can also have different environmental requirements (i.e. temperature, pH, oxygen) which must be balanced to allow for the coexistence of essential bacteria and archaea in the same system **(Boe *et al.*, 2010)**. Some differences in environmental requirements can be balanced by the bacteria in earlier degradation steps. For instance, some bacteria are able to consume oxygen before it can harm the archaea or other obligate anaerobes in the community **(Botheju & Bakke, 2011; Magdalena *et al.*, 2022)**. This function of bacteria improves overall degradation by allowing archaea to remain in the ecosystem. Another factor in-

fluencing digester performance is the hydrogen (H_2) partial pressure. An increase in H_2 partial pressure can disturb the metabolic equilibrium within a digester, and thereby, affecting certain microbial functions **(Braga Nan *et al.*, 2020)**.

Both temperature maintenance and fluctuations in temperature are particularly important physical conditions affecting AD bacteria **(Kim *et al.*, 2018; Nie *et al.*, 2021; Theuerl *et al.*, 2019)**. Thermophilic temperatures are generally considered to be more efficient for biogas production and are known to speed up certain metabolic reactions **(Levén *et al.*, 2007)**. For example, higher fermentation temperature can improve enzymatic activity and thus improves the hydrolysis rate, a critical bottleneck in AD **(Khan *et al.*, 2022; Nie *et al.*, 2021)**. However, thermophilic tem-peratures can also have strong negative effects on the growth and survival of certain bacteria which has consequences for the overall system stability. For example, high temperatures can contribute to an increase in levels of free ammonia which can become toxic to bacteria in large quantities **(Song *et al.*, 2004)**. Although the rate of chemical reactions in general increases with temperature, the critical functions (i.e. growth and survival) of the bacteria facilitating these chemical reactions may be restricted to certain optimal temperatures. Extreme temperatures conditions can also limit bacterial diversity which may lead to greater sensitivity of communities to temperature changes in AD (**Podar *et al.*, 2020)**.

Most of the control in AD is undertaken directly by the microorganisms themselves; however, factors affecting the performances of an anaerobic digester can be divided in three main classes: **(i)** feedstock characteristics; **(ii)** reactor design; and **(iii)** operational conditions **(Cioabla *et al.*, 2012)**. Given several advantages, the processing of bioenergy from waste via AD has many severe challenges. Therefore, understanding of the fundamental biological and physicochemical processes in AD is required to improve the technology.

External disturbances affecting bacteria processes include the rate of operation, retention time of digestate, and mixing. Additionally, the given reactor type and substrate used has been shown to have a strong link to taxonomic composition **(Abendroth *et al.*, 2015)**. Some system variables can in theory be manually changed at the digester level to prevent a problem from arising. However, other disturbances (e.g., increased salt concentration, acid accumulation, and elevated ammonia) can also occur through microbial community interactions and can be much more challenging to correct. Most notably, the fermentation that occurs in anaerobic digesters can result in the accumulation of VFAs which can be toxic **(Saha *et al.*, 2020)**.

The most common disorders of the biogas process are over-acidification **(Kleyböcker *et al.*, 2012)**. This problem implies a decline in digestate pH due to the accumulation of fatty acids, reflecting a kinetics imbalance between acid production and consumption rate and leading to a breakdown of the reactor buffering capacity **(Appels *et al.*, 2011)**. Consequently, archaeal communities in AD reactors facing acidification are quickly inhibited, leading to a decreased methane production **(Weiland, 2010)**. Excessive acidification could be avoided by optimising the C/N ratio using a co-digestion system or through the addition of trace elements **(Agyeman and Tao, 2014; Kong *et al.*, 2016)**. **Cerón-Vivas *et al.* (2019)** accessed the influence of pH and C/N ratio on methane yield of wastewater and observed a maximal biochemical methane production of 318 L CH_4/kgVSS with pH 7.5 and C/N ratio of 8.2±0.18. **Alavi-Borazjani *et al.* (2020)** have summarized the most common strategies used to control excessive acidification in AD within four ma-jor categories: **(i)** the use of additives, **(ii)** optimisation of the key parameters affecting AD, as for example decrease the organic loading rate **(iii)** modification of the process configuration, and **(iv)** co-digestion.

More detail on the physicochemical factors in Anaerobic Digestion is given in *chapter 6*, in which also the impact of elevated ammonia levels is discussed.

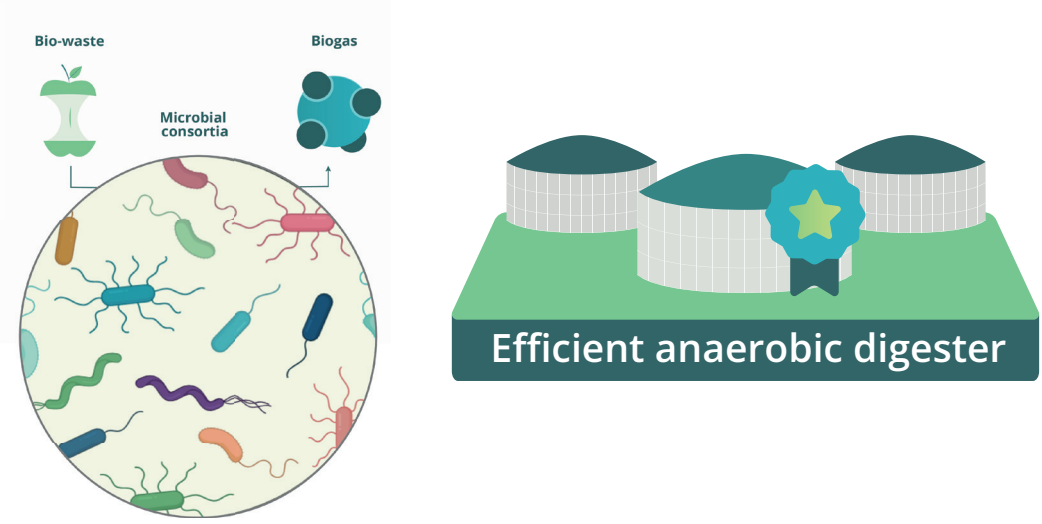


Figure 65: Microbiome analysis in Anaerobic Digestion will enable biogas producers to improve monitoring, troubleshooting and / or optimization of their systems (compiled by Bioclear earth b.v. & Scienseed).

Generating insight in the AD microbial ecosystem

Microbial ecosystems, generally referred to as microbiomes, have become a topic of interest in recent years due to their applications in a variety of fields such as agriculture, biotechnology, and particularly human medicine. Microbiome research is often necessary for understanding and improving the processes of larger biological systems **(Berg *et al.*, 2020)**. One such system that deeply relies on the microbiome is biogas production in AD's.

To better understand, control and optimize AD systems, it is apart from the physicochemical parameters also needed to better understand the composition of the microbial community, the abundances and diversity of the different taxa, the functionality of species, the interactions between microorganisms and the dynamics of these systems in relation to the physicochemical conditions and throughout time. Generating deep insight in the AD microbiome is therefore a key step that has been applied and optimized in EU MICRO4BIOGAS.

With the application of molecular biology-based approaches in microbial ecology, it has become increasingly evident that the diversity of microbial life in natural ecosystems far exceeds that which has been revealed by cultivation-based studies. Culture-independent methods rely on molecular methods to study microbes within their diverse environments. These approaches can give a better insight into the microbial community and its dynamics. Traditional cultivation techniques for the enrichment and isolation of microbes yield only a limited fraction, usually <1%, of all microorganisms present in the biosphere. Therefore, molecular tools give an overview about the full microbial diversity and their relative abundance in a specific ecological niche.

Polymerase chain reaction (PCR) amplification provides a fast and sensitive alternative to conventional culture techniques to identify and, in some cases, quantify microorganisms. This technique is based on the amplification of specific phylogenetic or functional marker regions of the DNA sequences that can be then sequenced to determine the microbial community composition. The small subunit rRNA genes (i.e., 16S rRNA) are regularly employed as phylogenetic marker to describe microbial diversity without the need of cultivation **(Dunbar *et al.*, 1999)**. The structure of the 16S-rRNA genes is defined by an alternation of highly-conserved and hypervariable regions making the 16S sequence an ideal proxy to achieve a trustworthy level of taxo-

nomic information. The conserved regions serve as way for PCR primers to adhere to the sequence while sequencing the variable regions identifies the microorganism. Therefore, the sequences can be used to infer taxonomic identifications based upon bioinformatics alignments against sequence databases (Caporaso *et al.*, 2011).

In the last decade, the next-generation sequencing (NGS) technologies have impressively accelerated research by enabling the production of large volumes of sequence data at drastically lower price per base, compared to the traditional Sanger sequencing method. Different NGS platforms have been developed, producing a massive amount of sequencing data. Of all the sequencing platforms available, Illumina is the most widely used. It is characterised by genera-tion of a large number of highly accurate reads (>99.5% accuracy). However, Illumina generates short reads (~75-300 pb), resulting in lower taxonomic resolution in some cases. Long-read se-quencing technologies (e.g., Pacific Biosciences and Oxford Nanopore Technologies) can overcome this limitation by being able to read DNA fragments longer than 1 Kbp. However, this comes at the cost of higher error rates, which continues to hinder wider adoption of these technologies (Latorre-Pérez *et al.*, 2022).

Several studies focused on the AD microbiome have recently been carried out as part of the EU MICRO4BIOGAS project and the microbiomes of many large-scale digesters are currently being reviewed. These results will not only expand our understanding of the AD microbiome but will also provide an improved database for further AD microbiome analysis.

Apart from the scientific aspects, it is envisioned by Bioclear earth that due to the increasing knowledge of the AD microbiome and the decreasing cost of AD microbiome analysis this will become an increasingly important tool for biogas producers to generate insight in their digester microbiology. With these insight process operators can start troubleshoot or optimize their digester systems through techniques such as inoculation, transplantation, biostimulation and bioaugmentation while monitoring the development of the microbial community in their sys-tem relation to the process performance over time (**figure 65**).

5.2 Bacteria

Bacteria are found globally in diverse environments and play many important roles in the func-tionality, stability, and productivity of different biological systems. Individual bacteria exist together along with other microbes in microbial communities made up of the interactions and relationships between individuals (Kato & Watanabe, 2010). Bacteria are known to support each other's growth, compete for resources, and even communicate with other community members (Shimoyama *et al.*, 2009). Microbial communities are further influenced by environmental factors that fluctuate over time (Berg *et al.*, 2020). Interactive dynamics along with external con-ditions promote the formation of a complex microbial ecosystem (Carballa *et al.*, 2015).

Role of bacteria in the various stages of AD

Distinct biochemical stages occur in AD, causing the degradation of organic matter in a series of steps (Saha *et al.*, 2020). Bacteria in AD form trophic relationships at different levels of degradation that feed into each subsequent step (Kleinsteuber, 2018). Here we address the roles of bacteria in the three key stages of AD: hydrolysis, acidogenesis and acetogenesis. As the stages already have been described in *5.2*, this chapter goes in depth on the bacteria involved.

Hydrolytic Bacteria: In the first stage of AD, primary fermentative bacteria convert complex biopolymers into soluble monomers and oligomers (Kleinsteuber, 2018). These bacteria release various enzymes which biochemically decompose organic material into smaller components that can be further degraded by other bacteria (Tabatabaei *et al.*, 2020). Hydrolytic bacteria are phylogenetically diverse; however, two phyla, namely, *Bacteroidetes* and *Bacillota* (previously *Firmicutes*), are the

two most dominant phyla in AD. The abundance of hydrolytic bacteria in AD depends on the type of inoculum, operating temperature, cell retention time and substrate characteristics (Nguyen *et al.*, 2018).

Hydrolytic bacteria in general are fast-growing and can utilize a range of substrates. This stage is relatively quick for simple polymers, but it can be more difficult for bacteria to degrade more complex substrates. For instance, plant material is often used as organic substrate for biogas production. Crop stover, forestry residues, food residues and wood scraps are all examples of plant-based biomass. These substrates all contain lignocellulose, a main component of the plant cell wall, which is very difficult to degrade (Lim *et al.*, 2020). Lignocellulose is comprised of three subcomponents: cellulose, hemicellulose, and lignin (Khan *et al.*, 2022). Cellulose can have different degrees of crystalline structure which vary its degradability. The higher the crystallinity index of cellulose, the more difficult it is to digest. Hemicellulose is considered the easiest to degrade compared to its fellow biopolymers, but it also contributes to the overall strength of lignocellulose by forming covalent bonds with lignin. Lignin in particular is considered highly difficult to degrade anaerobically due to its complex structure and additional bond to hemicellulose. The structural complexities of lignocellulosic materials limit the complete AD of plant-based matter and result in a lower methane yield (Khan *et al.*, 2022). As a result, hydrolysis is often considered a rate-limiting step of AD. Few bacterial species have been shown to break down lignin anaerobically, *Clostridium* being the most notable genus that has displayed this ability (Chukwuma *et al.*, 2021; Thomas *et al.*, 2014; Wang *et al.*, 2011). Notably, *Clostridium* is able to form specialized enzymatic structures called 'cellulosomes' which improve degradation of complex structures during hydrolysis (Tomazetto *et al.*, 2020). Bacteria that form cellulosomes are thought to be particularly important in AD due to the enhancement of lignocellulosic material breakdown which provides more resources in the later stages of AD (Chukwuma *et al.*, 2021). Lignocellulolytic enzyme activity also appears to have a correlation with higher temperatures. This was notably observed in the thermophilic Paenibacillus and Bacillus, which display a high amount of cellulase and lignin-modifying enzymes compared to mesophilic genera (Hemati *et al.*, 2018). Although lignin degradation by anaerobic bacteria has been reported, the exact mechanism for this process is not yet fully understood. Further investigation is necessary to improve the efficiency of this bottleneck (Tomazetto *et al.*, 2020).

Acidogenic Bacteria: In the second stage of AD, the simplified products of hydrolysis are fermented by acidogenic bacteria into hydrogen, carbon dioxide, alcohols, and fatty acids. The products resulting from acidogenesis that cannot be directly used by methanogens continue to the next digestion step; acetogenesis.

The acidogenic stage is usually the fastest step of AD and is not thought to be rate-limiting (Saha *et al.*, 2020). Acidogenic bacteria primarily belong to the phylum *Firmicutes*, and some genera identified in this stage include *Clostridium*, *Bacillus*, *Bacteroides*, *Enterobacteriaceae*, *Hydrogenoanaerobacterium*, *Paraclostridium*, *Anaerosalibacter*, *Tissierella*, and *Tepidanaerobacter* (Chang *et al.*, 2018; Nguyen *et al.*, 2019; Salama *et al.*, 2019). In general, there are fewer taxo-nomic studies that have focused on this stage, possibly because acidogenesis is rapid and not of concern for rate-limitation of methane production.

Fatty acids are converted into acetate and hydrogen at this stage by syntrophic fatty acid oxidizing (SFAO) bacteria (Salama *et al.*, 2019). Known SFAO genera include *Clostridia*, *Syntrophomonas*, and *Syntrophobacter* whereby only SFAO bacteria have been cultured to date all belong to the family *Syntrophomonadaceae* (Salama *et al.*, 2019). The acetate and hydrogen produced by SFAO bacteria can be utilized directly by acetogenic and hydrogenotrophic methanogens, respectively. Importantly, syntrophy between hydrogenotrophic methanogens and SFAO bacteria is required to maintain low hydrogen partial pressure (Salama *et al.*, 2019). In general, in this step, different microbio-

logical steps must be balanced and synchronised, otherwise the process can experience disturbance and accumulation of degradation intermediates such as volatile fatty acids (VFA). This accumulation causes a drop in pH which inhibits or stops methanogenesis completely.

Acetogenic Bacteria: In the third phase of AD, acetogenic bacteria further convert alcohols and acids, the products from acidogenesis, into acetic acid, hydrogen, and carbon dioxide **(Salama *et al.*, 2019)**.

Acetogenic bacteria are a highly phylogenetically diverse group compared to the other phases of AD with approximately 100 homoacetogenic species identified and phylogenetically classified in 21 different genera **(Drake *et al.*, 2006)**. The genera *Clostridium* and *Acetobacterium* make up the majority of known acetogens **(Schuchmann & Müller, 2014)**. Three species in particular have been investigated in detail: *Moorella thermoacetica*, *Acetobacterium woodii* and *Clostridium ljungdahlii* **(Schuchmann & Müller, 2014)**. *M. thermoacetica* is a thermophilic species historically used as the model strain for acetogenesis. As a result, all of the enzymes for the Wood-Ljungdahl pathway have been isolated and characterized in this species. Notably, *M. thermoacetica* contains cytochromes – specialized proteins involved in electron transfer – and does not require sodium ions for growth. *A. woodii* and *C. ljungdahlii* are both mesophilic species that grow well on hydrogen and carbon dioxide. In contrast to *M. thermoacetica*, these two species do not have cytochromes. *A. woodii* is dependent on sodium ions for growth, while *C. ljungdahlii* does not require sodium ions **(Schuchmann & Müller, 2014)**. In addition to these three well-investigated species, there are likely to be a large number of other acetogenic taxa, especially due to the broad phylogenetic presence of the function **(Drake *et al.*, 2006)**.

Syntrophic acetogenesis is the process in which VFA's are further biotransformed to form acetate, H_2, and CO_2 **(Venkiteshwaran *et al.*, 2016)**. In AD one the most difficult step is the fermentative degradation of short-chain fatty acids such as propionate and butyrate. Conversion of these metabolites to acetate, CO_2, formate and hydrogen is endergonic under standard conditions and occurs only if methanogens keep the concentrations of these intermediate products low. This reaction is dependent on instant H_2 removal by hydrogenotrophic methanogens, with subsequent production of CH_4. The syntrophic oxidation of propionate and butyrate to acetate is therefore coupled with hydrogenotrophic methanation. **(Müller, Plugge *et al.*, 2010)**. The enhancement of syntrophic degradation of propionate is thought as an ap-proach to improving AD efficiency in high ammonia AD processes. Butyrate and propionate degradation pathways include oxidation steps of comparably high redox potential, i.e. oxidation of butyryl-CoA to crotonyl-CoA and of succinate to fumarate, respectively, that require investment of energy to release the electrons as hydrogen or formate. Although investigated for several decades, the biochemistry of these reactions is still not completely understood. Genome analysis of the butyrate-oxidizing *Syntrophomonas wolfei* and *Syntrophus aciditrophicus* and of the propionate-oxidizing *Syntrophobacter fumaroxidans* and *Pelotomaculum thermopropionicum* reveals the presence of energy-transforming protein complexes. **(Müller, Plugge *et al.*, 2010, Müller 2016)**.

Two important groups of microbes involved in acetogenesis are syntrophic acetate-oxidizing bacteria (SAOB) and homoacetogenic bacteria. SAOBs convert acetate into hydrogen and CO_2 while homoacetogens do the opposite. Both of these bacteria groups produce or convert acetate through the Wood-Ljungdahl pathway which is considered to be the first biochemical pathway to have existed on earth **(Müller & Frerichs, 2013)**. The homoacetogens are often outcompeted by hydrogenotrophic methanogens for H_2. Importantly, SAOBs form a syntrophic relationship with hydrogenotrophic methanogens that convert the hydrogen into methane **(Kleinsteuber, 2018)**. SAOBs are thought to be slow growers which causes the SAOB-hydrogenotroph syntrophy to be at a disadvantage with respect to acetotrophic methanogens **(Westerholm *et al.*, 2016)**. Although the chemical stoichiometry is the same for both acetotrophic methano-

gens and SAOB-hydrogenotrophic syntrophy, the energy produced by the syntrophic process needs to be shared by two microbes instead of one **(Westerholm *et al.*, 2016)**. However, the SAOB-hydrogenotroph syntrophy is thought to become a dominant pathway under system conditions that would inhibit acetotrophic methanogens **(Pan *et al.*, 2021)**. For example, this syntrophy becomes important when digesters are faced with high ammonia, high volatile fatty acid concentrations, or high temperature **(Pan *et al.*, 2021; Westerholm *et al.*, 2016)**. Thus, SAOBs appear to be important specialized bacteria which can help respond to fluctuating digester conditions. Known SAOB species include *Clostridium ultunense*, *Syntrophaceticus schinkii*, *Tepidanaerobacter acetatoxydans*, *Thermacetogenium phaeum*, and *Thermacetoga lettingae* (aka *Pseudothermacetoga lettingae*) **(Hattori, 2008; Westerholm *et al.*, 2018)**.

Recent studies, some of them carried out by the MICRO4BIOGAS consortium, have identified new potential syntrophic acetate oxidizing bacteria (SAOB), including MBA03 *(Darwinibacteriales)*, DTU014 and Dethiobacteraceae **(Otto *et al.*, 2023; Puchol *et al.*, 2023)**.

Collaboration between micro-organisms

Mutualism, the interaction between microorganisms from which each species benefits, is widespread in various microbial ecosystems and is also important for the function of AD communities **(Zhu *et al.*, 2020)**. In aerobic environments, oxygen acts as an electron acceptor which is energetically very favourable and lowers the need for syntrophic interactions **(Morris *et al.*, 2013)**. Thus, some reactions can become more energetically difficult anaerobically because oxygen cannot be used as a terminal electron acceptor and energy yields are low. This limitation may promote more diverse energetic pathways and more syntrophic relationships in anaerobic digesters **(Morris *et al.*, 2013)**.

Bacteria often engage in symbiosis, which are interspecies relationships that driving both individual resource acquisition and overall community function **(Mutungwazi *et al.*, 2021)**. In AD, hydrolytic and fermentative bacteria, syntrophic bacteria, and methanogenic archaea associate via intricate symbiosis to allow the volumetric reduction and conversion of organic waste to methane **(Kleinsteuber, 2018)**. **Saha *et al.* (2020)**, reviewed the microbial symbiosis during anaerobic biomethanation followed by alteration in microbial communities in AD due to the feeding of various types of organic waste, and the impact of microbial alterations on interspecies networks and biomethanation. In this review, the authors showed that all steps are followed by each other in a symbiotic work **(Saha *et al.*, 2020)**. Substrates (as sugars, glycerol, and amino acids) produced by hydrolytic microorganisms are used in acidogenesis by fermentative bacteria **(Saha *et al.*, 2020)**. Besides this metabolic cooperation providing trophic benefits, there exists a commensalistic symbiosis of hydrolytic microorganisms with some host eukaryotes that have anaerobic conditions for instance the rumen of ruminants **(Wrede *et al.* 2012)**.

The dependent relationship between methanogenic archaea and bacteria is a form of symbiosis called syntrophy, and is the fundamental basis of methanogenesis. **(Morris *et al.*, 2013; Saha *et al.*, 2020)**. In general terms, syntrophy refers to a dependent interaction between two or more organisms where one partner provides a metabolic product that the other partner utilizes. This syntrophic activity forms a metabolic and community dynamic that would not occur if each microbe was acting independently **(Morris *et al.*, 2013)**. Syntrophy is essential to produce of methane and is well-described in the context of AD **(Goux *et al.*, 2015; Kouzuma *et al.*, 2015)**. In AD, syntrophy usually refers to the associated relationship between organisms based upon H_2 transfer from one to the other, and a H_2-producing organism that relies on H_2-consuming organisms for its growth. **Kamagta (2015)**, for example, shows a list of different bacteria and reac-tions involved in the degradation of low-molecule organic matter, syntrophic associations and bacteria in the methanogenesis step. Sometimes this term could be mixed with symbiosis **(Johnravindar *et al.*, 2021)**.

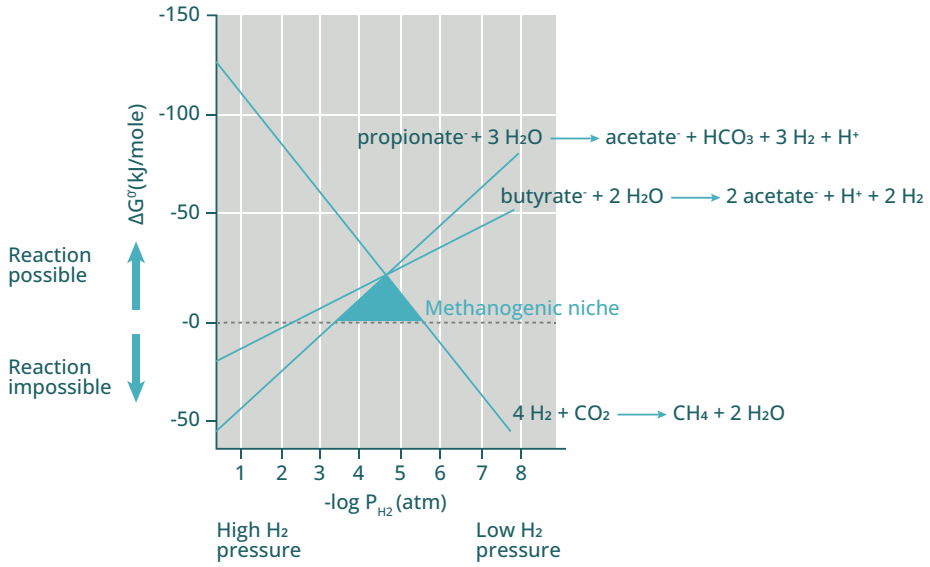


Figure 66: Free energy change as a function of the H_2 partial pressure. The operating window of Anaerobic Digestion is indicated by the blue triangle where the Gibbs free energy is negative, indicating spontaneous reaction is possible (Adapted from Van Lier *et al.,* 2008).

The products from acidogenic pathways (organic acids, short-chain fatty acids, alcohols, H_2, and CO_2) are used in different metabolic pathways **(Saha *et al.,* 2020)**. While the long-chain fatty acids are oxidized to acetate and hydrogen by acetogenic bacteria, the acids can be oxidized into acetate via syntrophic fatty acid oxidizers and syntrophic acetate oxidizers, that oxidize acetate into H_2 and CO_2 **(Saha *et al.,* 2020)**. Another symbiotic relationship during acidogenesis exists between lactic acid bacteria and butyrate-producing bacteria where the butyrate-producing bacteria form more butyrate from lactate and acetate, resulting in a higher hydrogen yield **(Detman *et al.,* 2019; Mutungwazi *et al.,* 2021)**. Due to the several associations present in the acidogenesis, it is visible that this is a product-determining step in AD processes where control and optimization strategies should be clarified.

Then, the conversion of the products from acidogenesis into acetate and H_2 by acetogenic bac-teria and obligate H_2-producing bacteria takes place in the acetogenesis step. The H_2 gas formed in the acetogenesis step can inhibit the action of acetogenic bacteria. However, the hydrogenotrophic bacteria in the methanogenesis step can consume and convert H_2 into methane. **Mutungwazi *et al.* (2021)** mentioned that there is a beneficial syntrophy between the acetogenic and hydrogenotrophic methanogens and that it is worthwhile to establish H_2 inhibition levels for different substrates through a profiling of the taxonomy and functional roles in AD. **Figure 66** shows the small operating window of AD based on thermodynamic analysis. Both the energy yields (y-axis) and the operating window of hydrogen pressure (x-axis) are relatively low. As a result, the resulting 'methanogenic niche', where all reactions can keep proceeding at the same time is relatively small **(Van Lier *et al.,* 2008)**.

Lastly, the methanogenesis step has 3 different routes. The survival of methanogens is largely dependent on the acetogens and acidogens as the conversion of simple monomers into VFAs, acetic acid, carbon dioxide and hydrogen depend on these two groups. The prerequisite substances (e.g. Hydrogen and acetate) for methanogenesis are produced by bacteria and create a syntrophic relationship between the methanogenic archaea and certain bacteria **(Kouzuma *et al.,* 2015)**.

Further, specialized groups of bacteria carry out compound conversions that can shift the

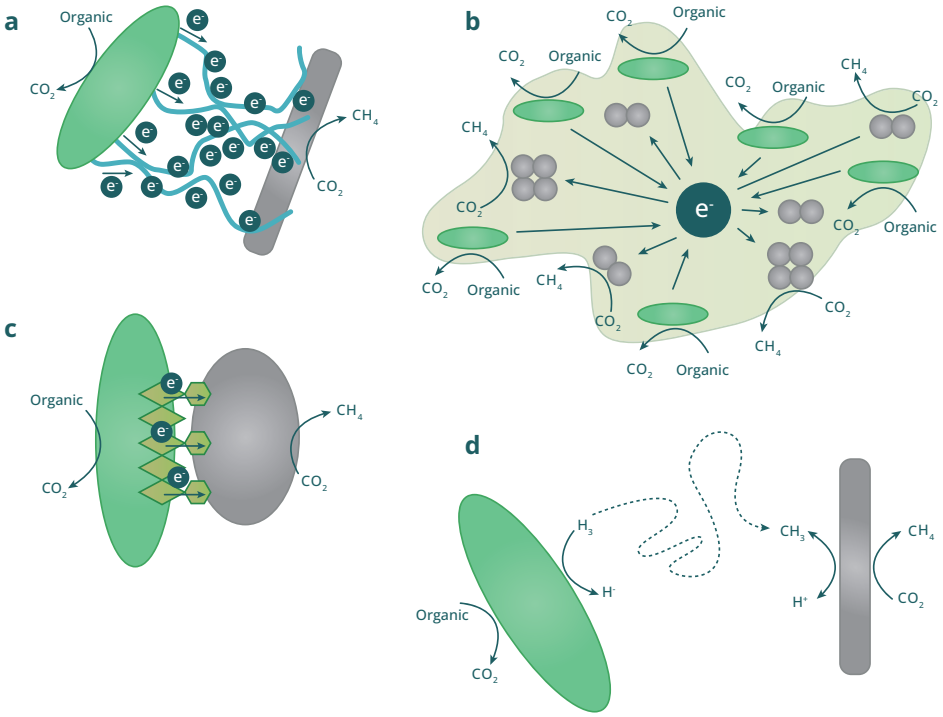


Figure 67: Potential mechanisms of DIET including (a) electron transfer through electrically conductive pili (in blue), (b) electron transfer through electrically conductive materials (in purple), (c) electron transfer between electron transport proteins and (d) comparison with MIET (d) in which diffusive transfer of H2 takes place (Adapted from Lovley *et al.,* 2017).

energetic favourability of methanogenesis pathways **(Kleinsteuber, 2018; Pan *et al.,* 2021)**. For example, syntrophic acetate oxidizing bacteria (SAOB) convert acetate into hydrogen while homoacetogenic bacteria convert the reverse. These bacteria produce essential substrates for both the production of methane through the two methanogenic pathways, and for the substrate conversions amongst themselves (such as hydrogen into acetate or acetate into hydrogen). In response to environmental changes or shifts in the microbial community, bacteria constantly adjust their resource utilization. Enhanced connectivity between different trophic levels provides more avenues for adaptability. **(Blair *et al.,* 2021; Carballa *et al.,* 2015; De Vrieze & Ver-straete, 2016)**. Syntrophic links in particular may be a key underlying mechanism driving the metabolic cooperation and stability of AD microbiomes **(Kouzuma *et al.,* 2015)**. Besides, the microbial ecology revolving around syntrophy is still poorly understood because only a few syntrophs have been isolated **(Schink and Stams, 2013)**. To elucidate the complex syntrophic acid degrading ecology, it is essential to investigate who participates in syntrophy in situ.

Electron Transfer

Interspecies electron transfer (IET) is an important component of AD in which electrons are transferred from bacteria to methanogens **(Saha *et al.,* 2020)**. Historically, IET was thought to only occur through an intermediate electron shuttle, either hydrogen or formate **(Wang *et al.,* 2021)**. This form of transport is called Mediated Interspecies Electron Transfer (MIET) and is re-garded as being inefficient and often unfavourable due to the high hydrogen content that could subsequently lead to the accumulation of VFAs **(Wang *et al.,* 2021)**. Furthermore, the concentrations of hydrogen and formate must

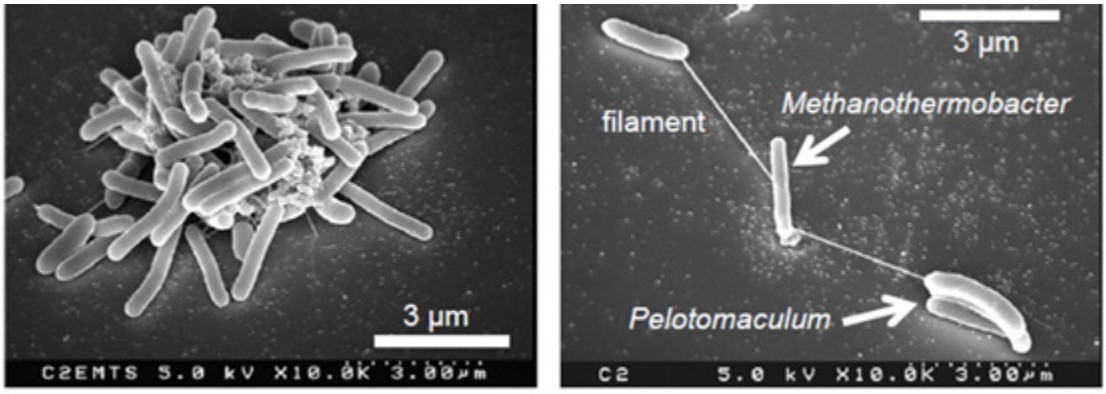


Figure 68: Scanning electron micrographs depicting DIET between bacteria (*Pelotomaculum*) and a methanogen (*Methanothermobacter*) in both co-aggregate (B) and mid-logarithmic (B) growth stages. The micro-organisms are shown being connected through the filament structure (B). Adapted from Kouzuma *et al.,* 2015.

remain low in order for this transfer reaction to be thermodynamically favourable, while to obtain higher diffusion rates high concentration are more desirable.

Direct interspecies electrons transfer (DIET) is a syntrophic metabolism in which free electrons flow from one cell to another without being shuttled by reduced molecules such as molecular hydrogen or formate. As more and more micro-organisms show a capacity for electron exchange, either to export or import them, it becomes obvious that DIET is a syntrophic metabo-lism that is much more present in nature than previously thought **(Dubé *et al.,* 2015)**. Direct interspecies electron transfer (DIET) is a relatively recent discovery and a very effective form of syntrophy in methanogenic communities. In this step, electrons transfer directly from electron donors to electron acceptors with fewer biological enzymatic reactions **(Summers *et al.,* 2010)**. Electrons are transported via mediation by a conductive material or with specialized bacterial cell structures (see **figure 67**). A theoretical modelling study concludes that the rate of DIET can be 8-fold the rate of Hydrogen-MIET, the main carrier for electron transfer. Nevertheless for-mate-MIET, less articulated in AD processes, was shown to be the fastest being 8-fold faster than DIET. **(Storck *et al.,* 2016)**.

The bacteria involved in DIET include mainly include *Desulfobacula, Desulfobacterium, Deferribacter, Geobacter sp., Geoalkalibacter, G. metallireducens, G. sulfurreducens, Thauera sp., Syntrophus sp., Pseudomonas sp., and Methanothrix sp.* **(Van Steendam *et al.* ,2019)**. Syntrophs engaged in conductive material-mediated DIET utilize materials such as granular activated carbon, biochar, graphene, iron oxide nanoparticles, and akaganeite **(Saha *et al.,* 2020)**. These conductive materials promote syntrophic IET by reducing energy expenditure by exoelectrogens **(Saha *et al.,* 2020)**. The concentrations of hydrogen and formate do not inhibit or support this process as these molecules are no longer required for electron transport. There are other potential advantages of DIET as well, particularly the rapid speed of direct transfer. For these reasons, there is much interest in understanding and potentially enhancing the DIET process to improve AD. It is thought that the syntrophic oxidation of VFAs is able to occur even when H_2 partial pressure is high through direct interspecies electron transfer (DIET) **(Wang *et al.,* 2021).**

Certain DIET bacteria are also able to transfer electrons directly to methanogens without the use of conductive materials. The mechanism for direct transfer is not yet fully understood, but the function is usually determined by the presence of conductive pili **(Wang *et al.,* 2021)** (**figure 68**). Other newly proposed mechanisms include the use of cytochromes as well as nanowires **(Wang *et al.,* 2021)**. Known exoelectrogenic bacteria that engage in this syntrophic partnership include *Geobacter, Pelotomaculum,* and *Syntrophus* **(Saha**

et al., 2020). Research into DIET continues to grow and it is well-established that this function is critical to the process of AD. However, the mechanism by which methanogens accept electrons directly from their syntrophic bacteria partners remains unknown. More investigation is needed to understand this syntrophy which could be of great importance for the end production of biogas.

It is suggested that DIET could be a new way to improve AD performance, and there are few reviews focused on comprehensively summarizing the mechanisms of DIET, the stimulation of DIET by ethanol and the effects of DIET mediated by conductive materials on anaerobic digestion performances (Gahlot *et al.,* 2020; Nguyen *et al.,* 2021). *Chapter 6* discusses more details on syntrophy and DIET.

Culture independent analysis

There are a variety of approaches used to assess the structure and diversity of microbiomes, as well as culturing techniques to observe specific strains in a laboratory setting. The isolation of an individual microbe allows for a definitive assessment of metabolism and functionality, as well as a starting point for further genomic study. However, experimental limitations, combined with the high phylogenetic diversity and the complex interactions within the AD microbiome, limit the ability to draw meaningful conclusions about the greater community from single isolates.

It has been estimated that only 1% of microorganisms found in anaerobic environments have been successfully characterized through isolation methods (Lim *et al.*, 2020). Furthermore, isolation approaches do not take into account the effect of community dynamics on an individual's functionality or role in the broader system. Additionally, certain bacteria from digesters may be difficult to cultivate in the laboratory due to oxygen requirements (i.e. obligate anaerobes) or reliance on a symbiotic relationship with another micro-organism (Lim *et al.,* 2020).

Alternative culture-independent methods utilize marker genes to differentiate various individual taxa within a single community. The markers primarily used correspond to the 16S ribosomal RNA (rRNA) gene, which can be used as a phylogenetic marker for both bacteria and archaea. The sequence of this gene is highly variable and contains enough information to reliably distinguish between taxa. Importantly, due to the common use of the 16S rRNA as a marker, databases have been developed to archive and centralize the phylogenetic information for various microbiomes (Lim *et al.,* 2020). Use of the 16S rRNA marker is widespread among microbiome research which makes findings highly accessible and comparable.

However, there are also limitations to this approach that must first be considered. One drawback is that this method requires the use of well-established reference sequences for species present in the AD microbiome. Even with a rich database, some microorganisms contain more than one copy of the 16S rRNA gene in their genomes, which hampers the calculation of relative abundances. Finally, the resolution of the 16S rRNA gene is limited, and it often allows for the identification of micro-organisms at the genus level (Klappenbach *et al.,* 2001, Větrovský & Baldrian, 2013).

Functionality of the microbiome is an important factor to consider when investigating a large microbial system like AD. Although taxonomic data can be indirectly linked to biological functions, isolation methods and phylogenetic markers cannot be used to directly analyze functionality of the species identified. To remedy this knowledge gap, functional gene markers can be used instead to identify functions of interest. Functional marker genes have been used previously in AD research. Most notably, the *mcr*A gene is found in methanogenic archaea and has been used as a marker for methanogenic ability. Other functional genes include fhs and acsB which code for two modes within the homoacetogenesis pathway (Lim *et al.,* 2020). Most functional genes are single-copy, making identification more accurate and providing, another advantage over 16S rRNA. Due to the complexity of microbi-

omes, functional marker genes can provide deeper insight into specific processes or pathways. In the case of AD research, this can provide particularly relevant information. Furthermore, multiple species may be able to perform the same function. By using marker genes, the prevalence of the function itself is revealed without reliance on species identification.

However, not every bacterial function is phylogenetically conserved. There are likely multiple genes that contribute to the same process that have arisen independently in multiple bacterial taxa. For instance, bacteria with functional roles in acetogenesis have been found across 23 different bacterial genera **(Schuchman & Muller 2014)**. Thus, acetogenic functionality is not a phylogenetically conserved trait and will have varying genetic markers associated with this function. This means that although the homoacetogenesis marker genes mentioned previously may identify some bacteria responsible for acetogenesis, these genes will not identify all taxa capable of this function. Because marker genes rely on existing knowledge of genes, findings may be phylogenetically biased and miss analogous functions from other groups.

In these cases, metagenomic sequencing is a good alternative. This technique is based on the sequencing of all DNA isolated from a sample without the need for prior amplification of specific marker genes. After assembly and binning steps, the genomes of the microorganisms present in the sample can be recovered and studied. In some cases, the analysis can be performed at the metagenome level, allowing the detection of sequences that are less abundant in the microbiome and cannot be associated with individual genomes. In any case, the functions encoded in the microbiome can be detected by homology-based searches (e.g., BLAST or DIAMOND), hidden Markov models (HMMs) or machine learning algorithms. Metagenomics also provides better taxonomic resolution than 16S rRNA gene sequencing. However, there are some limitations to metagenomic sequencing, including the lack of comprehensive genome databases and the difficulty in calculating relative abundances.

Community assessment

Bacteria within an anaerobic digester have various environmental and ecological requirements that fluctuate along with the digester conditions. Different digesters will foster different microbiomes depending on the feedstock, the loading rate, and other external conditions. Even if it was possible to observe the entire microbiome of an AD, disentangling the fine-scale dynamics of the ecosystem would remain a challenge. Looking at the bigger ecological picture instead can shed light on the underlying dynamics. For example, diversity is a measure used often by ecologists to determine the stability and resiliency of an ecosystem due to the number of trophic connections and redundant pathways **(Carballa *et al.,* 2015)**. A functionally diverse AD microbiome is often correlated with greater stability of the digester **(Carballa *et al.,* 2015)**.

However, diversity is not an easily comparable measure, and does not adequately represent the underlying dynamics of a system **(Shade, 2017)**. Similar to the differences between 16S rRNA markers and functional gene markers, the "true" diversity of a microbiome (i.e. the number of different species) does not take into account the functional diversity which may better reflects the resiliency of a digester community **(Carballa *et al.,* 2015)**. In other words, a community may contain highly diverse taxa, but perhaps is lacking in a few critical functions required for a stable AD process. Or a community containing all the necessary functionalities, the abundance of these organisms may be too low for providing sufficient productivity. Although diversity and abundance measures can provide a good guideline for microbiome changes within a single digester, high diversity or abundance alone is not enough to signal stability or productivity.

As mentioned previously, the AD microbiome contains intricate trophic connections and functional dependencies among bacteria. One evaluation method used often in ecology is the identification of keystone species which have been previously labelled as important for community function **(De Vrieze**

& Verstraete, 2016). Although there is not a standardized definition, keystone species are often determined based on their role in a function of interest, typically thought to be of critical importance for the broader ecosystem. Presence of a keystone species can then serve as an indicator of stability without need to reveal the full composition of an ecosystem (Berg *et al.*, 2020). However, keystone species may be challenging to detect and identify due to low abundance or lack of existing taxonomic classification.

Despite the recognition of importance and broad interest in microbiomes, these systems are incredibly complex. Even with constant methodological improvements, we currently lack the data and technology necessary to draw firm conclusions about the underlying dynamics of microbial ecosystems (Berg et al., 2020; Zarraonaindia *et al.*, 2013).

Microbiota are now widely recognized as being central players in the health of all organisms and ecosystems, and subsequently have been the subject of intense study. However, analysing and converting microbiome data into meaningful biological insights remains very challenging. Applying network theory has been shown to enhance understanding of higher-order interactions that occur within microbiomes. Network-based analytical approaches have the potential to help disentangle complex polymicrobial and microbe-host interactions, and thereby further the applicability of microbiome research to environmental and industrial applications and agriculture. (Layeghifard *et al.*, 2016). The combination of big-data on microbial consortia with network analysis and Machine Learning algorithms provides us with new toolboxes that can deal with the complexity of the microbial dynamics in AD.

Bioaugmentation of bacteria

Traditional approaches to improve biogas production have focused on either pre-treatment of feedstock substrate or post-treatment of gas to remove impurities. However, alteration of the AD microbiome is rapidly increasing in interest (Khan *et al.*, 2022; Ozbayram *et al.*, 2017; Tabatabaei *et al.*, 2020). The targeted manipulation of microbial activity is known as bioaugmentation and is typically approached by identifying a microbe or small consortia of interest that is believed to improve the function of the digester. Often the effects of this target microbe are amplified through some form of enrichment. The microbe is then introduced into the AD community, sometimes in combination with other methods of physical biogas enhancement (Tabatabaei et al., 2020). The three main functional steps that pose general bottlenecks limiting the performance of the AD process and that can be targeted with bioaugmentation are **1)** hydrolysis being limiting to conversion rate and biogas yield, **2)** acetate conversion to methane being limiting to loading rate and digester stability and **3)** hydrogen conversion to methane being limiting to the rate and stability of the fermentation step. Digester performance could be further improved by promoting DIET or by the selective removal of toxic components inhibiting micro-organisms involved in the conversion of substrates.

Due to the limited number of studies investigating the effects of bioaugmentation through the enrichment of pure cultures or microbial consortia, an optimal bioaugmentation methodology has not yet been firmly established. However, as each digester and microbial community is slightly different, there may be multiple applicable approaches. For instance, mixed findings exist regarding the bioaugmentation duration and application frequency of microbes into a new system (De Vrieze & Verstraete, 2016). Although some studies are able to observe shifts in overall community structure and see a positive impact on digester productivity, they are unable to reveal the actual functional contribution from the bioaugmentation (Yang *et al.*, 2016).

Bioaugmentation of bacteria may be particularly useful in the stages of AD where bottlenecks are thought to occur. As mentioned previously, hydrolysis is often considered to be rate-limiting due to the degradation limitations of lignocellulose (Chukwuma *et al.*, 2021; Lim *et al.*, 2020). Thus, there is much incentive to implement approaches to improve the breakdown of this material. Vari-

ous approaches have been considered and put into practice including thermal, chemical, and physical treatment of lignocellulosic materials (Khan *et al.*, 2022). A relatively new treatment approach is bioaugmentation through microbial consortia. Hydrolytic bacteria are able to break down insoluble polymers into soluble monomers by producing enzymes including amylase, cellulase, lipase, xylanase, among others (Saha *et al.*, 2020). This treatment approach utilizes the abilities of those bacteria to improve the downstream degradation of complex compounds and subsequent methane production (Khan *et al.*, 2022). However, there is a lack of standardization among laboratory studies and very few validations of microbial bioaugmentation in a full-scale reactor (Park *et al.*, 2008). Although bacteria play an important role in the functionality and stability of anaerobic digesters, more research is needed to develop bioaugmentation strategies utilizing bacteria.

Methanogens are often the desired targets of bioaugmentation to produce biogas due to their direct role in this function. However, methanogenic archaea have strict needs and are highly sensitive to environmental changes - particularly to oxygen stress – which makes them difficult organisms to manage in the lab (Khelaifia *et al.*, 2016). Due to this limitation, bacteria may be a more favourable group for laboratory bioaugmentation. Each step before methanogenesis contributes to further degrading the initial substrate for the final step where the penultimate products are converted into methane. Thus, any improvements by bioaugmentation to the bacteria in the first steps are thought to have a positive downstream effect on biogas production (Tabatabaei *et al.*, 2020).

Also, EU MICRO4BIOGAS explores techniques to optimize the microbiome of biogas production alternative to bioaugmentation. Those are the transplantation of digestates between digesters and biostimulation. The transplantation of digestates offers an affordable way to introduce a complete microbiome at a relatively high level of a few percent into the native digester microbiome. Biostimulation concerns changing the physicochemical conditions in the digester in such a way that certain species or functionalities are promoted. One route for biostimulation that is further explained in *chapter 6* is to introduce granulated activated carbon in the digester which not only provides a separate niche environment for methanogens, but also has a role in improving interspecies electron transfer.

5.3 Archaea

Archaea represent the third domain of life, alongside with *Bacteria* and *Eukarya*. Since the discovery of Archaea being a distinct domain in the late 1960s, scientists have come to recognize that this domain represents a major proportion of microbial biomass on the planet. The increase in knowledge on Archaea has expanded our view of the tree of life and early archaeal evolution, and has provided new insights into archaeal cultivation, manipulation and ecological roles.

Archaea

Archaea possess unique characteristics that distinguish them from bacteria. These features include novel membrane lipids based upon phytanyl ether lipids and information processing systems like those found in eukaryotes (Liu *et al.*, 2012). They were initially found in environments with extreme physiochemical conditions such as high salinity and high temperature, and habitats in which they evolved to metabolize compounds such as methane and hydrogen (Cabello *et al.*, 2004; Singh *et al.*, 2012). In addition to inhabiting extreme environments, archaea exhibit widespread distribution across diverse habitats ranging from terrestrial to aquatic environments.

Members of *Crenarchaeota* and *Euryarchaeota* are globally distributed, and some lineages, often uncultivated ones, are abundant in waters, soils and sediments (Bomberg *et al.*, 2008; Timonen and Bomberg, 2009; Schleper and Nicol, 2010). For *Euryarchaeota*, the role those ubiquitous methanogens carry out in the environment is well characterized. Formation of methane in the final degradation step of organic matter under anaerobic con-

ditions is an exclusive attribute of archaea. However, some *Euryarchaeota* participate in syntrophic anaerobic methane oxidation and are known as anaerobic methane oxidizers **(Orphan *et al.*, 2001)**. In relation to the nitrogen cycle, some cultured archaea (both *Euryarchaeota* and *Crenarchaeota*) are capable of denitrification and nitrogen fixation. Furthermore, ammonia-oxidizing archaea contribute greatly to the ammonia oxidation process. This is an important step of the nitrification pathway responsible for nitrogen leaching and N_2O emissions and thought to impede plant nitrogen use efficiency in agricultural systems. In addition, studies have suggested that some Euryarchaeota may also degrade hydrocarbons **(Laso-Pérez *et al.*, 2016; Dombrowski *et al.*, 2017)**, and they could also be involved in other biogeochemical processes such as sulphur and iron metabolism **(Ofre *et al.*, 2013)**. For example, several archaea utilize sulphur compounds as electron donors or acceptors for energy production **(Kletzin, 2007)**. In the case of iron, Fe(III) serves as terminal electron acceptor for anaerobic respiration by a variety of archaea, while Fe(II) operates as electron donor and/or energy sources for archaeal growth **(Dong *et al.*, 2021)**.

Bathyarchaeota, is an archaeal phylum of global generalists that are widespread in anoxic sediments, which host relatively high abundance archaeal communities and possess multiple important ecological functions. Furthermore, bathyarchaeotal members have wide metabolic capabilities, including acetogenesis, methane metabolism, and dissimilatory nitrogen and sulfur reduction, and they also have potential interactions with anaerobic methane-oxidizing archaea, acetoclastic methanogens and heterotrophic bacteria. These results have not only demonstrated multiple and important ecological functions of this archaeal phylum **(Zhou *et al.*, 2018)**.

Methanogenic archaea

Methanogenic archaea are a highly catabolically specialized group, and the only group of micro-organisms responsible for CH_4 production, a very potent greenhouse gas as well as a renewable fuel. Methane gas is produced under anoxic conditions in numerous natural environments such as oceans, estuaries, soils, and lakes **(Wen *et al.*, 2017)**. Its emission from natural environments such as wetlands, oceans, and sediments accounts for over 70% of atmospheric methane globally **(IPCC, 2007)**. *Archaea* are also present and can produce gas in multicellular organisms (i.e., rumen fluid and digestive tracts) **(Saengkerdsub and Ricke, 2013)**. Furthermore, methanogenesis can be encountered in geothermal environments where gases (carbon dioxide and hydrogen gas) are the substrates used by methanogens.

Despite their broad phylogenetic diversity and distribution in different habitats, methanogens metabolize a very narrow range of substrates. A major consequence of this specialization is that methanogens are dependent on other micro-organisms that act as electron donors, and are typically outcompeted for those electron donors by more efficient metabolic processes such as nitrate, iron, and sulphate reduction. The substrates can be converted by syntrophic interaction of

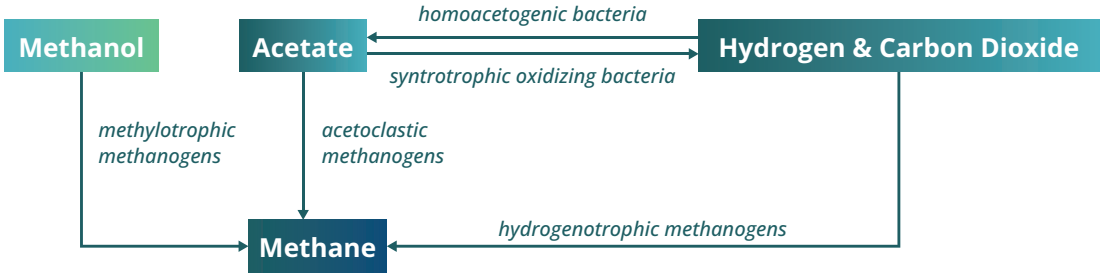


Figure 69: Overview of the main pathways leading to methane production.

methanogens and fermenting bacteria that closely cooperate in the degradation of fatty acids, alcohols, most aromatic compounds, and amino acids **(Worm *et al.*, 2010)**.

The biological formation of methane can be performed in three catabolic pathways (**figure 69**): hydrogenotrophic, growth on H_2 and CO_2 with CO_2 serving as both carbon source and electron acceptor; methylotrophic pathway, the reduction of the methyl group of methyl-containing compounds to methane; and acetoclastic pathway, the cleavage of acetate to CH_4 and CO_2. Central to all those pathways is the Wood–Ljundahl pathway and the most widely used substrate for methanogenesis is acetate, which accounts for two-thirds of the methane generated in environments. However, the hydrogenotrophic pathway comprises the largest phylogenetic diversity found in methanogens. During growth on H_2 and CO_2, CO_2 is first bound to methanofuran and thereby reduced to a formyl group. The formyl group is transferred to tetrahydromethanopterin (H4MPT) and reduced to a methyl group through the costs transferring 1.7 Na^+/CH_4 over the membrane **(Schlegel and Muller, 2011)**. Subsequently, the methyl-CoM undergoes a reaction with the thiolate anion of coenzyme B (HS-CoB), ultimately leading to the release of methane. In the methylotrophic pathway, small, methylated carbon compounds like methanol and methylated amines are used **(Kurth *et al.*, 2020)**. Three quarters of the methyl groups are reduced to methane whereas one quarter is oxidized to carbon dioxide **(Kurth *et al.*, 2020)**. Methanogenesis from acetate starts with the activation of acetate to acetyl-CoA, followed by an oxidation of acetyl-CoA to CO_2 and a coenzyme-bound methyl group that is reduced to CH_4 with electrons gained during the oxidation reaction **(Schlegel and Muller, 2011)**.

Our understanding of how methanogenic substrate utilization and energy conservation is still incomplete but is rapidly expanding through the application of molecular biology techniques such as metagenomics. These approaches can enable, for example, the discovery of hydrogenotrophic and acetoclastic methanogenesis genes encoded for different taxa.

Biology of methanogenic archaea

Taxonomically, all methanogens belong to the phylum Euryarchaeota, forming a diverse collection encompassing seven orders: *Methanobacteriales, Methanococcales, Methanomicrobiales, Methanopyrales, Methanomassiliicoccales, Methanocellales and Methanosarcinales*. The presence of the gene mcrA, accountable for methane production, characterizes these orders **(DasSarma *et al.*, 2009; Buan, 2020)**. The *Methanosarcinales* have the most diverse members, with many genera qualified for more than one methanogenesis pathway, up to all three methanogenesis pathways. Nevertheless *Methanosarcinales* as well has obligate hydrogenotrophic or obligate acetoclastic members **(Buan, 2020)**. Five of the methanogen orders (*Methanopyrales, Methanococcales, Methanobacteriales, Methanomicrobiales, and Methanocellales*) only contain hydrogenotrophic methanogens, while the *Methanomasiliicoccales* group is defined as having obligate methyl-respiring methylotrophic methanogens **(Buan, 2020)**. See below the biology of the selected groups based on their participation in the different pathways:

Hydrogenotrophic methanogenic archaea: The hydrogenotrophic archaea produce methane from H_2 and CO_2, from formate, or from H_2 and methanol. These archaea use only sodium ions rather than protons for chemiosmotic energy conservation **(Thauer *et al.* 2008)**. As hydrogenotrophic archaea can use a broad range of substrates it is possible to distinguish them. According to a phylogenetic analysis, methanogens can be divided into two major groups: Class I methanogens (*Methanopyrales, Methanococcales, and Methanobacteriales*) and Class II methanogens (*Methanomicrobiales, Methanocellales, and Methanosarcinales*). On the other hand, **Thauer *et al.* (2008)** have debated that methanogens can be divided into two classes based on the presence or absence of cytochromes, with *Methanosarcinales* alone possessing cytochromes. In this scenario, the *Methanomicrobiales* thus belong to the phylogenetic group of Class II methanogens, but to the physiological group of methanogens without cytochromes. That is why, some references, such as **Anderson *et***

al. (2009) show that three distinct classes of methanogens: the Class I methanogens, the Methanomicrobiales (Class II), and the Methanosarcinales (Class III) with *Methanococcales* and *Methanomasiliiicoccales* belonging to none of these groups.

Despite the ongoing and still unresolved discussion on the classification of methanogens, the six orders of methanogens involved in hydrogenotrophic methanation are further described (Thauer *et al.*, 2008, Michal *et al.*, Phymet2 2018, Silva Taxonomy Browser).

- ***Methanopyrales:*** includes only one species, *Methanopyrus kandleri*. This hyperthermophilic species, growing between 84 - 110 degrees at pH range of 5.5 - 7.0 is an obligate hydrogenotrophic archaea (Sowers, 2019; Kurr *et al.*, 1991, Huber and Stetter, 2001, Liu, 2010).
- ***Methanococcales:*** includes organisms that grow exclusively by CO_2 reduction with H_2 or in some cases use formate for growth and methanogenesis. This order includes several mesophilic *Methanococcus spp.*, moderately thermophilic *Methanothermococcus spp.*, and the extremely thermophilic genera *Methanocaldococcus* and *Methanotorris* that grows between 20 - 88 degrees at a pH range of 4.5 - 9.8 (Sowers, 2019).
- ***Methanobacteriales:*** includes organisms that grow by CO_2 reduction with H_2, CO, formate and C1-methylated compounds (Angelidaki *et al.*, 2011). Methanosphaera, for example, uses H_2 to reduce the methyl group of methanol instead of CO_2. They can grow between a range of 20 – 88 degrees and 5.0 – 8.8 pH (Liu and Whitman, 2008, Thauer *et al.*, 2008). Most occurring species in anaerobic digestion are *Methanobacter* and *Methanothermobacter*, the other members in this order are *Methanobrevibacter, Methanosphaera* and the hyperthermophillic *Methanothermus*.
- ***Methanomicrobiales:*** contains species that differ in their salt tolerance (range from non-halophilic to slightly halophilic), pH (between 6.1 - 8.1) and temperature (ranging from 15 to 60 degrees). Most species grow by CO_2 reduction with H_2, but some species also use formate or secondary alcohols such as ethanol, 2-propanol, 2-butanol cyclopentanol (Sowers, 2019; Garcia *et al.*, 2006, Liu and Whitman, 2008, Dianou *et al.*, 2001, Thauer *et al.*, 2008). Methanoculleus has an important role in anaerobic digesters as it is able to survive at a high free ammonia concentration (Xu *et al.*, 2021). Other members of this diverse order include *Methanospirillum, Methanolinea, Methanocalculus, Methanomicrobium*.
- ***Methanocellales:*** This order includes only one species, *Methanocella*. These organisms have all been isolated from rice fields (Sowers, 2019) and grow by CO_2 reduction with H_2 or by using formate in a range of temperature between 25 - 40 and pH of 6.5 - 7.8.
- ***Methanosarcinales:*** includes the most metabolically diverse species of methanogens. The species grow by CO_2 reduction with H_2, but they can also grow by methyl reduction with H_2, acetoclastic fermentation of acetate, or methylotrophic catabolism of methanol, methylated amines, and dimethylsulfide (Sowers, 2019). Their growth ranges from temperature of 1 - 70 degrees and from a pH range of 4.0 - 10. This group has *Methanosarcina* and *Methanothrix (Methanosaeta)* as most well-known genera, but also contains *Methanimicrococcus* and *Methanomethylovorans* amongst other members.

Methylotrophic methanogenic archaea: These archaea can be hydrogen-dependent or hydrogen-independent and they are limited to *Methanosarcinales* (including methylotrophic species from the following genera: *Methanosarcina, Methanomicrococcus, Methermicoccus, Methanomethylovorans, Methanohalophilus, Methanolobus, Methanococcoides*), the obligate methylotrophic *Methanomassiliicoccales* (including *Methanomassiliicoccus, Candidatus Methanoplasma* and *Candidatus Methanogranum*), and one

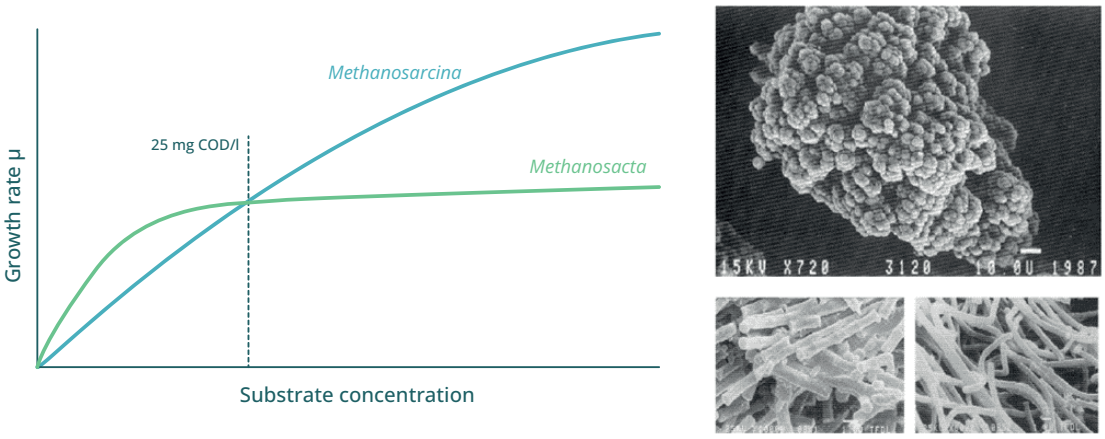


Figure 70: Curves of the acetotrophic growth of *Methanosarcina* and *Methanosaeta* adapated from Lier et al. (2008). The results presented in this figure are according to umax of (5.8 and 1.0 1/d) and the saturation constant (Ks) (30 and 300 mgCOD/l) for *Methanosarcina* and *Methanosaeta*, respectively. The picture on the right shows the morphology of *Methanosarcina* (top) and *Methanosaeta* (bottom).

species of Methanobacteriales **(Lang *et al.*, 2015)**. Among these, the hydrogen-dependent archaea use a mixed-mode of methanogenesis by amalgamating the hydrogenotrophic and the methylotrophic pathways. Notably, these archaea lack enzymes for the Wood-Ljungdahl pathway. As a result, they need methylated compounds and hydrogen as electron acceptor and donor, respectively **(Kurth *et al.*, 2020; Borrel *et al.*, 2016, Buan, 2020, Michal *et al.*, Phymet2, 2018)**.

Acetoclastic methanogenic archaea: Acetoclastic methanogens occur in only 2 genera: *Methanosarcina* (also considered hydrogenotrophic or methylotrophic) and *Methanothrix* (the only known obligate acetotrophic methanogens also referred previously in the literature as *Methanosaeta*). Methanothrix is well adapted to lower acetate concentration 7-70 μM), when in comparison with Methanosarcina species (minimum of ~1.2 mM) **(Smith and Ingram-Smith, 2007; Welte and Deppenmeier 2014)**. Conversely, Methanosarcina displays a relatively versatile nature, with a preference for utilizing methanol methylamine over acetate **(Boone *et al.*, 2001)**. Apparently the two genera employ different enzymes to catalyze the first step of acetoclastic methanogenesis while the core steps of methanogenesis are similar in the two genera **(Deppenmeier *et al.*, 2002, Smith and Ingram-Smith, 2007)**. The differences between the 2 genera refers to the electron transfer and energy conservation **(Smith and Ingram-Smith, 2007)**. From the conversion of acetyl-CoA to methane and CO_2 *Methanothrix* should earn enough energy to synthesize more than the expended two ATPs, while for *Methanosarcina*, the electrochemical gradient produced by the membrane-bound Ech hydrogenase is used for ATP synthesis during growth on acetate **(Smith and Ingram-Smith, 2007)**.

These differences between the two pathways, makes *Methanothrix* to have a higher affinity for acetate than *Methanosarcina* **(Smith and Ingram-Smith, 2007)**. More detail on difference in acetate kinase enzymes between the two genera is given in Chapter 6. **Van Lier *et al.*, (2008)** also showed in their review that *Methanosarcina* has a relatively high maximum growth rate (μ_{max}) while *Methanothrix* are kinetically characterized by a low μ_{max} in wastewater treatments (**figure 70**).

Cultivation of archaea

Initially, the investigation of archaea relied solely on low throughput laboratory cultivation techniques, whereas the more recent cultivation-independent techniques have enabled the direct investigation of archaeal ecology and diversity. Nevertheless, cultivation, i.e., growth on specific substrates, remains important as it is the final proof of metabolic activity and is required for detailed physiologic study. Although the majority of

microorganisms are not yet cultivable in artificial media as pure cultures, the combination of enrichment cultivation and molecular techniques can provide valuable insight into the function of microorganisms, often not possible using uniquely molecular tools.

Similar to the isolation of bacteria, archaeal cultivation includes three fundamental steps: sample collection, enrichment, and isolation. A variety of suitable basal media compositions are detailed in literature and scientific publications and further advanced cultivation methods have been utilized such as:

Anaerobic incubation using the Hungate method (Hungate, 1969): a roll tube technique was employed to cultivate a maximal portion of the organisms of the gingival crevice area of humans and other environments. This technique achieves an anaerobic state by flushing the local environment with oxygen-free gas so it can be used for the cultivation of methanogens. It requires gases such as H_2 in the case of hydrogenotrophic archaea.

Continuous cultivation: this method consist of an open operation system with continuous addition and discharge of a solution. Micro-organisms and the sterile nutrient solution are added homogeneously to the bioreactor, continuously, while they are transformed equivalently in the system. This method can be used to cultivate microbes, especially methanogens (Imachi *et al.*, 2011).

Ultrafiltration: is one of the most common and simple methods for the enrichment of small archaea. For example, Nanoarchaeota were physically isolated from their host Ignicoccus spheres by ultrafiltration through 0.45 µm pore-size membranes (Huber *et al.*, 2002).

Anaerobic cultivation methods diverge from classic microbiological techniques in several aspects. The designs of instruments are tailored to prevent any exposure of the cultivated organisms to oxygen as a concentration of 10 ppm is normally lethal, making this field challenging (Wolfe and Metcalf, 2010). Some laboratories are currently preparing specific culture media for each one of the various methanogenic archaea species (Khelaifia *et al.*, 2013). Furthermore, Wolfe (2011) presents a book chapter with basic techniques for the cultivation of methanogenic archaea in anoxic media. Here we have collected information on genus-specific cultivation related to their methanogenic pathway:

Hydrogenotrophic archaea: isolating hydrogenotrophic archaea remains a critical process because they require an optimal atmosphere (80% H_2 and 20% CO_2) and they grow slowly (Dridi *et al.*, 2011). Repeated gassing is necessary because the gaseous substrate in the headspace is consumed according to the specific gas uptake rate and the biomass concentration of gas-utilizing microbes (Hanišáková *et al.*, 2022). Furthermore, some species can be cultivated on agar plates without a H_2 atmosphere by using, for example, formate as a substrate (Long *et al.*, 2017). Another strategy suggested by Long *et al.* (2017), is the co-cultivation with bacteria. In this way, organic acids such as lactate, acetate, butyrate and propionate are consumed by bacteria and H_2 is gradually released, which is then utilized by hydrogenotrophic methanogens.

Acetoclastic archaea: Steinhaus *et al.* (2007) successfully isolated a pure culture of methanogenic Methanosaeta concilii using a microfluidics technique under N_2/CO_2 atmosphere conditions. Another isolation method was detailed by Janssen (2003), who described a simple method for the isolation of axenic cultures of members of *Methanosaeta* based on acetone- and isopropanol-utilizing bacteria that slowly ferment substrates to acetate, allowing *Methanosaeta spp*. to maintain the acetate concentration at levels below the threshold required for growth of *Methanosarcina spp*.

Methylotrophic archaea: with acetoclastic archaea, methylotrophic species are not dependent on H_2, so it is possible to cultivate them under a H_2-free atmosphere (Hanišáková *et al.*, 2022) with methanol, methylamine, dimethylamine, trimethylamine or dimethylsulfide as substrates. Lino *et al.* (2013) reported a methanogenic enrichment

culture derived from the sludge of an anaerobic digestion (AD) process, that contained a novel methanogenic archaeon.

Culture-independent analysis

Molecular techniques have proven to be highly effective for evaluating methanogens in several environments. As previously said, the most frequently used molecular marker is the 16S rRNA gene. For this a general prokaryotic primer may be used to gain insight in the complete community of both bacteria and archaea. However, as the abundance of archaea is relatively low compared to the abundance of bacteria in AD systems, most of the obtained reads will be assigned to bacteria. Therefore, a primer targeted specifically at archaea only may be applied to obtain an even more detailed insight in the archaeal community. A molecular marker that is even more specific for methanogenic archaea, is the gene encoding the α subunit of the methyl-CoM reductase (*mcrA*) **(Steinberg and Regan, 2009)**. The mcrA gene is thought to be highly conserved and specific for methanogens and several studies have used this gene as biomarker to characterise methanogenic communities in soils and anaerobic digesters **(Liu *et al.*, 2018; Alvarado *et al.*, 2014)**. Methanogenic phylogenies determined using the *mcrA* and 16S rRNA genes are largely congruent. Therefore, both genes have been used to elucidate the diversity and phylogeny of methanogens.

Bioaugmentation of methanogenic archaea

Bioaugmentation is a strategy to introduce micro-organisms with desirable properties into a biological system to increment specific microbial functions of interest. With that, bioaugmentation could accelerate the start-up of bioreactors, maintain their stability, and enhance the conversion rate of complex substrates. Archaea are slow growers with low biomass yields that produce explosive by-products. Nevertheless, because of the important and limiting role of archaea in AD, it is valuable to explore the potential impact of an improved archaeal consortium on biogas production as a first step before more thought can be given towards specific augmentation concepts. As it is notoriously difficult to culture methanogenic archaea, other strategies such as the transplantation of digestates or the biostimulation of archaea within the digester itself (by for example specific nutrients, light, electricity, electroactive carrier materials) may provide alternative routes that may be feasible. More details on the mentioned approaches are discussed in *Chapter 6*.

In recent years, bioaugmentation has also been applied to increase the biogas production of AD **(Li *et al.*, 2018; Nielsen and Angelidaki, 2008)**. As an example, bioaugmentation with an adapted hydrolytic consortium increased the biogas yield by 10 - 29% in the AD of maize silage **(Poszytek *et al.*, 2017)**.

Several studies show that bioaugmentation has being used to alleviate ammonia related problems in bioreactors **(e.g., Yan *et al.*, 2022)**. **Tian *et al.* (2019)** showed that bioaugmentation through an enriched culture, and a mixed culture composed 50/50 by *Methanoculleus thermophilus* and the enriched culture improved methane yield and decreased the volatile fatty acids. Similarily, bioaugmentation with certain strains (the SAO *Clostridium ultunense* and the hyrogenotrophic archaea *Methanoculleus*) has proven to be an effective way to alleviate ammonia inhibition **(Fotidis *et al.*, 2014)**. Furthermore, **Yang *et al.* (2019)** used different micro-organisms (including obligate acetoclastic methanogens, facultative acetoclastic methanogens, hydrogenotrophic methanogens and syntrophic acetate oxidizing bacteria) in AD to mitigate the ammonia inhibition. The authors concluded that bioaugmentation with the hydrogenotrophic methanogen *Methanobrevibacter smithii* was the optimal choice to improve the methane production **(Yang *et al.*, 2019)**. Besides ammonia limitation, the review by **Nzila *et al.* (2016)** shows that many other bioaugmentation strategies and inocula (bioaugmentation with either single or mixed micro-organisms) can be used to overcome other problems found in bioreactors such as low temperature limitation, toxicity induced by O_2 or recovery time following overload, leading to an increase in biogas formation.

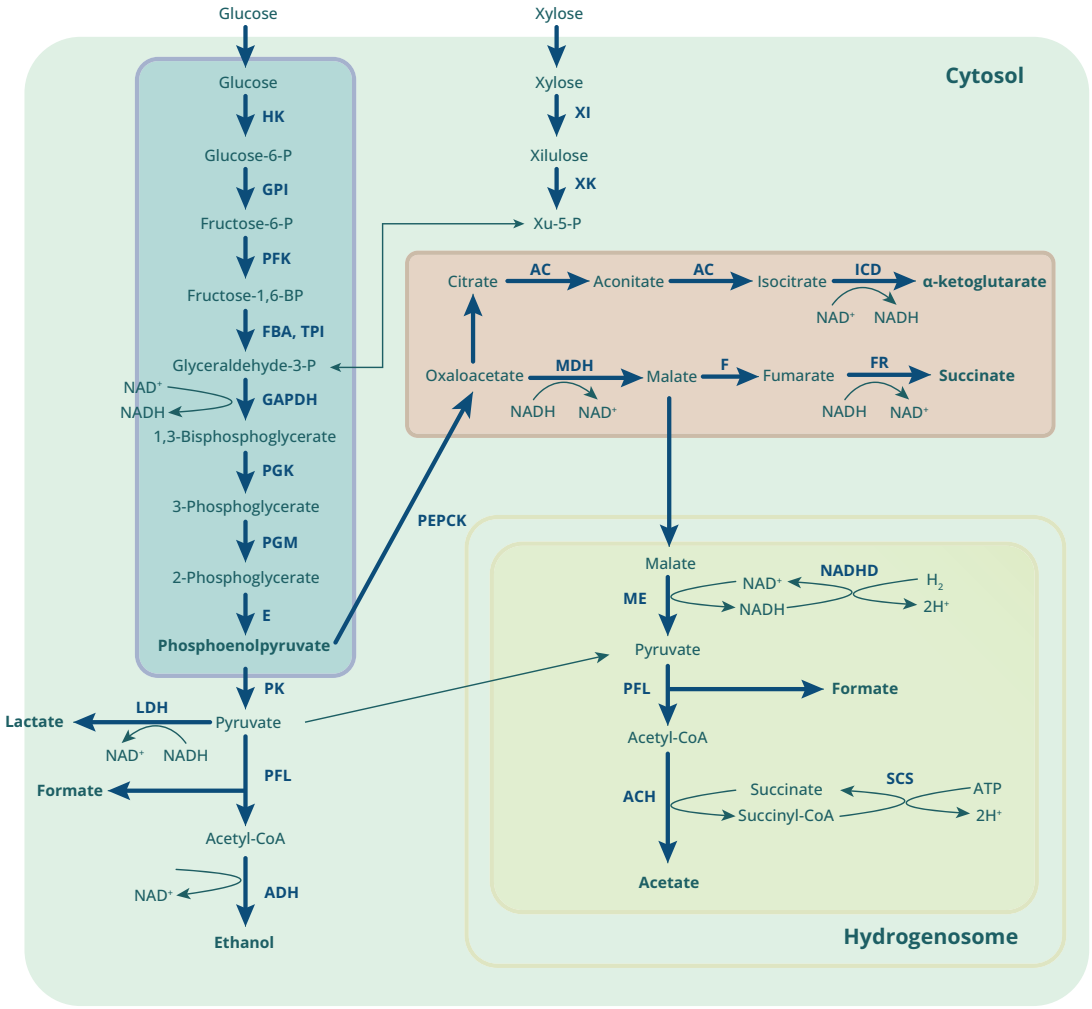


Figure 71: Metabolic pathway for glucose and xylose in anaerobic fungi. Adapted from Li *et al.* (2021). A complete explanation of all enzymes involved can be found in the same paper.

Nonetheless, not all bioaugmentation cases lead to a positive impact on digestion performance and despite the merits of bioaugmentation, the main concern regarding bioaugmentation, several challenges and concerns have arisen. In one example, in a continuous AD system, the injected micro-organisms for bioaugmentation are probably washed out ultimately due to the daily fed-withdraw mode **(Romero-Güiza *et al.*, 2016)**. **Yang *et al.* (2019)** shows a list of different publications that have obtained negative results when applying bioaugmentation strategies. It must be noted that methanogens of *Methanosarcina* have proven to be more robust and appropriate in bioaugmentation to increase methanogenesis phase **(Yang *et al.*, 2019)**.

5.4 Eukaryotes

While prokaryotic communities in AD are commonly studied, the eukaryotic community is poorly known. Furthermore, most research focusses on microscopic determination or first-generation sequencing **(Priya *et al.*, 2008a; Priya *et al.*, 2008b; Bhaskaran *et al.*, 2016; Matsubayashi *et al.*, 2017)**. Research found that, out of the total number of microbial rRNA in AD, 0.1 – 1.4% accounted for eukaryotes. Although the number of eukaryotes cannot precisely be determined because of the heterogeneous rRNA gene copy numbers,

it shows that there is a eukaryotic community in AD (Matsubayashi *et al.*, 2017). This chapter first describes the group of anaerobic fungi (*paragraph 5.4.1*) and then continues with other eukaryotes (*paragraph 5.4.2*) that are informally classified as protozoa.

5.4.1 Anaerobic fungi

Biology of anaerobic fungi

Anaerobic fungi of the phylum Neocallimastigomycota mainly inhabit the digestive system of mammals and reptiles with a fibrous diet. They have been found worldwide and it appears that host phylogeny is more important than geographical location (Haitjema *et al.*, 2014; Gruninger *et al.*, 2014). Despite their relatively low abundance in these animals, they are one of the key players in degradation of lignified materials, where they colonize the fresh feed within minutes. Mitochondria are completely absent in anaerobic fungi. For respiration, they use mitochondria-related organelles named hydrogenosomes. Anaerobic fungi perform mixed acid fermentation and monosaccharides are fermented into hydrogen, carbon dioxide, formate, acetate, lactate, ethanol and succinate (**figure 71**) (Haitjema *et al.*, 2014; Gruninger *et al.*, 2014; Hackstein *et al.*, 2019; Vinzelj *et al.*, 2019).

Anaerobic fungi excrete a large range of fiber-degrading enzymes, including cellulases, hemicellulases, pectinases, esterases, glycoside hydrolases, carbohydrate esterases, glycosyl transferases, polysaccharide lyases, carbohydrate esterases, and carbohydrate-binding modules. Together with their ability to penetrate plant tissues with their rhizoids, anaerobic fungi can effectively degrade fibers (Haitjema *et al.*, 2014; Cheng *et al.*, 2018).

Cultivation of anaerobic fungi

Anaerobic fungi are obligate anaerobes, and are usually cultivated in stationary conditions in a liquid medium at 39 °C, but growth can be observed between 33 and 41 °C (Wilken *et al.*, 2019). Generally, they are grown in Hungate tubes on either soluble or insoluble carbons source, with 100% CO_2. Overgrown substrate is preferred as inoculum, as it reduces lag time in comparison to inoculation with free zoospores. Another growth technique is with agar roll tubes. Reducing agents such as sodium sulfide or L-cysteine hydrochloride are added to ensure a negative redox level. The redox indicator resazurin is used to check the redox level (Haitjema *et al.*, 2014).

Anaerobic fungi can be isolated directly from rumen, but spores can also be obtained from faeces. They can be isolated by making serial dilutions of an anaerobic fungi-containing material and inoculating the dilutions in an antibiotic-containing growth medium with an insoluble carbon source such as grass, hay, or wheat straw. Growth can be observed either by gas production or visible biomass growth (Haitjema *et al.*, 2014).

Culture-independent analysis

Molecular analysis of anaerobic fungi raises problems, as anaerobic fungi have the lowest GC value of all sequenced fungi, with an overall average of 25% GC, and with non-coding regions that can display as low as 16% GC (Wilken *et al.*, 2019).

Previously, the 18S rRNA gene was mostly used for anaerobic fungi phylogeny, but this gene is highly conserved, making it unusable for determining below order level. Therefore, alternatives such as the ITS1 (internal transcribed spacer 1) and the D1/D2 region of the large ribosomal subunit (LSU) have been developed. At this moment, the ITS1 region remains the most used in fungal phylogeny (Vinzelj *et al.*, 2019; Edwards *et al.*, 2017).

Three sets of primers were developed by Dollhofer *et al.* (2016), to study the quantity, diversity and activity of anaerobic fungi in AD. In an anaerobic digester fed on cattle slurry and maize silage, they used qPCR primers on the 18S rRNA gene and found up to 10^9 DNA copies/ml of anaerobic fungi (Dollhover *et al.*, 2017). The correlation of DNA

copy numbers and cell biomass, however, is complicated as it is influenced by factors such as growth phase. For phylogeny, they used a primer paired targeting the large ribosomal subunit (LSA, 28S rRNA), detecting the genera Caecomyces, Piromyces, and Anaeromyces.

Anaerobic fungi in AD

Although the presence and long-time survival of anaerobic fungi in digesters is not often studied, it is known that they are present in digesters fed with manure **(Dollhofer *et al.*, 2017)**. However, it has been reported that anaerobic fungi were not found in anaerobic sludge digesters **(Matsubayashi *et al.*, 2017; Hirakata et al., 2019)**.

Using the same primers discussed in the previous section, **Dollhofer *et al.* (2017)** studied the presence and activity of anaerobic fungi in biogas plants. Up to 10^9 rRNA gene copies/ml were found in biogas plants fed with cattle manure or slurry. Because presence of anaerobic fungi doesn't mean that they are metabolically active, the presence of anaerobic fungi GH5 endoglucanase mRNA was also measured as an indication of cellulose degradation activity. It was found that only two biogas plants showed transcriptional activity, with the highest having only $1.8x10^2$ transcripts/ml, which is 10^3-fold lower than what they found in cattle rumen **(Dollhofer *et al.*, 2016)**. Furthermore, they could only isolate one anaerobic fungi of the genus Piromyces from all biogas plants **(Dollhover *et al.*, 2017)**. This suggests that the anaerobic fungi get imported with the fresh manure, but they are not able to settle in the anaerobic digester.

Symbiosis and syntrophy

Research has revealed that anaerobic fungi can form stable co-cultures in vitro with methanogenic archaea in which the fungi's metabolites are consumed by the archaea. Notably, studies have shown that acetate and hydrogen production were upregulated, while the production of ethanol and lactate were downregulated in an anaerobic fungus in co-culture with a methanogen. Acetate and hydrogen are the main substrates for methanogens **(Li *et al.*, 2021)**.

Bioaugmentation of anaerobic fungi

The recalcitrance of lignocellulosic material is one of the rate-limiting steps in AD. In several studies, biogas production was either accelerated or increased by performing bioaugmentation with an anaerobic fungus and a methanogen, improving the degradation of lignocellulosic materials, including paper, fibre, leaves, cellulose, ryegrass stem, rice straw, wheat straw, corncob, and corn core **(Cheng *et al.*, 2018; Li *et al.*, 2020; Li *et al.*, 2021)**. However, most of these studies focus on aseptic systems, whereas an anaerobic digester contains a rich diversity of micro-organisms.

In a study conducted by **Prochazka *et al.* (2012)**, experiments with anaerobic sludge, and a complex carbon source, with the addition of 0,1g/l dry mycelium of the anaerobic fungi *Anaeromyces spp.*, an increase in biogas production was observed. Although a relatively large amount of mycelium was added, the anaerobic fungi could not be detected after 7 days, indicating that the fungi could not settle in the anaerobic reactor **(Prochazka *et al.* (2012)**.

Akyol *et al.* (2019) studied the ability of *Orpinomyces sp.* to increase biogas production from various lignocellulosic materials with anaerobic seed sludge or cow manure. They found that, in all cases, methane production was both accelerated and increased, and cellulose loss was increased. Furthermore, they found that the bacterial community was heavily influenced by the fungal bioaugmentation, whereby the augmentated trials showed a significantly lower diversity. This shows that addition of an anaerobic fungi affects the process **(Akyol *et al.*, 2019)**.

Experiments performed by **Dollhofer *et al.* (2018)** with bioaugmentation of anaerobic fungi *Neocallimastix frontalis* in methane production of lignocellulosic biomass, showed that biogas production was increased and accelerated after hydrolytic pretreatment.

However, anaerobic fungi activity and viability decreased over time (Dollhofer *et al.*, 2018).

Outlook

Although Anaerobic Fungi provide a highly relevant and potentially significant contribution to anaerobic digestion, in many studies, the presence and long-term survival of anaerobic fungi in anaerobic fermenters is rarely considered. For bioaugmentation in an agricultural biogas plant, establishment of the anaerobic fungi in the anaerobic digester would be the most ideal case. Otherwise, the fungi must be added at periodic intervals. In this latter scenario a better understanding of the interaction between the fungal and bacterial populations and the effect on biogas production is needed. A challenge towards practical application is obtaining active fungal cultures at sufficient cell density.

5.4.2 Other eukaryotes (protozoa)

With anaerobic fungi as the best studied eukaryotes in AD, the role of other eukaryotes such as protozoa has been greatly overlooked. Protozoa are a highly diverse group of single cell eukaryotes that are not plants, animals or fungi. They live in a wide variety of habitats, feed by heterotrophy, often show animal like behaviour such as motility and predation and lack a cell wall such as found in plants and algae. As protozoa are polyphyletic, not sharing common ancestors, they are distributed amongst various supergroups of micro-organisms within the eukaryotes and cannot be considered as a separate taxon.

The protozoan role is generally reported as antagonistic to bacterial growth from grazing activity and thereby unfavourable to bacterial growth and substrate degradation. However it also has been reported that grazing and flocculation activity of anaerobic ciliates lead to an overall stimulation of anaerobic activity and to an increased turn-over rate in wet anaerobic landfill sites (Priya, 2007; Fenchel 2010). These contradicting indications suggest more study is needed on the

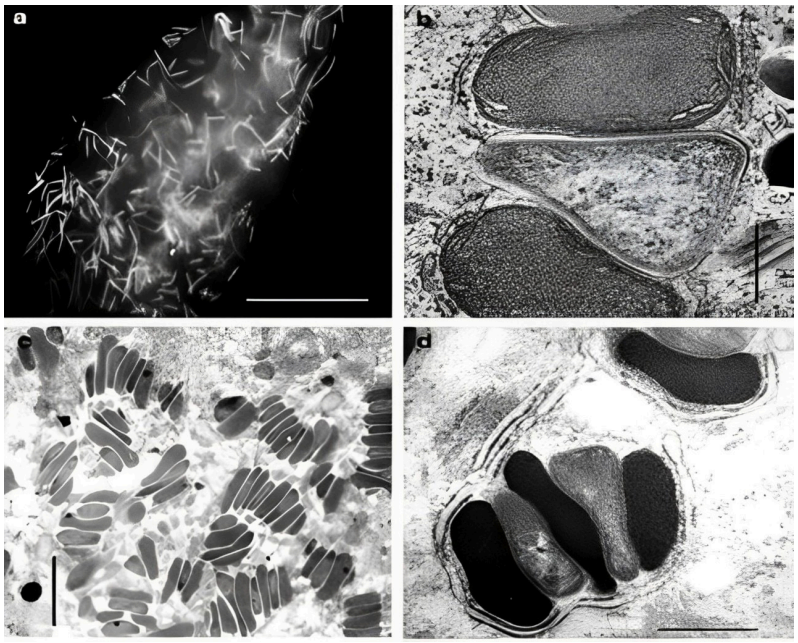


Figure 72: Fluorescence of methanogens in Metopuspalaeformis; scale bar: 10 µm. (b) A methanogen sandwiched between two hydrogenosomes in Metopuscontortus; scale bar: 0.5 µm. (c, d) Stacks of alternating hydrogenosomes (darker) and methanogens in Plagiopylafrontata: scale bars 5 and 0.5µm, respectively. Figure adjusted from Fenchel & Finlay (2018).

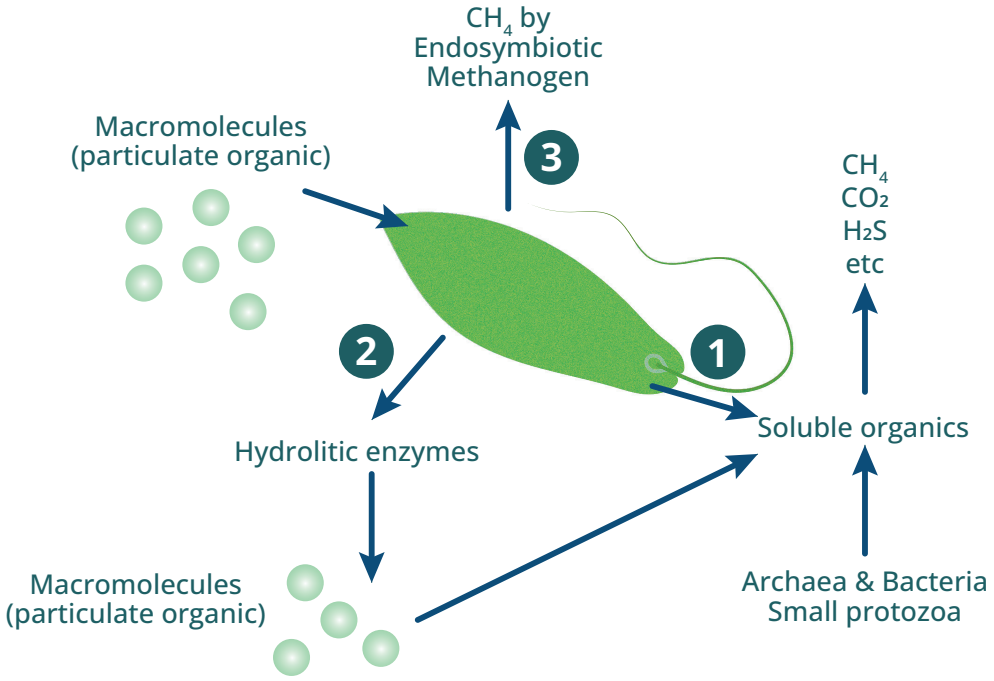


Figure 73: Hypothetical scheme: the role of protozoa in anaerobic digestion of complex organics (Adapted from Prabhakaran *et al.*, 2016).

role of protozoa in relation to abundance and activity of bacteria.

Additionally, protozoa may have a functional role in the biodegradation process. The significance of protozoa in anaerobic environments has been less studied with respect to their role in biodegradation. In rumen protozoa has been reported to enhance the degradation of organic matter by direct uptake. Many anaerobic protozoa have been identified, which contain endosymbiotic methanogens **(Embley and Finlay, 1993; Fenchel and Finlay, 2018)**. Mostly found in ciliates, they are also occasionally present in other eukaryotes such as amoebes and flagellates. The basis of the symbiotic relationship between ciliates and methanogenic archaea lies in the ciliate's metabolism. Instead of mitochondria, ciliates have hydrogenosomes, where they ferment pyruvate into acetate and hydrogen gas. Without methanogenic endosymbionts, this hydrogen gas can accumulate into concentrations high enough to thermodynamically inhibit pyruvate fermentation. In the symbiosis with methanogens, the hydrogen is consumed quickly and the fermentation reaction is feasible (**Figure 72**). The number of methanogens in ciliates varies, with 300-400 individual cells in *Metopus-palaeformisto* and 6,000-10,000 in *Metopus-contorus*, but with a relatively constant total volume of approximately 2% of the host. The methanogens and hydrogenosomes are disk shaped and stacked with alternating methanogens and hydrogenosomes, ensuring a close physical contact (**Figure 73**) **(Fenchel and Finlay, 2018)**. On the significance of this relationship, Fenchel and Finlay wrote:

"Taking into consideration (1) that methanogenic symbionts do not apparently occur as free living, (2) that they have many special adaptation to life as endosymbionts, and (3) that aposymbiotic ciliates apparently cannot be re-infected with methanogens, it seems to indicate that the endosymbiotic methanogens have approached the status of organelles." **(Fenchel and Finlay, 2018)**.

The quantitative role of endosymbiotic methanogens in protozoa in methane production in natural habitats is estimated relatively low calculated to be at 3% of total methane pro-

duction. In environments rich with sulphate and organic matter, the role is bigger, with experimentally measured values of on average 20% up to 80% of total methane production **(Fenchel and Finlay, 2018)**. However, the role of protozoa with endosymbiotic methanogens in AD has not yet been studied.

In a study done on a lab-scale UASB, **Priya *et al.* (2008a)** studied the community dynamics of protozoa by observation and determination by microscope. They could identify the ciliates *Metopus, Cyclidium* and *Colpoda*, and the flagellates *Menoidium, Rhyncomonas*, and *Bodo* on genus level. Correlations of up to R^2 = 0.92 between gas production and protozoa concentration were found. They propose this effect might come due to hydrolytic enzyme excretion, or CH_4 production by endosymbiotic methanogens **(Prabhakaran *et al.*, 2016)**.

In a comparable experiment by **Priya *et al.* (2008a)**, in a lab-scale continuous stirred tank anaerobic reactor fed with sodium acetate or sodium oleate, it was found that during the start-up phase, up to 10^6 protozoa per ml were observed, mostly ciliates and flagellates. A correlation between COD degradation and ciliate density was found **(Priya *et al.*, 2008a)**.

Matsubayashi *et al.* (2017) cloned 164 eukaryotic 18s rRNA genes out of five anaerobic digester samples. After sequencing, they found 66% fungi, 14% *Alveolata*, 11% *Viridiplantae*, 6% *Metazoan*, 1% *Amoebozoa*, 1% *Rhizaria*, and 1% *Stramenopiles*. An interesting group they found were the *Cryptomycota* or *Rozellomycota* **(Matsubayashi *et al.*, 2017)**.

Culture-independent analysis

It is clear that the research of eukaryotes in AD is still in its infancy and that many blind spots exist. The two methods, determination by microscopic observation and next-generation sequencing, both have their drawbacks. The determination of eukaryotes based on microscopic observation is time-intensive and sensitive to observer bias. Next generation sequencing, however, has a primer bias **(Kounosu *et al.*, 2019)** and often insufficient databases.

Additionally, taking in account the close genetic relationship between free-living and endosymbiotic methanogens **(Beinart *et al.*, 2017)**, NGS cannot discriminate between the two groups of methanogens. It must be mentioned that the studies working with microscopic determination also did not study the endosymbionts.

Outlook

Anaerobic protozoa can provide a highly relevant and potentially significant contribution to the anaerobic digestion process. However the presence and contribution of this diverse group of eukaryotes in anaerobic fermenters is rarely considered in studies on the microbiome of these systems. Their method of action could be in the direct biodegradation of substrates as well as the in the recycling of cells and nutrients through grazing. Before considering biostimulation and/or bioaugmentation of anaerobic protozoa, first a better understanding needs to be built on the current role of this group of organisms in the AD microbial ecosystem. Reliable analysis and interpretation still provide bottlenecks to be overcome in analysing protozoan presence, abundance, diversity and functionality. Apart from this, it is also important to uncover their interaction with the other micro-organisms present. Especially the impact of grazing action on the bacterial population and the role of endosymbiotic methanogens in the bioconversion of substrates should be mentioned.

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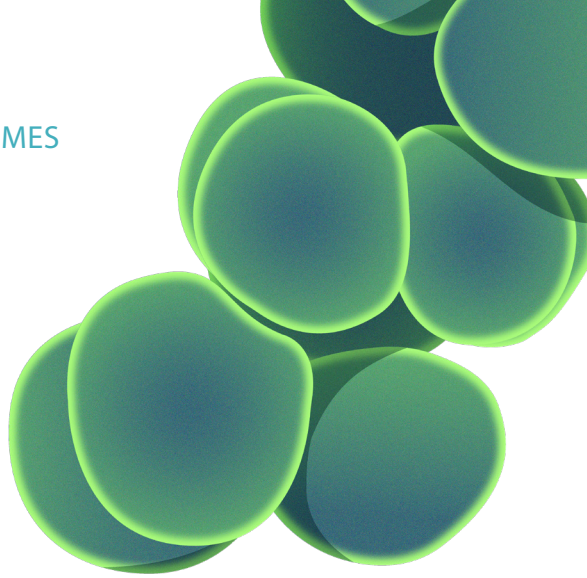

06

Manipulability of anaerobic microbiomes

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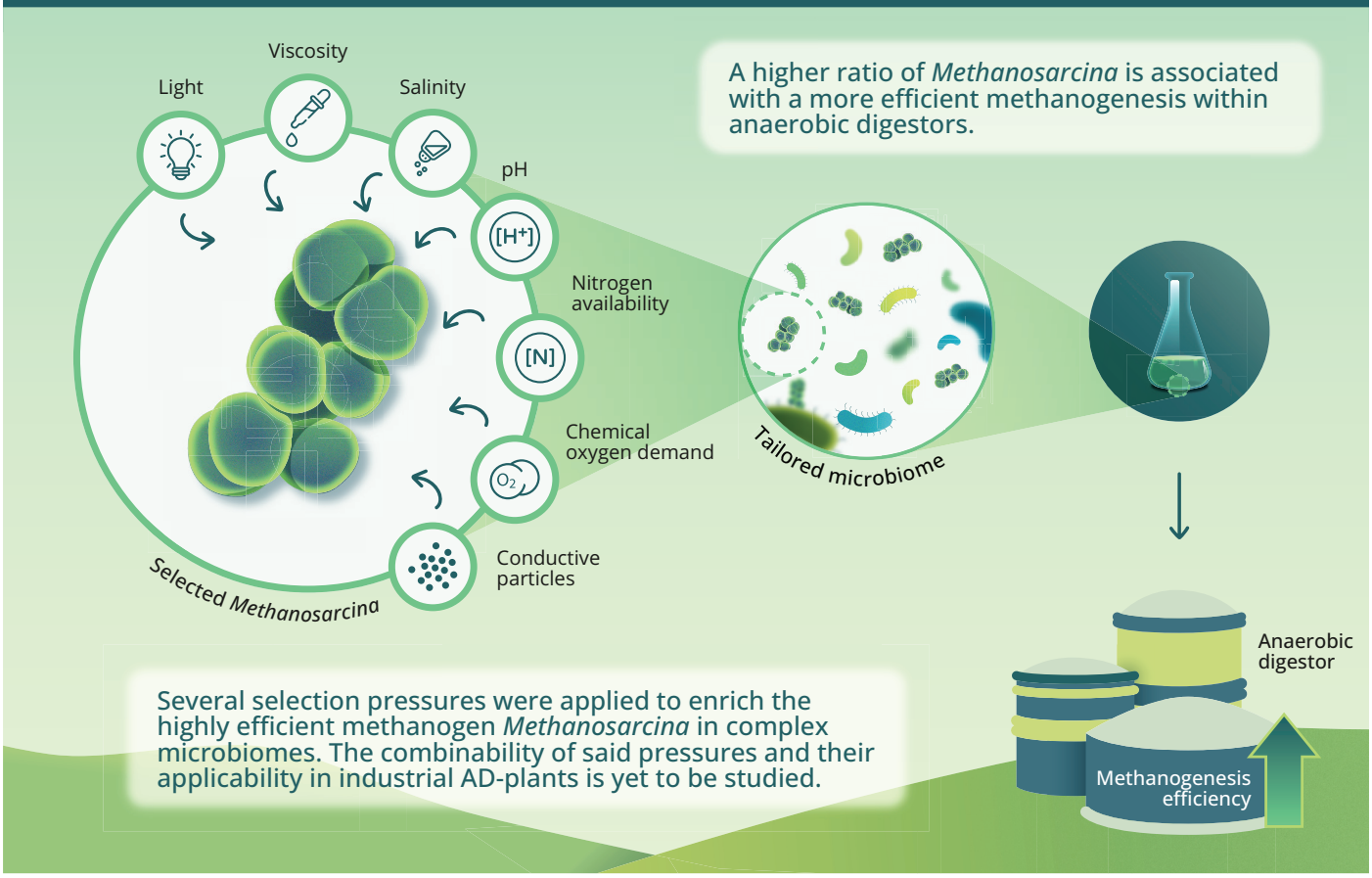
Abstract:

The chapter presented here comes largely from the postdoc thesis by Christian Abendroth. The work was submitted as a cumulative work. It addresses selected examples of microbiome engineering, especially with focus on the methanogen Methanosarcina and the phenomenon of direct interspecies electron transfer (DIET). The importance of *Methanosarcina* as a high-performance methanogen is highlighted. Multiple selection pressures are highlighted, which might allow the enrichment of *Methanosarcina*. A very promising selection pressure, which has been addressed only scarcely in the existing literature is light. Based on few outstanding research articles, a hypothesis is proposed, which indicates a relation between *Methanosarcina*, light and DIET. Apart from this, further options for microbiome engineering are discussed briefly. This includes the combination of dark fermentation and anaerobic digestion based on a two-stage approach. Two-stage digestion might open-up the possible production of hydrogen and multiple organic acids next to methane. Apart from this, pre-treatment is highlighted as a tool for microbiome manipulation as well. For example, MAP-precipitation (Magnesium-Ammonium-Phosphate

precipitation) and N-stripping could help to control the N-level in anaerobic digester plants, which also affects the underlying microbiome. Although microbiome manipulation is of high interest for the biorefinery industries, it is difficult to control due to the underlying complexity. In this regard, the present chapter de-scribes the complexity of anaerobic microbiomes in respect to taxonomic profiles and microbial interaction patterns. This goes hand in hand with statements from **Abendroth *et al.* (2022)** given in a recent conference article. Pascal Otto, Christina Dornack and Christian Abendroth co-authored this conference article and presented it in the frame of MICRO4BIOGAS. This focus of this article is microbiome manipulation as well. With the title "microscopic heroes of the biogas branche", the article was presented on the German "*Rostocker Bioenergieforum*". To make this work accessible to a broader audience, a translated version has been merged with parts from Christian Abendroths postdoc thesis. The article deepens the understanding of the complexity of anaerobic microbiomes and highlights multiple microbes, which are promising targets for microbiome engineering. It relates to anaerobic fungi, the so-called CRP-group (nanobacteria), bacteriophages and unicellular eucaryotes. Also, the extraordinary energy metabolism of anaerobic microbiomes is highlighted. Some organisms in anaerobic microbiomes work at the energetic minimum, which is also important for microbiome manipulation, especially in respect to syntrophic interaction.

Anaerobic microbiomes and their functionality within AD-plants can hypothetically be reprogrammed and tailored through an increase in the ratio of *Methanosarcina,* influenced by diverse parameters.



6.1 Introduction

The chapter presented here comes largely from the postdoc thesis by Christian Abendroth. The work was submitted as a cumulative work. The introduction and the discussion part itself have not yet been published and are therefore well suited as part of the present roadmap. Although the postdoc thesis itself was not part of the MICRO4BIOGAS project, it fits very well with the goals set in the project, especially regarding the application of light and conductive particles to manipulate anaerobic microbiomes. Therefore, it was decided to be included in a marginally adapted form.

Based on collaboration of Pascal Otto, Christina Dornack and Christian Abendroth, a conference article was presented in the frame of MICRO4BIOGAS, which addresses microbiome manipulation as well. With the title *"Microscopic heroes of the biogas branche"*, the article was presented on the German *"Rostocker Bioenergieforum"*. To make this work accessible to a broader audience, a translated version has been merged with the here presented parts from Christian Abendroth's postdoc thesis. The original title of the conference article by **Abendroth *et al.* (2022)** is *"Mikroskopische Helden der Biogasbranche"* and it can be found in *"Schriftenreihe Umweltingenieurwesen Band 110. Tagungsband - Dialog Abfallwirtschaft & 16. Rostocker Bio-*

energieforum", 233 – 241, 2022. https://doi.org/10.18453/rosdok_id00003615.

6.2 The AD microbiome

For many people, the word microbiome may seem highly innovative at this point. The word is a combination of the terms "Micro" and "Biome", which is related to a characteristic microbial population in a defined habitat. We find microbiomes everywhere around us. In the soil, in the air, in water and in countless sub-niches. In recent years, a number of popular scientific books have appeared on this subject, especially with regard to medicine. Examples are *"The Silent Power of the Microbiome"* by Jens Lauterzeller (2021), *"The Microbiome"* by Robynne Chutkan (2017) or *"Intestinal bacteria as a key to health"* by Katharina Zschocke (2019). In fact, the microbiome has been researched since the 1990s. It was introduced in 1988 by Whipps et al. in connection with plant diseases. Research into the microbiome gained momentum with the development of technologies for high-throughput sequencing ("next generation sequencing"), which first came onto the market in 2005 **(https://the-dna-universe.com, visited on March 18, 2022)**. From that point on, it was possible to read thousands of DNA sequences in a short time. This allowed an insight into the microbial diversity of numerous microbiomes. Microbiomes are now being studied in all areas of the world, including water **(Bruno *et al.*, 2018)**, the soil **(Geisen, 2021)** and the air **(Leung *et al.*, 2021)**. Increasingly unusual places are being explored for the occurrence of microorganisms. There are scientific journals that focus exclusively on specific microbiomes. Examples of this are the journals *"Microbiome"*, *"Human Microbiome Journal"* or *"Environmental Microbiomes"*. The IG Nobel Prize for Ecology was recently awarded for research into the chewing gum bacteriome **(https://improbable.com/ig/winners/#ig2021, accessed on 03/18/2022)**. In addition, interest in anaerobic microbiomes, such as those found in biogas plants, is increasing. Search queries on common databases such as Web of Science or NCBI already list several thousand entries under the keywords "anaerobic digestion" AND "microbiome". The AD-microbiome is due to the high number of microbial species in combination with its technological relevance and its potential for a biobased industry a great playground for microbiome researchers all over the world. A recent review by **Berg *et al.* (2008)** mentions the money that is spent on microbiome research, stating that US$1.7 billion were spent during the last decade in medical research. Microbiome associated research activity is less intense in the field of agronomy for now. However, **Berg *et al.*** highlights that *"Agricultural products based on the microbiota are one of the fastest growing sectors"*. In this relation, a tremendous effort is made to understand the microbiomes that are involved in AD-processes. A simplified overview of the structure of anaerobic microbiomes can be found in the works of **Sundberg *et al.* (2013)** and **Abendroth *et al.* (2015)**, in which microbiomes from sewage sludge digestion and co-digestion are distinguished. Additionally, **Abendroth *et al***. highlights differences between CSTR systems and leach-bed systems. Both authors distinguish between bacterial and archaeal key-players. In simple terms, it can be said that bacteria are responsible for the first 3 AD phases (hydrolysis, acidogenesis, and acetogenesis). Methanogenic archaea are key players in the last phase (methanogenesis). Bacteria hydrolyze complex polymers in the first phase and convert them step by step into organic acids during acidogenesis. Eventually, all organic acids are converted into acetate, which is the main substrate for methanogenesis **(Robles *et al.*, 2018)**. In the first three stages, the following bacterial phyla are abundant: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Chloroflexi, Spirochaetes, Thermotogae and Synergistetes. Among them, particularly Chloroflexi and Proteobacteria are more abundant in sewage sludge digestion than in co-digestion **(Sundberg *et al.*, 2013; Abendroth *et al.*, 2015)**. Spirochaetes are abundant in leachate from leach-bed systems and in sewage sludge digesters as well. In contrast, Spirochaetes are not abundant in CSTRs, very like due to the high percentage of total solids (TS). Usually, CSTRs show very high ratios of Firmicutes and Bacteroidetes.

The latter one is abundant in sewage sludge digestion and leachate from leach-bed digestions as well. **Sundberg *et al.*** and **Abendroth *et al.*** describe further that among the genera of methanogenic archaea, particularly *Methanobacterium, Methanoculleus, Methanospirillum, Methanothrix,* (also known as *Methanosaeta*), *Methanobrevibacter, Methanothermobacter, Methanomethylovorans* and *Methanosarcina* are abundant. Among them, *Methanothrix, Methanothermobacter* and *Methanomethylovorans* are especially abundant in sewage sludge digestion. In contrast, *Methanobacterium, Methanoculleus, Methanosarcina* as well as *Methanobrevibacter* seem to be abundant in co-digesters. Again, **Abendroth et al.** describes differences between leachate from leach-bed systems and CSTRs. While *Methanoculleus* is particularly common in CSTRs, *Methanosarcina* dominates the leachate from leach-bed reactors. *Methanosarcina* stands our due to its metabolic diversity, as well as due to its robustness against multiple stressors, especially low pH, high concentrations of COD, high concentrations of salt and ammonia **(De Vrieze *et al.*, 2012)**.

6.3 Microscopic heroes of biogas plants

The heart of every biogas plant is the sludge it contains. Biogas plants may be technically more sophisticated, but the essential work steps of biogas formation remain with the microorganisms involved. New reactor configurations or processing techniques can of course help to make the work of these small workers, of which there are countless, easier. A good orientation value is 10^{10} microorganisms per milliliter **(Hardegen *et al.*, 2018)**. This means that more microorganisms are present in one milliliter of anaerobic sludge than there are people on our planet. In a 1000 m^3 system, there would already be 10^{19} microorganisms. If we manage to use this immense workforce sensibly, numerous employees would be ready on a microscopic scale to make our plans for a growing bioeconomy a reality. In recent years, some exotic microorganisms have been discovered in biogas plants, which could be relevant for research and industry in the future. A few outstanding examples are presented below.

Life at the energetic minimum: In recent years, some interesting microorganisms have been found, which make biogas plants a particularly exciting habitat. A topic that is relevant, especially for biochemists, is the enzyme "ATP synthase": So-called "syntrophic" microorganisms, which live at the energetic minimum, occur in anaerobic sludge. The key enzyme for providing energy in all living beings is ATP synthase. Put simply, it works like a hydroelectric turbine, which is driven by protons instead of water. Three protons are needed for each energy equivalent of ATP (adenosine triphosphate). There are two unusual features in the biogas process. In syntrophic microorganisms, the concentration gradient for protons is sometimes so small that three protons are not enough to produce energy. Nevertheless, ATP formation takes place. It is assumed that, contrary to general opinion, if the concentration gradient is small, ATP formation with a translocation of four or five protons is possible **(Schink & Stams, 2006)**. In addition, a special form of the enzyme is observed in the methanogenic archaea, which is driven by sodium instead of protons. Only a few microbial representatives are known to use this special ATP synthase. A detailed consideration of this was given in 2008 under the title *"The past and present of sodium energetics: May the sodium-motive force be with you"* **(Mulkidjanian *et al.*, 2008)**. The Na^+-dependent ATP synthase and deviating amounts in the proton translocation make biogas plants an exotic habitat, which could give us more insight into the energy metabolism in living beings in the future.

Restricted metabolism, but efficient in degradation? Bacteria of the CRP group (*"Candidate Phyla Radiation"*) are also of interest to biochemists. Here, the *Gracilibacterium* (BD1-5) can be highlighted. This has recently been observed in the mining of petroleum contamination in the Gulf of Mexico **(Quéméneur *et al.*, 2019)**. Due to its small size, it is called nanobacteria. It is not viable on its own, impor-

tant metabolic pathways are missing and it is assumed that it is docked to other bacteria and kept alive by their metabolic products. The fact that other bacteria go to the trouble of keeping *Gracilibacterium* alive while degrading poorly degradable substrates could be linked to special degradative abilities of these nanobacteria. No concrete data are known about this. In this context, the *Gracilibacterium* becomes interesting for the biogas industry, since it was found in association with methanogenic archaea **(Quéméneur *et al.*, 2019)**. Would it be possible that *Gracilibacterium* is involved in the degradation of complex substrates in the biogas process in a methanogenic microbiome?

Anaerobic fungi: The existence of anaerobic fungi has only been known since the 1970s. **(Hess *et al.*, 2020)**. This is surprising against the background that these are easily recognizable under a conventional light microscope and have a very characteristic growth form. It is surprising that these so-called *"Neocallimastigomycota"* are involved in syntrophic processes. Syntrophic processes between archaea and bacteria have been described in a large number of articles, but anaerobic fungi have hardly been discussed. Nevertheless, it has already been pointed out in the literature that the enrichment of anaerobic fungi in biogas plants (bioaugmentation) would make it possible to increase the process output. In addition to their involvement in syntrophic processes, a possible increase in the degradation of lignocellulose is also pointed out **(Dollhover *et al.*, 2015)**. A key component here is a highly specialized cellulosome. A cellulosome refers to a multi-enzyme complex, which is largely unexplored with regard to anaerobic fungi. The dynamic use of the cellulosome is still outstanding in anaerobic fungi. It is found anchored to the cell wall where it is actively carried into the substrate by hyphae. Anaerobic fungi combine this degradation strategy with free (non-anchored) cellulosomes and a variety of so-called CAZymes (carbohydrate active enzymes) **(Hess *et al.*, 2020)**.

Electroactive microorganisms: In 2005, **Reguera *et al.*** in the journal *"Letters"* of the publishing group *"Nature"* addressed the microbial ability to transmit electrical energy through cable-like structures. These so-called nanowires were first described for bacteria, although the presence of electrically conductive pili has now also been demonstrated in archaea **(Walker *et al.*, 2019)**. It is now known that it is even possible to transfer electrons without nanowires, provided conductive structures are available (such as magnetite). Microorganisms adhere to conductive structures to chemically reduce other organisms (e.g., methanogenic archaea) via these structures. This mechanism was described in an article by **Cheng & Call (2016)**, among others, and is referred to in specialist literature as the direct interspecies electron transfer (DIET) effect. The great advantage of directly passing on electrons is that syntrophic bacteria pass on the electrons necessary for methane formation to archaea without releasing hydrogen (H_2) in the process. This is advantageous for the process, since syntrophic degradation can be inhibited at high H_2 partial pressures. The inhibitory influence of hydrogen can be minimized by the DIET.

Biofilms and nanostructures adapted to impurity: In 2021, the authors of this chapter were involved in a practical study in which the capillary carbon CarboFerm® was used in industrial biogas plants **(Heitkamp *et al.* 2021)**. According to the manufacturer LUCRAT GmbH, this is a conductive carbon, which is interspersed with the smallest capillaries. The use of this supplement resulted in a process improvement in the form of a reduction in the concentration of organic acids. This result was completed by analyzing the underlying taxonomic profiles. The search for typical microorganisms for the DIET effect followed. Contrary to what the authors expected, the genus *Methanoculleus* predominated among the methane producers directly in the capillary carbon. This genus has not previously been associated with DIET. In this context another interesting study from **Salvador *et al.* (2017)** can be highlighted. **Salvador *et al.*** achieved an increase in performance based on carbon-based nanotubes (*"carbonanotubes"*). The increase in performance was demonstrated in pure cultures at the metabolic level and no evidence of DIET was found. This

raises the question of whether the capillary carbon used (CarboFerm®) in **Heitkamp *et al.* (2020)** was related to similar effects as in Salvador et al. There is still no precise answer to this question. This is due to the fact that it is very difficult to differentiate between adsorption-based effects, DIET and nanostructure-based effects.

In the study by **Heitkamp *et al.*** it is also interesting that the comparison of the taxonomic profiles implies an adaptation to disturbing substances. In a direct comparison to the surrounding mud, there was a significant increase in the amount of Halobacterota, Halanaerobiaeota and Acidobacteriota. This indicates harsh conditions due to the accumulation of salts and acids. All other groups of microorganisms typical of biogas plants were also present. Thus, the high evolutionary pressure in the immediate vicinity of biochar could favor the formation of robust microorganisms in biogas plants.

Methanogenic eukaryotes: In 2019, a study was published in the journal Nature Microbiology with a description of the so-called iron-only nitrogenase **(Zheng *et al.*, 2018)**. Among other things, this enzyme enables the species *Rhodopseudomonas palustris* to form methane. This result was unexpected since the described ability is usually attributed to archaea and not to bacteria. It may be all the more surprising that we now know that eukaryotes, i.e. organisms with a cell nucleus, are also involved in methane formation. In 2017, a paper by **Matsubayashi *et al.*** showed that in four different stages of rotting, the microbiome consisted of 0.1% to 1.4% eukaryotes. A high biodiversity was found with the representatives for Fungi, Alveolata, Viridiplantae, Amoebozoa, Rhizaria, Stramenopiles and Metazoa. In 2019, Nature Publishing Group's journal Scientific Reports found that the eukaryotes Cyclidium, Platyophrya and Subulatomonas correlate with chemical oxygen demand (COD) and suspended solids concentration in upflow anaerobic reactors (UASB reactors) **(Hirakata *et al.*, 2019)**. This indicates that eukaryotes are actively involved in degradation processes. This assumption is supported by the observation that methanogenic endosymbionts have been found in some eukaryotes. An outstanding example is the species *Metopus palaeformis*. These are unicellular eukaryotes (protista), which are assigned to the phylum Alveolata. The authors Fenchel and Finlay found numerous methanogenic archaea and organelles for storing hydrogen (hydrogenosomes) inside these protozoa. The specific function of eukaryotes in anaerobic microbiomes is largely unexplored, with the presence of hydrogenosomes and methanogenic archaea indicating syntrophic relationships.

Bacteriophages: In addition to the methanogenic eukaryotes, bacteriophages in methanogenic microbiomes are also largely unexplored. Nevertheless, there are a few articles that deal with bacteriophages. In 2019, **Heyer *et al.*** investigated the proteome of biogas plants. 0.4% of the proteins analyzed were traced back to phages. Against the background that phages are significantly smaller than most other microorganisms, the result of **Heyer *et al.*** concludes that there is a high proportion of bacteriophages in the anaerobic microbiome. The authors further describe a hypothetical mechanism by which bacteriophages lyse both auxotrophic and prototrophic microorganisms. Auxotrophic microorganisms that cannot synthesize all the metabolites necessary for life would benefit from this. However, the role of bacteriophage appears to be more complex. In a study by **Zhang *et al.*** from 2017, a correlation between bacteriophages and prokaryotes was found. In a multivariate comparison, 40.6% of the fluctuations in the diversity of prokaryotes could be traced back to bacteriophages. Bacteriophages thus appear to have a key role in the structure and dynamic behavior of anaerobic microbiomes. This leads to the question of whether we will eventually be able to target phages to manipulate anaerobic microbiomes.

6.4 Is the microbiome programmable?

The previous chapter makes it clear that the medium "sludge from a biogas plant" is a real treasure trove with regard to exotic microorganisms. This raises the question of how we can specifically benefit from this knowledge in the future. Without a doubt, these findings are of high academic interest, but could there be any industrial benefit beyond that?

Even if a targeted neofunctionalization of microbiomes in biogas plants according to the state of the art has so far hardly been used, there exists an extensive knowledge about optimal nutrition and chemical process parameters. An important parameter for example is the C:N:P:S ratio, which, according to **Weiland (2009)** is optimal at an ratio of 600:15:5:1. Carbon and nitrogen are the basic elements that build up organic matter and cellular structures. Sulfur is needed for the growth of methanogens and several amino acids. Phosphate is an essential energy carrier **(Nsair *et al.*, 2020)**. Apart from these basic elements, there is a wide range of other nutrients that are important and extensively studied. The review article by **Nsair *et al.*** has recently summarized many process parameters such as optimal values for ammonia, sulfides, light and heavy metals (e.g., Calcium, Cobalt, Molybdenium, Nickel, Zinc, Iron and other). Apart from the optimal feedstock composition, it is of high importance to choose the right environmental parameters. The pH is usually kept around 7 and especially low pH-values are inhibiting involved methanogenic archaea. Moreover, it was found that there is a correlation between pH, undissociated volatile fatty acids (VFAs) and process inhibition. Especially undissociated VFAs have an inhibitory effect. At lower pH-values a higher ratio of a given VFA is protonated. Hence, a given acid concentration can cause varying pH-dependent inhibition **(Kroiss and Svardal, 2005)**. To prevent a low pH-value, the organic loading rate needs to be chosen carefully and different loading rates need to be applied for different substrates. To give here an example: agricultural waste is, according to **Nasir *et al.*, (2020)** slower degraded than food waste, as it contains higher amounts of lignocellulose. In turn, VFAs are faster accumulated during the degradation of food waste, which in turn can result in a pH drop. A low pH increases the concentration of protonated VFAs and eventually inhibits the process **(Kroiss and Svardal, 2005)**. When nitrogen enriched substrates, such as chicken manure, are applied, high ammonia concentrations prevent low pH values, which allows methane formation even with unusual high substrate concentrations **(Abendroth *et al.*, 2015)**. Hence, the sensitivity of AD plants to VFAs also depends on the buffer capacity. Although ammonia can stabilize the pH, this does not mean that methanation at high level of ammonia occurs effectively. In fact, ammonia is a known inhibitor for anaerobic digestion. It can pass the membrane of several microorganisms (e.g., *Methanobacterium formicicum* or *Methanospirillum hungatei*), which in turn leads to a loss of cytoplasmic K^+ ions **(Yenigün and Demirel, 2013)**. Another substance that is important for the buffer capacity is bicarbonate. Even if high concentrations of bicarbonate can have inhibiting effects as well **(Lin *et al.*, 2013)**, bicarbonate buffer systems are generally acknowledged as important for the AD process **(Kasali *et al.*, 1989; Lin *et al.*, 2013)**. The bicarbonate buffer system can be created just from process water and carbon dioxide, which is formed during fermentation. In fact, it's the same buffer system as in our blood, which is, however, strongly affected by pH. A pH < 7.35 or > 7.45 can rapidly impair its buffering capacity **(Rhoades and Bell, 2012)**. A low pH shifts the equilibrium of the buffer system in the direction of the CO_2, so that the buffer is driven out. As low pH-values impair the puffer system and result in higher levels of protonated (inhibiting) VFAs, sharp drops in the pH value should be avoided as far as possible. Because of this, the German biogas industry elaborated a new parameter, the so-called FOS/TAC. It describes the ratio of organic acids (FOS) to the inorganic buffer capacity (TAC). The FOS/TAC can now also be found in international research articles, so that this parameter is developing into an in-

ternationally recognized process parameter (Lili *et al.*, 2011; Costa *et al.*, 2016). Finally, it needs to be highlighted, that the ratio of dissociated and undissociated substances is also affected by temperature. The literature describes AD-processes at thermophilic-, mesophilic-, and as well at psychrophilic temperatures (Kroiss and Svarland, 2005). Psychrophilic temperatures are particularly interesting with respect to high ammonia levels. As lower temperatures can shift the equilibrium from ammonia to the less toxic ammonium, literature discusses the suitability of anaerobic digestion at low temperatures. The positive effect of lower ammonia levels at lower temperatures is counteracted by the fact that the lower temperature also lowers the rate constant for methanogenesis (Garcia and Angenent, 2009). On the other hand, one can conclude that especially thermophilic AD-plants could be sensitive to ammonia-stress. The approaches to optimizing fermentation processes described here only scratch the surface of what is known about the influence of chemical parameters on the process. This section could probably be expanded into an entire book. Nevertheless, this section clearly indicates the immense knowledge about adaptation strategies that help to set high-performance processes in motion, even if the underlying microbiome is treated as a black box.

6.5 Microbiome engineering

It is a tempting idea to modify complex microbiomes to achieve a specific function. AD microbiomes could become faster and resilient, the risk of germs could be reduced, or new valuable substances could be produced instead of methane. The goal of high-value products from anaerobic digestion is partially in reach, even without fully resolving the AD-black box. This can be explained as follows: If particularly high-volume loads with easily digestible substrates are used, low pH values can be achieved, which inhibits methanogenic archaea. It is thus possible to carry out a separate hydrolysis/acidification without the accumulated metabolites being broken down by methanogenesis. Such an approach allows the enrichment of medium-length carboxylic acids (De Groof *et al.*, 2019) and hydrogen (Wang and Yin, 2018). Multiple articles have already demonstrated the possibility of combining dark fermentation with subsequent methanation (Abendroth *et al.*, 2017, Wang *et al.*, 2018, Wainaina *et al.*, 2019). If further fermentation- and pretreatment steps are applied, it is even possible to enrich valuable fatty acids such as caproic acid (Chen *et al.*, 2017).

It is further possible to combine methanogenic stages with pure culture approaches. An example is the production of succinic acid. It has been demonstrated that biogas can be purged with the help of the bacterium Actinobacillus succinogenes. If biogas is injected into a separate fermentation stage, A. succinogenes can capture CO_2. The fixed CO_2 is then co-fermented with sugar, which improves the efficiency of A. succinogenes in producing succinic acid (Gunnarsson *et al.*, 2014).

Apart from the two-stage approach, which separates methanogenesis from the phases of biogas formation, researchers are also interested in questioning, whether the methanogenic stage itself could be optimized. Even if there are only a few articles on this so far, some authors have tried to add powerful microorganisms directly to the methane stage (bioaugmentation). To give here a few examples: Mulat *et al.* (2018) used the strain *Caldicellulosiruptor bescii* to improve methane production from lignocellulosic biomass. Montusiewicz *et al.* (2020) used a microbial consortium from Yellowstone National Park (USA), which tends to an intracellular accumulation of heavy-metal ions to manipulate heavy metal concentrations. Mutschlechner *et al.* (2020) used a soil derived inoculum, which improved the methane production and counteracted common process failures.

Despite that there are some promising examples in respect to bioaugmentation, it remains questionable whether a long-lasting affect can be achieved on industrial scale. For microorganisms it might be difficult to

assert themselves in an alien environment against organisms that have already adapted to the domestic biome. In this relation, a recent article about bioaugmentation states that *"At present it is still unclear which factors determine the success of such a treatment"* **(Müller and Mahro, 2001)**. To make bioaugmentation a reliable method, new approaches and strategies are required. **Thompson *et al.* (2005)** in their work highlight that modern approaches in molecular biology and analytical chemistry allow a more targeted search for microorganisms that are suited for bioaugmentation. According to **Thompson *et al.*** this *"information-based approach"* is already more efficient than the former *"black-box"* approaches. Modern methods of molecular biology indeed had a major impact on the understanding of AD microbiomes. For just over a decade, next generation sequencing is being applied, beginning with the Roche 454 sequencing technology in 2005 **(Selzer *et al.*, 2018)**. This ushered in a flood of research articles trying to break down the black box of anaerobic microbiomes piece by piece. This major advancement is also highlighted by a recent article from **Laczi *et al.*, (2020)** entitled *"New Frontiers of Anaerobic Hydrocarbon Biodegradation in the Multi-Omics Era"*, where OMICs is a suffix that denotes the analysis of a population of elements with similar characteristics. Recent literature describes the application of metagenomics **(e.g., Bertucci *et al.*, 2019; Becker *et al.*, 2020)**, proteomics **(e.g., Bargiela *et al.*, 2015; Zhang *et al.*, 2015)**, transcriptomics **(Maus *et al.*, 2016; Gruninger *et al.*, 2018)** or metabolomics **(e.g., Bargiela *et al.*, 2015; Artegoitia *et al.*, 2017)**.

6.6 Resolving the AD black-box

As knowledge about taxonomic profiles of anaerobic microbiomes progresses, so does functional understanding. An important recent finding is the ability of several microorganisms to directly exchange electrons between each other. Since 2005 first research articles appeared, which indicated the existence of microbial nanowires that can transport electrons **(Reguera *et al.*, 2005; Gorby *et al.*, 2006; Reguera *et al.*, 2006)**. The discovery that by using conductive particles DIET is also possible without microbial nanowires, spread a gold rush mood for agents that can be used to stimulate direct interspecies Electron Transfer (DIET). Many of the tested materials are highlighted in a recent review by **Martins *et al.*, (2018)**. A particularly exotic article by **Beckmann *et al.*, (2016)** describes the synthesis of novel phenazine crystals, which are stored between microorganisms and there promote the direct exchange of electrons. The promising prospects for the use of DIET are based on an integrated model with syntrophic reaction communities. The review article from **Schink and Stams (2006)** gives a comprehensive insight into syntrophic relations within anaerobic microbiomes. It describes that the degradation of several metabolites, such as acetic acid, propionic acid, butyric acid, or other metabolites occurs under thermodynamically unfavorable conditions, with very little energy gain for the involved microorganisms. From a thermodynamic point of view, the reverse reaction that consumes hydrogen is preferred. However, if hydrogen is actively removed from the system, the hydrogen consuming reverse reaction can be inhibited. In syntrophic communities, so-called hydrogenotrophic methanogens form methane from hydrogen and carbon dioxide, which ensures low levels of hydrogen. In turn, this allows the degradation of metabolites, such as acetic-, propionic-, or butyric acid. The hydrogen that is generated when these acids break down serves as a mediator for electrons. With the help of microbial nanowires, the electrons to be given off can be passed on to hydrogenotrophic methanogens without actually releasing hydrogen. This in turn, facilitates the degradation of the respective VFAs **(Xu *et al.*, 2018, Zhang *et al.*, 2019)**. The concept of DIET also explains positive effects that are observed when electricity is used for bio stimulation. In 2005 the concept of so-called microbial electrolysis cells (MECs) has been introduced by two institutions in parallel **(Liu *et al.*, 2005; Rozendal and Buisman, 2005)**. The idea behind MECs is that the breakdown of organic substances leads to an excess of electrons, which can be transferred to an anode. With a low starting voltage (0.2 - 0.8V),

these electrons can be conducted to a cathode. The degradation processes also lead to the release of protons, which diffuse towards the cathode. A catalytic surface (usually platinum) allows electrons to be transferred to the protons to generate hydrogen **(Kadier *et al.*, 2016)**. Although the idea was originally developed for the synthesis of hydrogen, it is now known that the microbial methane formation can also be stimulated with the help of electrodes **(Yanuka-Golub et al., 2019)**.

6.7 Combined application of *Methanosarcina* and DIET

6.7.1 General remarks on Methanosarcina

Current research suggests that *Methanosarcina* may be an efficient high-performance methanogen. In the present chapter several articles address the enrichment of the genus *Methanosarcina* **(Hardegen *et al.*, 2018; Abendroth *et al.*, 2020; Heitkamp *et al.*, 2021)**, which is why a separate sub-section is dedicated to this genus at this point. The methanogen *Methanosarcina* is a genus of methanogenic archaea belonging to the phylum Euryarchaeota (NCBI: txid2207). While mainly bacteria play a role in the acidification stages (as discussed in the previous chapter), methanogenic archaea are the key players in methanogenic stages. Currently, three metabolic pathways for methane formation are known (KEGG, map0068), whereby methane from H_2 / CO_2 (hydrogenotrophic pathway), acetate (acetoclastic pathway) or methanol / methylamines / methyl sulfide (methylotrophic pathway) is produced **(Vanwonterghem *et al.*, 2016)**. Methanogens, which are described as particularly common and dominant in biogas plants, belong to the genera *Methanoculleus, Methanosarcina, Methanobrevibacter, Methanobacterium, Methanothermobacter* and *Methanosaeta* **(Abendroth *et al.*, 2015; Sundberg *et al.*, 2013)**, whereby it should be mentioned here that *Methanosaeta* was renamed *Methanothrix* some time ago **(Garrity *et al.*, 2011)**. Even if the literature already provides a lot of information on methanogenic archaea, the causes that lead to the accumulation of certain methane-forming archaea are not sufficiently investigated. The genus *Methanoarcina* has been of particular interest for some time, as some species of this genus dominate all metabolic routes of methane production **(Buan *et al.*, 2011)**. *Methanosarcines* form very characteristic clumps, which allow a good differentiation from other methane producers. They are often described as non-motile **(e.g., Shimizu *et al.*, 2011; Kern *et al.*, 2016; Oshurkova *et al.*, 2020)**, although it was suggested that they can move due to gas filled vesicles **(Meader *et al.*, 2006)**. In addition, there are indications in the literature that species of the genus *Methanosarcina* are high-performance organisms which could be of particular interest in the industry.

6.7.2 Industrial relevance of *Methanosarcina*

Methanosarcina is a genus that can be found in many biogas plants. Above all, they are found in high-performance fermenters, which use co-substrates to achieve a high concentration of chemical oxygen demand (COD) and organic acids **(Abendroth *et al.*, 2015)**. In contrast, the genus *Methanothrix* (formerly *Methanosaeta*), is mainly found in digestion towers of sewage treatment plants **(Sundberg *et al.*, 2013; Abendroth *et al.*, 2015)**. The activated sludge used as a substrate can be classified as low-energetic compared to substrates such as corn silage or sugar beet silage. This is due to the fact that over 90% of the COD is broken down in the activated sludge process **(Fang *et al.*, 1993)**. A simple explanation for the dominance of the genus *Methanothrix* in digestion towers is that it is able to absorb acetic acid more efficiently, as is the case with the genus *Methanosarcina* **(De Vrieze, 2014)**. This can be explained very well on the biochemical level.

While *Methanothrix* metabolizes acetate efficiently at a concentration of KS = 0.5 mM, this value is between 3 - 5 mM for *Methanosarcina*. *Methanosarcina* thus absorbs acetate much more inefficiently but metabolizes it more efficiently. The reason for this lies in the different activation mechanisms for acetate. *Methanthrix* uses the acetyl-CoA synthase for this, while *Methanosarcina* uses the acetate kinase. The maximum speed for the acetate kinase of *Methanosarcina thermophila* is 660 µmol mg^{-1} min^{-1} and the acetyl-CoA synthase of *Methanothrix soehngenii* reaches 55 µmol mg^{-1} min^{-1} **(Jetten *et al.*, 1991)**. *Methanosarcina* is therefore about 12 times faster than *Methanothrix*.

As early as 2012, in a review article by **De Vrieze *et al.*** under the title *"Methanosarcina: The rediscovered methanogen for heavy duty biomethanation"*, the genus *Methanosarcina* was highlighted as a high-performance organism for biogas plants. Key aspects to be mentioned in this context are robust behavior at high concentrations of salt and ammonium. It was further emphasized that *Methanosarcinales* are active in a broad temperature range and that, as already mentioned above, they are robust with high loading rates. Since then, several other beneficial characteristics have been described for the genus *Methanosarcina*. These include, for example, the ability to use diverse substrate sources **(Lackner *et al.*, 2018)** and the degradation of some toxic substances, such as tetramethylammonium **(Chen *et al.*, 2017)** or phenols **(Poirier *et al.*, 2018)**. Moreover, *Methanosarcina* shows a high robustness against nanoparticles made of silver oxide, titanium oxide or zinc oxide **(Eduok *et al.*, 2017)**. Another special ability of *Methanosarcina* is the direct interspecies electron transfer (DIET), which is often described for genera *Methanosarcina* and *Methanothrix* **(Martins *et al.*, 2018)**. Since the genus *Methanothrix* is more likely to be found in methane reactors with low levels of COD and organic acids, the industrial relevance of DIET in connection with the genus *Methanosarcina* should be pointed out.

6.7.3 Increasing the rate of direct interspecies electron transfer (DIET)

Interspecies electron transfer is the prerequisite for methanogenesis. The H_2 interspecies transfer (HIT), in which hydrogen is used as an electron carrier, has been particularly well investigated **(Sieber *et al.*, 2012)**. An alternative to HIT is direct electron transfer between species (DIET). For a direct transfer of electrons between bacteria and *Methanosaeta* (*Methanotrix*) *harundinacea*, cellular outlets with metal-like conduction properties for electrons are necessary **(Rotaru *et al.*, 2014)**. In contrast to **M. harundinacea**, DIET has been described several times for the genus *Methanosarcina*, which is based on the use of inorganic, extracellular metal surfaces **(Kato *et al.*, 2012; Liu *et al.*, 2012)**. An example is conversion of ethanol to acetate with the aid of ferrihydrite, as described by **Tang *et al.* (2016)**. In the respective work it was observed that depending on whether phosphate is added or not, the addition of ferrihydrite leads to the formation of magnetite or vivianite. During the degradation of ethanol to acetate by *Geobacter* there is an excess of electrons. Using magnetite or vivianite, *Geobacter* can conduct the surplus electrons via magnetite or vivianite to *Methanosarcina*, which is using the electron for the chemical reduction of CO_2.

DIET and Methanosarcina's ability to interact with extracellular, metallic, non-biological surfaces offer interesting application possibilities in industry. **Kato & Igarashi (2018)** described an acceleration of methanogenesis in methane reactors by adding iron oxide and sulfate. A similar observation was made by **Ye *et al.* (2018)**, where the addition of 20 g L^{-1} red mud (*"red mud"*) accelerated methane accumulation by 35.5%. At this point it should be emphasized that **Ye *et al.*** also observed a positive effect on hydrolysis & acidification.

However, the ability by *Methanosarcina* to DIET is not only relevant for the biogas industry. There are authors who highlight

Methanosarcina in connection with biological remediation. For example, **Holmes *et al.* (2018)** described that *Methanosarcina* has a high potential to contribute to uranium reduction in uranium-contaminated soils when acetate is added (Acetate-Promoted Groundwater Bioremediation). In connection with the interaction between *Methanosarcina* and metallic surfaces, *Methanosarcina* is therefore also described as being an indicator organism for contamination **(Pei *et al.*, 2018)**.

In a biogas plant, the microbial diversity is very high. About 300 different species can explain 80% of the process biocenosis of a biogas reactor **(Kirkegaard *et al.*, 2017)**. This high diversity of microorganisms forms a robust microbiome, which makes it difficult to accumulate a certain species on purpose **(Abendroth, 2018)**. Finding influencing parameters for the enrichment of the genus *Methanosarcina* is therefore a great challenge. In this context, it is helpful to know that different types of sludge from digestion plants naturally have a different profile of methanogenic archaea **(Abendroth *et al.*, 2015)**. The fact that digested sewage sludge accumulates species of the genus *Methanothrix* can be explained by the fact that *Methanothrix* is better suited to the uptake of acetic acid in low concentration ranges than is the case with the other genera. A value of 3000 mg acetic acid was given as the limit value **(De Vrieze, 2014)**. Above this acetic acid concentration, the high-performance methane-forming agents *Methanoculleus* and *Methanosarcina* occur more intensely, whereby the genus *Methanosarcina* is particularly interesting due to its ability for direct interspecies electron transfer. However, as viscous sludge tends to accumulate the genus *Methanoculleus* (for which DIET was not described so far) this raises the question how a *Methanosarcina* prevalence can be achieved with high acid loads?

A hint can be found in the publication by **Abendroth *et al.*, (2015)**: In contrast to thick sludge from stirred tank systems, the dry matter content (TS) in the leachate from leach-bed digesters is significantly reduced. In recent work by **Hardegen *et al.*, 2018**, there is an indication that the dry matter content has an influence on the profile of methanogenic archaea. A low percentage of total solids seems to favor the accumulation of the genus *Methanosarcina*. **Hardegen *et al.*** gradually increased the chemical oxygen demand (COD) in digested sewage sludge by adding liquid and solid co-substrates. In line with the expected value, there was a population shift. Digested sewage sludge charged with liquid biomass showed an increase in *Methanosarcina*. According to the work by **Abendroth *et al.* (2015)**, one could have expected an enrichment of the genus *Methanoculleus* in the experiments fed width solids by **Hardegen *et al.*** This expected population "shift" did not materialize, which might be explained by an accumulation of inhibiting substances. Since the presented experiments by **Hardegen *et al.*** did not reach a "steady state", the continuously changing conditions could also have caused a stressful situation that favored the accumulation of the genus *Methanosarcina*. As already described above, the genus *Methanosarcina* is efficient compared to other methane producers in dealing with stressful conditions. This statement is also in line with a recent publication by **Town and Dumonceaux (2016)**. Reactors that entered acidosis, were reactivated with an inoculum. This occurred with an increase in the genus *Methanosarcina*.

A first application for the observations made by **Hardegen *et al.*** could be given in a slowly increasing loading rate in sewage sludge digesters in combination with the use of conductive particles. The loading rate might be increased by switching from mono-digestion to co-digestion using additional co-substrates, which was also the case in **Hardegen *et al.* (2018).** In relation to the application of DIET, however, it would still have to be demonstrated that the processes described by **Hardegen *et al.*** results in a prevalence of the genus Methanosarcina that persists over a longer period. Since the interspecies electron transfer has been described for both the genera *Methanosaeta (Methanothrix)* and *Methanosarcina* **(Rotaru *et al.*, 2014)**, its use by adding electrically conductive particles is conceivable, regardless of whether additional biogenic co-ferments are used in addition to the sewage sludge, albeit the process would be much more efficient, if *Methano-*

sarcina would be enriched. A disadvantage of a targeted enrichment of *Methanosarcina* would be that there would have to be a continuously high concentration of organic acids. This contradicts the goal of maximum degradation and would require subsequent digestion stages to minimize the C-load. A solution here could be an anaerobic baffled reactor (ABF). ABF reactors are separated in several baffles. To start with, a high loading rate can be applied, which is decreasing continuously. The system is known to be resistant to an organic shock load, shows improved biomass retention and lower sludge yields **(Barber and Stuckey, 1999)**.

Apart from sewage sludge digestion (water treatment) it would also be interesting to refurbish existing high-performance biogas plants (typical co-digesters that ferment agricultural residues, food waste or municipal waste). One hurdle here is the typically high concentration of cells of the genus *Methanoculleus*. An exception is the leach-bed digestion, as leachate is typically enriched in *Methanosarcina*. However, systems with highly viscous sludge (continuous stirred tank reactors of plug flow fermenters) are favored by the genus *Methanoculleus* **(Abendroth *et al.*, 2015)**. The existing literature so far does not describe any approaches to suppress *Methanoculleus* in favor of the genus *Methanosarcina* in such systems. With regard to a targeted application of DIET, it would make sense to achieve a prevalence of the genus *Methanosarcina* in solids enriched reactors, fixed beds or biofilters. One way to achieve this is the application of very harsh conditions. As already described above, *Methanosarcina* shows a robust behavior against low pH-values, high concentrations of COD, salt, and ammonia. A possible experimental approach here would be the use of stressful situations (e.g., temperature shocks, cyclical changes in nutrient conditions or cyclical fluctuations in pH values), which are carried out directly in fixed beds, solid-rich reactors, fixed beds or biofilters. Particularly high ammonium content could be of interest here, since **Dai *et al.* (2016)** described an enrichment of the genus *Methanosarcina* in a laboratory experiment, using dehydrated TS-rich sewage sludge as a mono-substrate and causing ammonium stress (5,000 mgN L^{-1}). In this relation, the work from **Heitkamp *et al.*** must be highlighted. **Heitkamp *et al.*** applied biochar on multiple industrial CSTRs. Several of them had high nitrogen concentrations and three of them had even higher concentrations as described by **Dai *et al.*** (>5,000 mgN L^{-1}). But still, Heitkamp et al. described a surprisingly low amount of *Methanosarcina*. An explanation for this observation might be the fact that **Dai *et al.*** used digested sewage as seed sludge, which is typically enriched with the genus *Methanothrix*. With increasing loading rates, *Methanosarcina* has a selection advantage compared to *Methanothrix*. As CSTR reactors as the ones investigated by Heitkamp et al. are typically enriched in Methanoculleus **(Abendroth *et al.*, 2015)**, it might be harder for *Methanosarcina* to compete with *Methanoculleus*. It needs to be highlighted that the enrichment of *Methanosarcina* was possible in the work by **Dai *et al.*** despite high concentrations of solids, which contrasts with the works by **Abendroth *et al.* (2015)** and **Hardegen *et al.* (2018)**. In summary, it can be said at this point that it becomes clearer which selection pressures influence *Methanosarcina*, albeit this question has not yet been finally clarified. One should also ask oneself whether the combination of particularly harsh conditions (high concentrations of COD, salt, and ammonium) is the right method for enriching the genus *Methanosarcina*, as this could nevertheless reduce the overall efficiency of the system. Therefore, the search for suitable selection pressures to enrich the genus *Methanosarcina* continues. The addition of conductive particles itself could already improve the conditions for *Methanosarcina*, which is discussed in the following subsection.

6.7.4 Light harvesting and electroactive microbiomes

Another influencing factor that seems to favor *Methanosarcina* is light. In the second publication of the present section, a red biofilm was metagenomically analyzed. The biofilm developed unexpectedly on the inner wall of a transparent biogas reactor **(Abendroth *et al.*, 2020)**. At first, it was assumed that it could be algae. However, since

no DNA amplification of typical eukaryotic gene sequences was possible, the taxonomic profile based on 16S-rRNA gene amplicon high-throughput sequencing showed that the red colour can come from phototrophic bacteria. Typical phototrophic bacteria are sulfur bacteria, purple non-sulfur bacteria, green sulfur bacteria, and green and red filamentous anoxygenic phototrophic bacteria **(Frigaard, 2016)**. In the case of the red biofilm and based on 16S-rRNA gene sequencing, the genus *Rhodopseudomonas* (purple nonsulfur bacteria) in particular was highly abundant. Metagenomic sequencing indicated later that particularly *Rhodopseudomonas feacalis* was present **(Abendroth *et al.*, 2020)**. Several reports can be found on microalgae in relation to anaerobic digestion **(e. g., Ermis *et al.*, 2020; Xia and Murphy, 2016)**. However, those studies only refer to algae that are grown on digestate that had already left the methanogenic digester. Therefore, the study by **Abendroth *et al.*** is generally in agreement with the existing literature on the fact that no eucaryotic algae were found. In relation to phototrophic bacteria, one can now ask the question, which positive effects could be brought about by light in biogas reactors. One positive effect could be an oxygen release, as oxygen is a typical product from photosynthesis. In a recent study, the general belief that oxygen is toxic to biogas reactors has been refuted. It was emphasized that communalization of aerobic and anaerobic microorganisms is possible, and that the oxygen can even have a positive effect on the process efficiency **(Botheju and Bakke, 2011)**. In the presented work from **Abendroth *et al.* (2020)**, no biochemical processes have been described that indicate an oxygen release. In fact, a study from 2016 states that none of the known anoxygenic phototrophic bacteria can oxidize water **(Hanada, 2016)**, which makes it very unlikely that oxygen is naturally produced in biogas reactors. One might suspect that aerobic phototrophs could possibly be enriched under microaerophilic conditions. However, it has been shown in the case of known aerobic phototrophs that they require high oxygen concentrations, and that the photosynthesis rate is severely restricted when the amount of oxygen decreases **(Yurkov and Beatty, 1998)**. It is therefore highly questionable whether phototrophic bacteria could cause a release of oxygen. On the other hand, a recent study needs to be mentioned, which highlights the O_2 production by cyanobacterial mats. These mats are not fully understood as they involve a complex metabolic network resulting from a highly diverse microbial community **(Dick *et al.*, 2018)**. In addition to releasing oxygen to naturally induced microaerophilic conditions, cyanobacteria could also contribute to decrease the H_2S concentration. At H_2S concentrations > 200 µM, *Cyanobacteria* show the capability of performing a sulfide-dependent photosynthesis **(Cohen *et al.*, 1986)**. With H_2S having a molar mass of 34.082 g mol^{-1}, 200 µM corresponds to 0.034 mg L^{-1}. In practice, significantly higher H_2S concentrations are conceivable, with process inhibition at 22 mg L^{-1} in thermophilic and 50 mg L^{-1} in mesophilic reactors to be expected **(Haghighatafshar *et al.*, 2012)**. In the case of the work from **Abendroth *et al.* (2020)**, no *Cyanobacteria* were found in the phototrophic biofilm analyzed. However, it is known that *Cyanobacteria* can be present in anaerobic digesters. For example, **Abendroth *et al.* (2017)** described a high abundance of *Cyanobacteria* in separated acidification stages, although they were not able to differentiate between *Cyanobacteria* and chloroplasts from biomass. **Heitkamp *et al.* (2021)** observed *Cyanobacteria* as well. While indeed 0.3% of the observed reads corresponded to chloroplasts (PCC-6307), 1.6% of the reads were assigned to the *Cyanobacterium Cyanobium* **(Heitkamp *et al.*, 2021)**. In this relation it can be highlighted that a recent study found *Cyanobium* to be very sensitive to salt. With increasing concentrations of iron- and magnesium sulfate, *Cyanobium* was not able to survive **(Ermis *et al.*, 2020)**. In this relation it is of particular importance that in the here presented study by **Heitkamp *et al.*** *Cyanobium* was only increased in the sludge fraction, but not on the biochar that was used as a supplement, and which tends to adsorb salt and other inhibiting substances. Therefore, the study by **Heitkamp *et al.*** indicates that a combination of light and adsorptive materials could support the enrichment of *Cyanobacteria* such as *Cyanobium* in anaerobic digesters.

Besides H_2S, ammonia and ammonium are common inhibitors within anaerobic digestion. In general, ammonia and ammonium can only be degraded in the presence of oxygen. Wastewater treatment plants therefore activate sludge with oxygen, which not only improves the efficiency in removing substances that are difficult to break down, but also reduces the number of potential pathogens **(Aghalari *et al.*, 2020)**. An exception is the so called anammox bacteria. Anammox bacteria can use nitrite or nitrate instead of oxygen for the oxidation of ammonium **(Kuenen, 2008)**. Apart from ammonia oxidizing bacteria also ammonia oxidizing archaea are known **(He *et al.*, 2018)**. Therefore, the view that ammonia / ammonium oxidation takes place exclusively anaerobically has meanwhile been rejected. With the discovery of new ways of breaking down ammonium, one might wonder whether light could also play a role in its breakdown. Although only analyzed in freshwater, a recent study by **French *et al.* (2012)** describes that white and blue light seems to rather inhibit both, ammonium oxidizing bacteria and archaea. However, it must be considered that the microbial density in anaerobic digesters is much higher than in freshwater. Therefore, the involved microbial community might be very different. Also, DIET might play a role. Even if it has not yet been investigated and is very hypothetical, it would be conceivable that ammonia/ammonium oxidizing microorganisms are protected by pigmented microorganisms and receive electrons from them via conductive structures. In support of such a hypothetic syntrophic community, it can be emphasized that syntrophic reaction communities seem to play an important role in the breakdown of nitrogen-rich substrates **(Yan *et al.*, 2020)**. Although it is involved in nitrogen fixation rather than ammonia/ammonium breakdown, the recent characterization of the so-called iron-only nitrogenase should be emphasized. This nitrogenase could play an important role in such hypothetical electroactive, phototrophic communities. It can use electrons that are released from thiosulfate or the citric acid cycle to reduce carbon dioxide to fix nitrogen and to produce at the same time methane **(Zheng *et al.*, 2018)**. **Zheng *et al.*** has shown this for the iron-only nitrogenase of the bacterium Rhodopseudomonas palustris. This article by **Zheng *et al.*** was not only an innovation in that it showed the possibility of bacterial methane formation, but it also indicates the metabolic complexity in phototrophic reactions. Moreover, the activity of the iron-only nitrogenase is coupled to carbon dioxide reduction, which might help to increase the methane ratio in anaerobic digesters. In this relation it is an outstanding discovery that a recent study describes a direct interspecies electron transfer between *Rhodopseudomonas palustris* and *Geobacter metallireducens*, as this directly links phototrophic activity to electroactivity **(Liu *et al.*, 2021)**. In this relation it needs to be highlighted that the red biofilm, which was investigated by **Abendroth *et al.* (2020)** was enriched in the genus Methanosarcina. The importance of *Methanosarcina* for electroactive syntrophic communities and its relation to DIET has been explained in detail in the previous chapter. Therefore, the high ratio of *Methanosarcina* in phototrophic biofilms might indicate that there is a metabolic linkage between light harvesting microorganisms and electroactive, methanogenic archaea as well, although this has not been demonstrated so far. An increased ratio of *Methanosarcina* under the influence of light was already highlighted my **Tada *et al.* (2006)**. While the study by **Abendroth *et al.* (2020)** was focused on a metagenomic characterization of the underlying biofilm, **Tada *et al.*** analyzed in detail the process performance of anaerobic digesters under the influence of blue light, stating an approximately 2.5-times higher methane productivity after 10 days.

In summary, it can be said: Even if the above assumptions are still hypothetical and further research is necessary, there is increasing evidence that the combination of light and electroactive microbiomes represents a realistic scenario for the cultivation of anaerobic high-performance microbiomes.

6.7.5 Flexible interaction patterns – and the question of the programmability of AD-microbiomes

Based on a recent article by **Schwan *et al.* (2020)**, a futuristic and hypothetical outlook for anaerobic microbiomes can be made at this point. Will we achieve programmability of anaerobic microbiomes in the future? Understanding microbial interactions and their influence on the composition of taxonomic profiles is important to answer this question. As described further above, **Hardegen *et al.* (2018), Abendroth *et al.*, 2020, Heitkamp *et al.* (2021)** presented amongst others different strategies on how to influence anaerobic microbiomes. Strategies on how to manipulate anaerobic microbiomes are addressed in multiple research articles in different research fields. Further exemplary works are about aerobic granular sludge, the production of bioplastics and biofuels, the digestive system of humans and animals and anammox. In this context, the catchphrase *"designer microbiomes"* can now also be found in the literature **(Strous and Sharp, 2018)**. With the advancing microbiome research, scientists started to investigate more and more on influencing factors for the formation and manipulation of anaerobic microbiomes. This increasingly involves new innovative molecular biological approaches, such as high-throughput sequencing for RNA, DNA, or proteins. In the context of the present previous sections, various influencing factors were discussed, such as pH, ammonium/ammonia, light and others. However, results that address the manipulability of anaerobic microbiomes are often based on an empirical adjustment of chemical and physical parameters of the AD black box and based on these results, attempts are made to find explanations for the effects that occur upon changing environmental conditions. A recently published study by **Lim *et al.* (2020)** entitled *"The microbiome driving anaerobic digestion and microbial analysis"* illustrates the increasing efforts to understand anaerobic process biocenosis. Multiple techniques are mentioned, which are now applied, such as *"Metataxonomics, Metagenomics, Metaproteomics, Metametabolomics and Culturomics"*. Many articles now use these methods and the data set on given microorganisms is growing immeasurably. However, considerations on microbial interactions are often not implemented or only shortly addressed. An exception are syntrophic relations, which is also the case for the study by **Lim *et al.* (2020)**. Methods that are used to resolve syntrophic relations are for example isotope analysis, analysis of gene-expression and transcriptomics, electrochemical analyses, fluorescent in situ hybridization (microscopy), analysis for the redox state of cells b quantifying NAD^+/NADH and ATP. Those methods were recently combined in an experimental approach to show the synthrophic relation between *Rhodopseudmonas palustris* and *Geobacter metallireducens* **(Liu *et al.*, 2021)**. Another example is an article from **Gorbi *et al.* (2006)**, which is one of the first articles dealing with the discovery of conductive nanowires. A combination of scanning- and transmission electron microscopy, scanning tunneling microscopy and tunneling spectroscopy, fluorescent microscopy, mutagenesis, ferrihydrite-reduction assays and MFC assays were necessary to give evidence for the direct interspecies electron transfer. In their work, Gorbi et al. have shown the ability for DIET by *Shewanella oneidensis, Synechocystis* PCC6803 and *Pelotomaculum thermopropionicum.* It was a highlight that the latter two could do this without being dissimilatory metal-reducing bacteria, which also showcases the importance of microbial nanowires for microbial interactions. **Liu *et al.* (2021)** and **Golbi *et al.* (2006)** both, worked with pure cultures and nevertheless a considerable methodological effort was necessary to prove the interactions of the organisms involved. If one considers the methodological effort that is necessary to detect microbial interactions between selected pure cultures, it seems almost impossible to resolve the interaction of all microorganisms in an AD microbiome. To give a couple of numbers here: **Kirkegaard *et al.* (2017)** compared 32 full-scale biogas plant and found that the core microbiome comprises 300 OTUs. If microorganisms outside of the core-microbiome are considered as

well, then the number increased into thousands. If the formula *((n!/(n-k)!k!)* is applied to 300 OTUs, which are forming the core-microbiome (n) and, for the sake of simplicity, limits the maximum number of possible interaction partners that interact with each other to 10 (k), there are 1.39832023324176 × 10^{18} possible interaction variants. To study microbial interactions with so many possibilities, new approaches and methods are required. Many scientists have recognized this problem already and are trying to solve this problem based on sequencing results in combination with multivariate statistics. A good example is a recent study by **De Vrieze *et al.* (2018)**. Co-occurrence networks were predicted, and functional predictions were possible. Correlation analyzes have shown a very diverse correlation pattern for the dependence of individual microorganisms on individual process parameters with the help of a heat map. For the respective microorganisms, very different correlations were found on the RNA and DNA level. Similar results were observed for the alpha- and beta diversity of the DNA and RNA, although the differences between DNA and RNA were much higher for Bacteria than for Archaea. De **Vrieze *et al.*** also applied non-metric distance scaling (NMDS) based on UniFrac distances, which is not a measure for the abundance, but for the relative relatedness, which includes phylogenetic distances. NMDS resulted in the finding that DNA and RNA profiles clustered far away from each other in the case of bacteria. On the other hand, they were close to each other in the case of archaea. This statistical result was interpreted in such a way, that the functional community structure remains rather similar, while shifts occur especially in the community composition. This was explained by the hypothesis that multiple organisms can occupy the same niches and that multiple organisms are engaged in many metabolic pathways at the same time. De Vrieze et al. explained further that the community composition depends strongly on multiple chemical process parameters. Using a so called "canonical correspondence analysis" in combination with a "PERMANOVA", it was revealed that the archaeal community is primarily shaped by temperature, pH, TAN, free ammonia, conductivity, VS and TS, although Na^+, K^+, propionate and total VFA had a strong impact as well. The bacterial community behaved slightly differently, with temperature, pH, TAN, free ammonia, conductivity, Na+, K^+, VS and propionate being the main factors. Although less strong, there were other important factors, which were TS, Mg^{2+}, acetate, butyrate and total VFA. Although hard to comprehend for non-statisticians, works such the one from De Vrieze et al. are crucial to get a deeper understanding of anaerobic microbiomes. There are other studies, which are also applying multivariate statistics on AD processes and especially co-occurrence studies are used to predict microbial clusters of microorganisms, which might be physically or metabolically integrated with each other. A few exemplary studies that can be mentioned here come from **Rui *et al.*, (2015), Ziels *et al.* (2018)** or **Orellana *et al.* (2019)**. Some of these studies are not just performing co-occurrence studies based on 16S-rRNA gene amplicon sequences, but they include metagenomes, which was for example the case in the study by **Orellana *et al.* (2019)**. Involving metagenomes allows the interpretation of the underlying metabolic pathways. Since the same genes in different organisms often show slight deviations in their DNA sequence, it is even possible to assign genes to specific organisms. With bioinformatic databases, such as the Kyoto Encyclopedia of Genes and Genomes (KEGG), it can finally be checked how complete metabolic pathways are mapped by the respective organisms. This knowledge can in turn be used to check which organisms could complement each other at the metabolic level. Applying multivariate statistics on metagenomic data sets, **Orellana *et al.*** has shown for example, that there are two main clusters in household biogas digesters. At the functional level, cluster I was dominated by acetotrophic processes and by primary, Clostridium-associated fermentation processes. The second cluster was dominated by acetogenic and hydrogenotrophic processes, as well as primary fermentation processes by *Spirochaetes, Bacteroidales,* and *Clostridia*.

In addition to experimentally quite complex studies in pure culture and multivariate comparative analyzes based on metagenomes

and 16S rRNA sequences, it is also a sensible option to include computer models of population dynamics in evaluations of sequence data. However, hardly any research results have been published in relation to biogas production. Two articles were found that presented online tools that allow the implementation of 16S-rRNA gene amplicon sequences in the so-called Lotka-Volterra model **(Shaw *et al.*, 2016; Kuntal *et al.*, 2019)**. The Lotka-Volterra model is a model that predicts predator-prey interactions and is generally known in respect to the *"rabbit-wolf graph"*. Considering the fact that the tools for applying the Lotka-Volterra model to sequence data have been available since 2016, it is surprising that this tool has so far hardly been used in the biogas sector. To close this gap, **Schwan *et al.*, 2020** published an article, which can now be regarded as one of the first articles that apply the Lotka-Volterra model in relation to biogas production. The special feature of this model is that the formula contains a microbial interaction coefficient, which indicates whether microorganisms behave more competitively or more symbiotically.

At this point it should be emphasized again that works like that of **De Vrieze *et al.*, or Orellana *et al.*** focus on statistical interpretations of empirical process fluctuations. One can ask now whether all these interaction studies based on pure cultures or high-throughput characterizations like the one from De Vrieze et al. allow us now to manipulate anaerobic microbiomes in a more targeted manner? At least they give us a glimpse. In previous chapters of the present thesis, various influencing factors were discussed, which were analyzed with high-throughput data or pure-culture approaches as well. The combined interpretation of all these studies might enable a new function of anaerobic microbiomes: The combination of conductive particles, light, low viscosity, low pH, high concentrations of COD, VFAs, salt and ammonia could give rise to formation of phototrophic, electroactive high-performance microbiomes, although this possibility has not yet been proven.

Another approach, which is also somewhat more targeted than the mere variation of process parameters, is the addition of microorganisms enriched separately in pure culture (bio-augmentation). In this relation, an interesting approach was published by **Kovács *et al.* (2013)**. As hydrogenotrophic archaea are important for methane formation, the hydrogen forming bacteria *Caldicellulosiruptor saccharolyticus* and *Enterobacter cloacae* were added to lab-scale digester experiments. **Kovács *et al.*** observed a higher biogas productivity, which is surprising as higher levels of hydrogen might also inhibit syntrophic relations. Although in case of the hydrogen forming bacterium *Enterobacter cloacae* a long-lasting effect with a stable cell number was observed **(Ács *et al.*, 2015)**, bio-augmentation is a process with an uncertain outcome. A good example of this is a work by **Strang *et al.* (2017)**, which enriched a cellulose consortium from a biogas process that increased methane formation by 22-24% in augmented biogas reactors. After the 4 most common species (*Thermoanaerobacterium thermosaccharolyticum, Caldanaerobacter subterraneus, Thermoanaerobacter pseudethanolicus* and *Clostridium cellulolyticum*) were isolated and enriched, the positive effects in augmented reactors were reduced by half compared to the effects by the original consortium. This shows that the metabolic complexity is still beyond our understanding. The work of Strang et al. also shows that even microorganisms that occur in very small quantities can make an important contribution.

Another important influencing factor is the competitiveness of the microorganisms involved. As explained earlier, it is a probable scenario that added microorganisms are repressed by the native microorganisms of the underlying microbiome. In order to overcome this hurdle, it is of particular importance that selection pressures are used which, in particular, favor the added type of microorganisms. The following examples can be given here: **Strang *et al.* (2017)** added lignocellulolytic bacteria using lignocellulolytic biomass as selection pressure, as such biomass degrades only very slowly. In addition to lignocellulolytic biomass, fats are also poorly degradable. This can therefore also be seen as a selection pressure, which

Cirne *et al.* (2006) used for bioaugmentation with fat-hydrolyzing bacteria. Another useful selection pressure is the slow rate of growth for syntrophic bacteria. Syntrophic bacteria usually grow very slowly but are still of central importance for methanogenesis or the breakdown of fatty acids. Their supplementation can therefore reduce the long start-up phase of reactors. Especially at high ammonium concentrations, syntrophic reaction communities seem to be important. **Westerholm *et al.* (2012)** inoculated successfully syntrophic acetate-oxidizing bacteria into a process with high ammonia concentration. This has two advantages. The enrichment of slowly growing syntrophs is accelerated and at the same time fermentation is facilitated with high ammonium contents.

Based on the examples listed and the selection pressures on which they are based, it becomes clear that the majority of bioaugmentation attempts are aimed at increasing the degradation efficiency and/or overcoming process disturbances. Due to unfavorable process conditions or poor degradability, the required selection pressure is usually already given. Bioaugmentation can shorten the time it takes for the microbiome to adapt to the prevailing conditions, and it can also prevent the reactor from crashing prematurely.

The question arises as to whether targeted functional changes are possible without poor substrate degradability or without having process disturbances. In terms of direct electron transfer between species, this is certainly the case. A recent article from **Salvador *et al.* (2017)** indicates further that carbon-nanotubes improved digesting conditions without showing any sign of direct interspecies electron transfer. This highlights that apart from DIET there might be further and yet unknown effects on the molecular level, which might be used to refurbish future AD-microbiomes. At this point it should be emphasized again that anaerobic microbiomes represent a treasure trove that we have barely opened so far. Building on this fact, **Kleeberezem *et al.*** titled a 2015 published review article with the question: *"Anaerobic digestion without biogas? "*. **Kleeberezem *et al.*** mention multiple metabolites of high value: xylitol, glucaric acid, 1,2-propanediol, 1,3-proanediol, 3-hydroxypropionate, L-lactate, 2,3-butanediol, L-alanine, ethanol, butanol, acetone, butyrate, adipic acid, acetate, PHB, citrate, itaconate, L-glutamate, putrescine, succinate, fumarate, malate, caprolactam, cadaverine, L-isoleucine, L-methionine, L-threonine, L-lysine, L-asparate. It would be a significant innovation if we could manipulate microbiomes to produce selected metabolites of the above as needed. To achieve this, we need completely new approaches to program and neofunctionalize microbiomes. And for this neofunctionalization a deeper understanding of anaerobic microbiomes is needed. In this context, the results of the Lotka-Volterra modeling from **Schwan *et al.*, 2020** can be highlighted. Starting from the same microbiome, several parallel experiments were carried out. These experiments were subjected to different chemical stressors. For some of the most abundant microorganisms, the interaction coefficient was determined from the Lotka-Volterra equation. Interestingly, it turned out that the interaction coefficient can indicate competitive or symbiotic relationships, or no relationship at all, between two given species, depending on the particular stressor. If the question of the neofunctionalization of anaerobic microbiomes is raised, this finding is important because it shows that a certain combination of microorganisms to be added in combination with varied selection pressures may only function under very defined reactor conditions. Apart from the fact that Schwan's work further complicates the high complexity of anaerobic microbiomes, a new approach to microbiome manipulation can also be devised based on the Lotka-Volterra model. If a certain target organism is introduced into a microbiome, Lotka-Volterra modeling based on 16S rRNA data could be used to search for microorganisms that have a strongly positive interaction coefficient with the target organism. The corresponding microorganism could now also be enriched. This "accompanying" organism could now be co-inoculated with the target organism. This method is hypothetical at first and should be explored further in the future. Should it prove to be true, this could considerably increase the chances of success in domesticating microorganisms in an alien environment. In this regards, also the application of machine learning algorithms could be supportive.

6.8 Pre-treatment of substrates

As already highlighted in the previous sections, a two-stage anaerobic biogas process with an acidic preliminary stage is the key to the enrichment of metabolites, which could be of central importance for the growing bioeconomy. A recent article by **Ramm *et al.* (2020)** is focused on N-removal technologies from high-strength liquor received from dark fermentation (two-stage methanation). The results presented in the work from **Ramm *et al.* (2020)** show that both MAP precipitation and N-stripping are suitable methods for the treatment of acidic high strength liquors resulting from substrate pretreatment from two-stage digesters. At this point the legitimate question could be asked whether N-stripping or MAP precipitation can also be used economically. In this relation it can be pointed out that similar N-removal technologies as described by Ramm et al. are already being used in industry, although they are only applied in separate fermentation stages. In this relation, a recent online statement by the European Biogas Association (EBA) says: *"The Green Energy farm holding of Chiari (BS – Italy) is an example of a success story. In fact, the company has a 1MW biogas plant mainly fed with chicken droppings and connected to a BTS Biogas stripping system which has made it possible to reduce nitrogen levels by up to 60%."*. The mentioned company is just one of multiple companies that can be found in this relation. In this context, however, it should also be noted that the maintenance costs for N-elimination technologies in acidification stages could possibly turn out differently than they are for methane stages. But still, the metabolites that might be retrieved from dark fermentation stages are much more valuable than just methane. A comparison of the monetary value of different metabolites was done by **Kleerebezem *et al.* (2015)**. To improve comparability, **Kleerebezem *et al.*** related the gain to the electrons that are contained in the respective metabolites. In June 2013, methane had a value of 0.8 EUR kmol-e^{-1}. In contrast, hydrogen or ethanol would both yield 2.0 EUR kmol-e^{-1}, and poly-hydroxybutyrate (PHB) would even yield 12.4 EUR kmol-e^{-1}, which is already 15.5-times as much revenue as from methane. Although this was not demonstrated yet, the high revenue from other metabolites than methane makes it appear likely that possible extra costs for the building and maintenance of a respective dark fermentation stage would be covered by the higher revenue obtained for the respective metabolites.

In this relation someone could ask why the N-elimination in two-stage systems should be carried out in the acidification stage and not in the methane stage as it is already done. This question cannot be answered with absolute certainty, but some clues from the current literature are given. First of all, the proposed technology from Ramm et al. was able to increase the acid formation by up to 19%. If one considers the increased revenues for the metabolites of dark fermentation compared to methane, 19% additional yield can already make a big monetary difference. In this relation, it is important to note that the proposed technology did not only improve the conditions for bacteria due to lowered nitrogen contents, but it is possible to selectively inhibit methanogenic archaea. With inhibited methanogens the long-term stability of the high-strength liquor produced might be improved as well. Another advantage, which has not yet been investigated in this context, is increased hydrogen formation. Many publications show that harsh conditions, in particular temperature shocks, can increase hydrogen formation, as for example shown in the works from **Magrini *et al.* (2020)** and **Alibardi *et al.* (2012)**.

It was also shown recently that magnesium can increase the efficiency for hydrogen formation, as it is an important co-factor for many enzymes. **Cardoso *et al.* (2014)** found the hydrogen formation to be optimal at magnesium concentrations between 1.2 and 1.6 g L^{-1}. As magnesium is one of the substances added for MAP precipitation (Ramm et al. applied 89 g L^{-1} of magnesium chloride hexahydrate), this method could also increase the magnesium content in a separated acidification stage. Unfortunately, the magnesium contents as not measured

after the application of the MAP precipitation in the high-strength liquor produced. However, it was noted that that concentration of all trace elements that were measured decreased, and that in parallel the conductivity increased. This indicated that the MAP precipitation increases the concentration of magnesium during the fermentation process.

It could also be advantageous to use larger amounts of sodium hydroxide (50 g L^{-1}) during the N-stripping to shift the pH value to a more basic level. A recent study by **Salmón *et al.* (2018)** has shown that alkaline treatments are well suited for CO_2 capture with carbonate produced simultaneously. Therefore, the N-removal technology suggested by Ramm et al. might also be used for the purification of produced hydrogen, although this has not been experimentally tested so far.

Following the hypothesis that the proposed N-removal technology in the present study could be used to increase the hydrogen formation, this would in turn affect the acid formation as well. It is known that the hydrogen partial pressure in the gas phase affects the ratio of different VFAs in the process. Higher partial pressures of hydrogen inhibit the hydrogenase enzyme, which in turn changes the metabolism, resulting in higher levels of butyric acid **(Dwidar *et al.*, 2012)**. Although butyric acid is a wanted and valuable product for the industry, as described by Dwidar et al., in higher concentrations it affects methanogenic fermentation negatively **(Kroiss and Svarland, 2005)**. The literature describes several technologies that allow efficient separation of VFAs from fermentation processes, such as ion exchange processes, solvent extraction, electrodialysis **(Murali *et al.*, 2017)** or adsorption **(Reyhanitash *et al.*, 2017)** processes. **Yousuf *et al.* (2016)** tried to improve the separation process or VFAs, reaching up to 74% of removal efficiency. But even with such high efficiencies, a substantial amount of butyric acid might enter the subsequent methanation stage that is used to digest the residues from the fermentation. Therefore, to improve the methanogenic digestibility of fermentation residues that are enriched in propionic- or butyric acid, further improvements are necessary. One possible solution could be the enrichment of *Methanosarcina* in combination with an increased rate of direct interspecies electron transfer (DIET).

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07

The case of Aras de los Olmos: a public initiative

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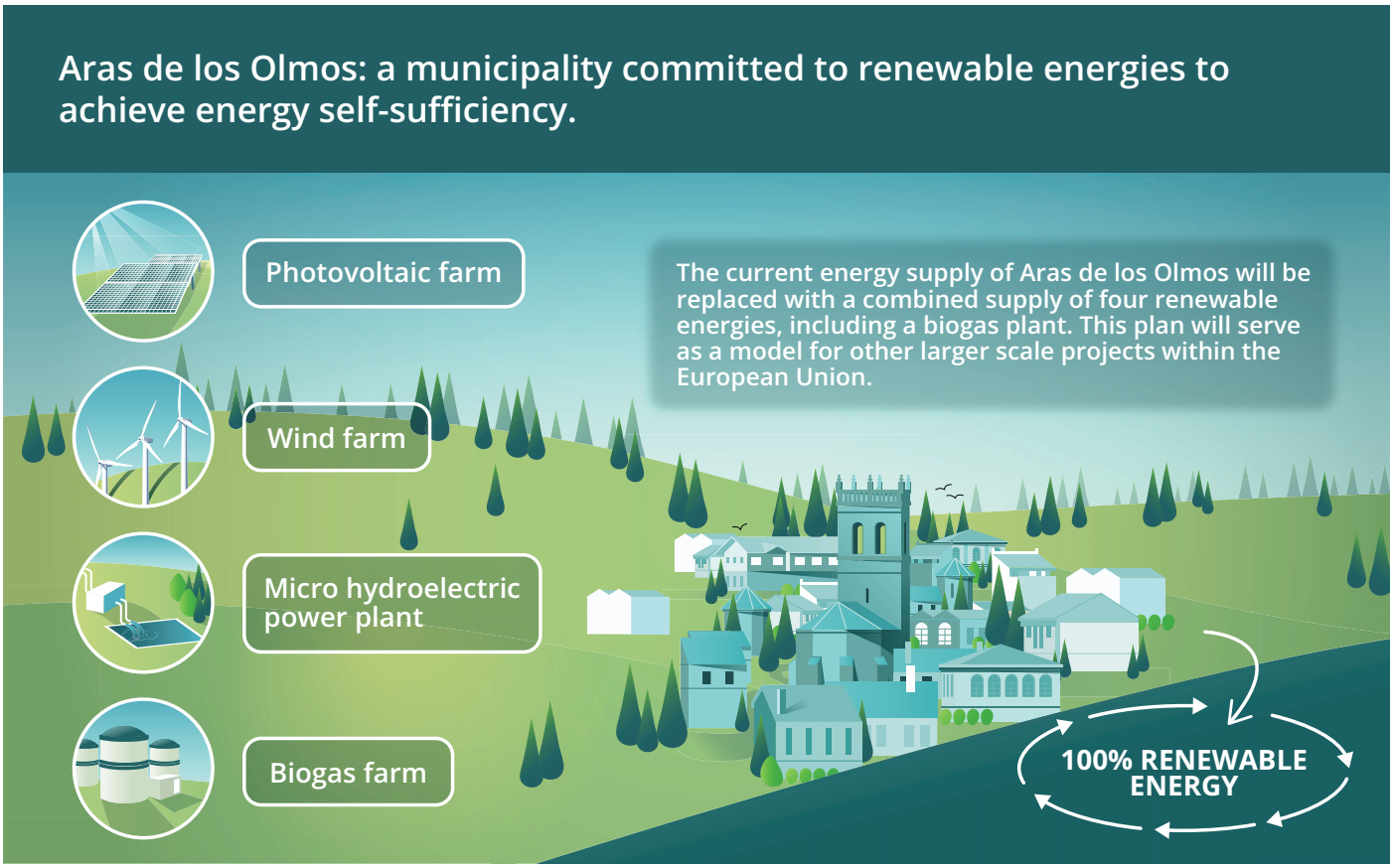
Abstract:

This chapter explains the experience of the municipality of Aras de los Olmos with the objective of serving as a guide for future projects in European rural municipalities.

Due to problems related to electricity supply, which cause continuous power outages, the municipality decided to have its own renewable energy source and generate 100% of its consumption. To achieve this purpose, the town hall developed an ambitious project consisting of a complex of four renewable energy plants: solar PV, wind, hydraulic, and biogas. In this chapter, the actions carried out and the installations to be done in the territory are mentioned in detail. The MICRO4BIOGAS project is necessary to increase the efficiency of the plant and the electric energy generated by it. At the same time, the participation of Aras de los Olmos in the project is fundamental to scale up the results from the laboratory scale to the industrial scale, with optimized bacteria for the biodigestion of waste. Finally, reference is made to the regulations that need to be complied with in order to obtain the necessary administrative permissions.



7.1 Town description

7.1.1 Features

Aras de los Olmos (**Figure 74**) is a town in the Serranía of the province of Valencia, in the heart of the Alto Turia. It is located 98 kilometers away from the capital (Valencia City) at an altitude of 936 meters above sea level. It has a continental Mediterranean climate, with cold winters and very mild summers. Snow is usually seen several times a year (**Figure 75**). Aras is situated in the northwest of the province (**Figure 76**), between two rivers: the Turia and the Arcos. Like most mountainous villages, it has an irregular surface. While it has some plains in the central part, the mountainous areas predominate, with deep ravines. The heights in the area exceed 1,300 meters above sea level. Within the same municipality, about 5 km from the town of Aras, there is a very small village called Losilla. From Losilla, you can observe the waterfall of the river Arcos, the "Sierra del Javalambre," and the old Central mill. Aras had a population of 374 inhabitants in 2021, but during the summer season, the population increases to well over 1,000 inhabitants. The main economic activities are based on livestock and agriculture.

In November 2018, the area on which the Astronomical Observatory of Aras de los Olmos sits was accredited as a Starlight Tourist Destination. This certification acknowledges the high quality of the night sky in this territory. The sky of Aras de los Olmos is among the best watchtowers in the world for observing the stars (**Figure 77**).

Figure 74: Aras de los Olmos: A bird's-eye view.

Figure 75: Snowfall Aras de los Olmos.

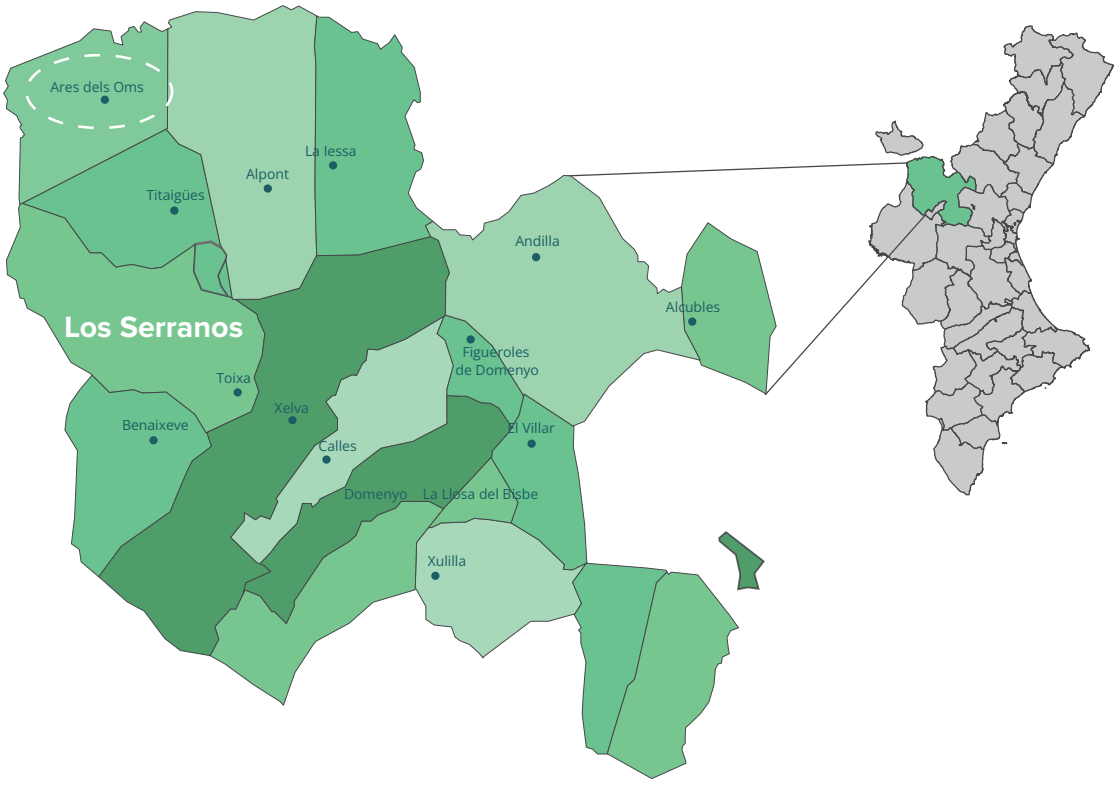


Figure 76: Map of Aras de los Olmos.

Figure 77: Astronomical Observatory of the University of Valencia in Aras de los Olmos.

7.1.2 Grid

The electricity grid in the municipality belongs to SERSUMA ("Servicios y Suministros Municipales Aras, SL"), which is a public entity under the jurisdiction of the Ayuntamiento de Aras de los Olmos. SERSUMA supplies electric power to Losilla and Aras de los Olmos. The electric line has an approximate length of 11.9 km and operates at 20 kV. It consists of two parts: the first part runs from El Collado (a village belonging to Alpuente municipality) to Losilla (5.2 km), and the second part extends to Aras with a length of approximately 6.7 km. Occasionally, there are connections to dispersed customers, mainly farms, some of which have photovoltaic production. The majority of the consumption is concentrated in the town of Aras, accounting for 90% of the total, while the remaining 10% is in Losilla. The high voltage distribution line operates at 20 kV and uses LA56 conductors (currently designated as 47-AL1/8-ST1A). The majority of this line traverses mountainous terrain in an area prone to storms and strong winds. These factors contribute to an unreliable electricity supply in Aras, with frequent interruptions, voltage instability, and service disruptions, which are not suitable for the demands of the 21st century.

7.2 Situation of Aras de los Olmos

7.2.1 Covenant of mayors

Following the adoption of the EU Climate and Energy Package until 2020 in 2008, the European Commission initiated the Covenant of Mayors to support local authorities in implementing sustainable energy policies. Building upon the success of the Covenant of Mayors, the Mayors Adapt initiative was launched in 2014, using the same governance model. It invited cities to make political commitments and take action to proactively address the inevitable effects of climate change. In late 2015, these two initiatives merged to form the new Covenant of Mayors for Climate and Energy. This updated covenant aligned with the EU's 2030 targets and adopted a comprehensive approach to both climate change mitigation and adaptation. The signatories share a common vision for 2050: to accelerate the decarbonization of their territories, enhance their capacity to adapt to the impacts of climate change, and ensure their populations have access to safe, sustainable, and affordable energy sources. Aras de los Olmos is a signatory town of this pact, demonstrating its commitment to the environment and future generations, as well as to the well-being of its territory and people.

7.2.2 SERSUMA SL

Considering the issues outlined in the previous point regarding electricity supply, SERSUMA SLU intends to collaborate with the Ayuntamiento of Aras de los Olmos to develop a project focused on renewable energies and energy efficiency in the town. A series of actions led by the Ayuntamiento Aras de los Olmos have been initiated to achieve the town's environmental objectives and address the electric service problems. These supply issues have resulted in a lack of electricity in the municipality, sometimes lasting for consecutive days during storms. These problems have a negative impact on the community as a whole and particularly affect local businesses. Access to electricity supply and internet connection is essential for rural development and the sustainability of village life. Therefore, it is of utmost importance to find a solution to these challenges in order to prevent depopulation and promote growth in rural areas.

7.3 Renewable Energy Implementation Center

7.3.1 Actions

As a result of the aforementioned considerations, and with the objective of the Ayuntamiento of Aras de los Olmos to implement a project focused on renewable energies and energy efficiency, they commissioned the GEDER group, Department of Electrical Engineering, in 2016 to conduct a Viability Study for the Integration of Sustainable Energy Resources in Aras de los Olmos. This marked the beginning of the process for establishing the **Renewable Energy Implementation Center** in the area.

Subsequently, the Ayuntamiento of Aras de los Olmos engaged the GEDER group to draft all the preliminary projects for the installations, including four plants for the production of electrical energy - a wind power plant, a photovoltaic plant, a hydroelectric plant, and a biomass plant. In June 2017, they submitted a Start Request to the Department of Agriculture, Rural Development, Climate Emergency, and Ecological Transition of the Comunidad Valenciana for the processing of a Special Plan for the Implementation of Renewable Energies in Aras de los Olmos. The Request for Initiation comprised the Initial Strategic Document and the Draft of the Special Plan for Renewable Energies of Aras de los Olmos.

In February 2018, the GEDER UPV group conducted the Environmental Impact Assessment to determine the direct or indirect significant effects of the project on the environment and to make decisions to prevent and minimize such effects. On February 28, 2019, the Environmental Assessment Commission issued a Scoping Document for the Strategic Environmental and Territorial Study, which included considerations to be established in the preparation of the study and the Preliminary version of the Special Plan. Following this, Aras de los Olmos continued the procedure by preparing the Preliminary Version of the Special Plan and the Strategic Environmental and Territorial Study. The Special Renewable Energy Plan of Aras de los Olmos aims to replace the current energy supply of the town and its village of Losilla with a combination of four renewable energies - wind turbines, solar panels, hydropower, and biogas - which together will achieve self-generation of the total consumption of the town.

The implementation of the Renewable Energy Center is aimed to be environmentally and urbanistically viable, and the town plans to present it to European instances to access financing instruments as signatories of the Covenant of Mayors for Climate and Energy. Additionally, the Landscape Integration Study is part of the documentation accompanying the Special Plan for the Implementation of Renewable Energies in Aras de los Olmos. The purpose of the study is to analyze and evaluate the landscape impact of the necessary installations for the Renewable Energy System.

Through the Special Plan for the Implementation of Renewable Energies and its mandatory Strategic Environmental and Territorial Study, modifications to the Urban Development Regulations are included to reinforce, make explicit, and enable the achievement of the pursued objectives. This means that, simultaneously and through almost identical procedures, the General Structural Urban Development Plan of Aras de los Olmos has been implemented. The plan aims to abandon the criteria of urban growth, which is based on occupying new territories, and instead focus on sustainable growth by utilizing all existing resources in urban land. This sustainable model can be achieved through the implementation of a renewable energy system that would meet the town's energy needs. Consequently, the Special Plan for the Implementation of Renewable Energies is currently being processed. It is important to note that on December 10, 2015, the Wildlife Service reported that the proposed

General Structural Plan would not have significant effects on the Natura 2000 Network. Therefore, it is considered that the project does not require a detailed assessment of its effects on the Natura 2000 Network. The Ayuntamiento of Aras de los Olmos has made all the relevant documentation available for public participation and consultation, as legally required. In this regard, on June 12, 2020, the Plenary of the Ayuntamiento initially approved the Plan. On June 29, 2020, the approval was published in the *"Diari Oficial Generalitat Valenciana" No. 8845*, initiating a 45-day period of public information, as mandated by regulations. The plan is currently being processed. It should be taken into account that if the Special Plan is not implemented, there will likely be an increase in CO_2 emissions into the atmosphere, equivalent to the consumption of the entire population during regular and holiday periods. This has negative implications in terms of combating climate change.

In conclusion, this project represents a pilot experience in the peninsular part of Spain aimed at achieving a zero-energy balance in a municipality. We believe that the effects of implementing this project can be minimized through corrective measures and are more than offset by the benefits for the environment in general and climate change in particular. (A reduction of approximately 1,200T. CO_2/year in emissions is estimated) Through the combination of various renewable energy systems such as wind, solar, hydro, and biogas, a permanent energy supply that meets the municipality's needs is intended. Although our case involves a municipality with a large area but a small population, this project is developed with the intention of becoming a model to be followed in other locations and promoting the widespread use of renewable energies at all levels.

7.3.2 Installations

To achieve the objectives outlined above, based on the Special Plan for the Implementation of Renewable Energies in Aras de los Olmos and the available natural resources, the following installations are necessary:

Photovoltaic farm: Solar energy has proven to be the most suitable source for meeting the majority of energy demands. A 700 kW photovoltaic park has been installed to capitalize on solar radiation, ensuring that it provides a significant portion of the required energy. The plant has the following specifications: it comprises 1,080 bifacial photovoltaic solar panels made of monocrystalline material, each with a power output of 700 Wp. These panels are mounted on a structure equipped with single-axis solar tracking technology, enhancing their efficiency by following the sun's trajectory. The installation includes six inverters rated at 110 kW each, along with one 40 kW inverter. These are connected to a 20kV/400V step-up transformer. Since the plant is designed to operate off-grid, the inverters have adjustable power factors and can deliver up to a maximum of 700 kW of power. The facility occupies an area of 14,000 m^2. Additionally, a newly constructed 20kV power line links the solar power plant to an existing line operated by Sersuma, ensuring smooth energy distribution.

Wind farm: The wind farm will consist of two 100 kW wind turbines. These turbines will be three-bladed machines with synchronous generators, multipliers, pitch angle control systems, and variable speed operation. The base of the wind farm will house the transformation center for connection to a 20 kV line.

Micro Hydroelectric Power Plant: The hydraulic resource from the Arcos River has also been considered for the Aras energy complex. The micro hydroelectric power plant will serve both as an energy storage and generation component, with a turbine power of 200 kW and a pumping power of 180 kW. The plant will include the following elements: an upper asphalt concrete reservoir of raft type, with a surface area of 8,100 m^2 and a storage capacity of 24,000 m^3 of water, allowing for 48 hours of turbination; a lower reinforced concrete tank with a capacity to store 4,000 m^3 of water, enabling 9.6 hours of turbination; two Pelton-type turbines, each with a hydraulic power of 100 kW; two synchronous generators capable of operating in parallel with the grid and in isolation, allowing control of reactive power delivery, voltage regulation,

and synchronization with the grid; and three 60 kW vertical centrifugal pumps for pumped storage. These elements will be connected to a 20/0.4 kV transformation center to link the installation to the 20 kV line.

Biogas plant: To address the technical challenges posed by the uncontrollable and fluctuating nature of solar and wind power generation, which could destabilize the local electricity system, the inclusion of a biogas plant is essential. Biogas offers controllable generation, making it an attractive and important component of the system. Therefore, a 200 kW biogas plant is planned to serve as a base generation and cover the demand during periods when other renewable resources are insufficient. The plant will consist of the following components:

- A 93 m^3 collection basin for storing raw materials for 3 days.
- A 925.15 m^3 digester, capable of producing 977 m^3 of biogas per day. The digester will be covered by a double-layer elastic membrane for biogas storage equivalent to 12 hours of production.
- A desulfurizer and dryer to enable the use of biogas in a 200 kW internal combustion engine, which will drive the synchronous generator for electricity generation.

Smart Grid: The Aras energy project, comprising the four described generation facilities, will form a microgrid that requires efficient management and control. To achieve an autonomous system capable of disconnecting from the grid, meeting the municipality's total energy demand, and ensuring efficient and reliable operation, a complex control system is necessary to transform the electrical network into a smart grid. Key aspects of developing this control system include:

- Challenges in predicting demand due to diverse consumption patterns.
- Difficulties in predicting actual generation capacity due to uncertainties associated with the different generation systems.
- The need for a manageable base generator that ensures stability when disconnected from the grid.
- The requirement for a large energy storage system.

Consequently, the development of a complex control system with automatic primary level control and secondary control from the control center will turn the microgrid into a smart grid.

MICRO4BIOGAS: The participation of the Ayuntamiento of Aras de los Olmos is crucial for the development of one of the manageable energy sources emphasized in the plan. Unlike wind and solar, energy from biogas can be obtained on demand. With the involvement of Aras in the project, the municipality will take part in a pioneering trial involving the use of optimized bacteria for waste biodigestion. This scientific strategy, led by MICRO4BIOGAS, aims to improve knowledge of biogas production and set an example for other countries. The project also presents a potential solution to the farming community's challenge in managing slurry and complying with European Commission regulations. Aras de los Olmos has pig, rabbit, and poultry farms, and this model enables controlled waste treatment, mitigating the environmental impact of natural decomposition. Following biogas production, the waste can be repurposed as agro-ecological fertilizer, and the excess water from the process will be used for irrigation. Consequently, this project serves as a benchmark for circular economy practices and rural development. It's important to note that financing is required for each installation, and administrative actions have been taken to facilitate future construction in conjunction with point 2 of this document. A summary of the project is provided in **figure 78**.

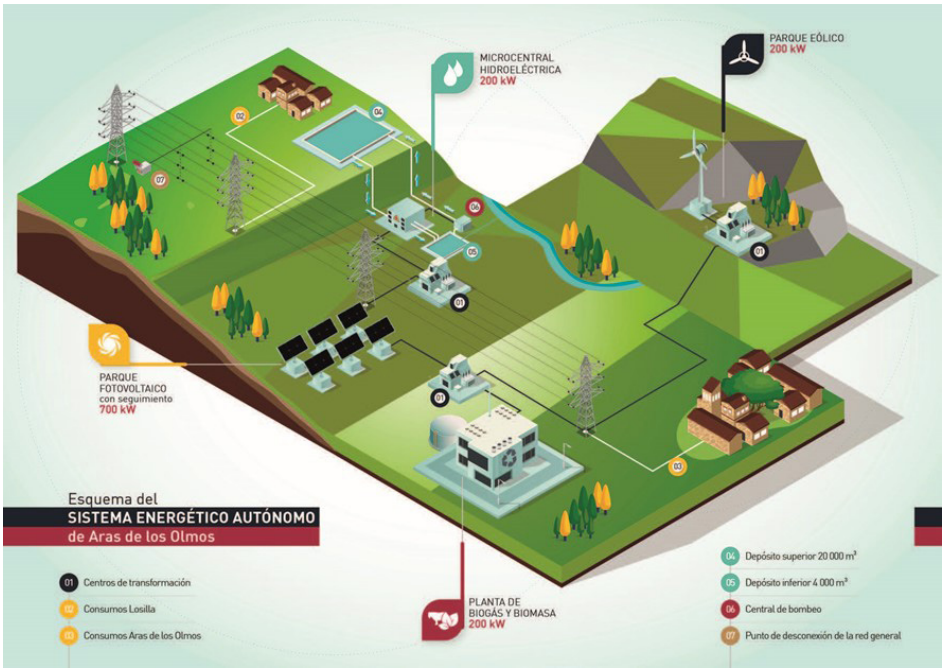


Figure 78: Resume of Renewable Energies in Aras de los Olmos project.

7.4 Relationship with the different organizations

European organizations: The Ayuntamiento has a relationship with the European Commission, which serves as a funding organization. The European Commission has provided a budget to the Ayuntamiento for the implementation of the MICRO4BIOGAS project. It has also provided funding to different ministries, from which the Ayuntamiento of Aras de los Olmos has benefited through grant and subsidy applications. The Ayuntamiento maintains relationships with various universities and European entities that serve as sources of research for the MICRO4BIOGAS project and its related activities.

State organizations: The Ministry for Ecological Transition and the Demographic Challenge (MITECO) plays a significant role in providing financial instruments to the Ayuntamiento. The construction of the biogas plant, among other financing needs, is co-financed by MITECO and the General Directorate for Ecological Transition of the Generalitat Valenciana. Additionally, the IDAE (Institute for Energy Diversification and Saving), an organization under MITECO, provides funding for technologically innovative and replicable projects. It participates in financing the Aras energy project through its support for local entities transitioning to a low-carbon economy. The Ministry of Territorial Policy and Public Function manages relations between the Government of Spain, the Autonomous Communities, and local entities. In the case of Aras de los Olmos, it facilitates communication between the government, the Valencian Community, and the City Council.

Autonomous community organizations: At the regional level, the "Conselleria de Agricultura, Cambio Climático, Medio Ambiente y Desarrollo Rural" through the "Direcció General de Medi Natural i d'Avaluació Ambiental" and the "Conselleria de Vivienda, Obras Públicas y Vertebración del Territorio" are involved in the processing of environmental and strategic evaluations necessary for the approval of a Special Plan of Renewable Energies in Aras de los Olmos. The "Conselleria de Economía, Industria, Turismo y Ocupación-Conselleria d'economia sostenible, sectors productius, comerç i tre-

ball" grants administrative authorization for the installation of a transformation center. The Ayuntamiento has had to engage with these bodies to fulfill requirements and formalities necessary to achieve its objectives. The "Diputación de Valencia" particularly the Areas of Environment and Roads, oversees the roads in the municipality of Aras de los Olmos and their potential impact from the project. The neighboring Ayuntamientos, especially Arcos de las Salinas, are relevant for the hydraulic plant aspect of the project. Consultations are conducted with national or regional companies that provide water, electricity, gas, telephony, and telecommunications services to obtain reports on project needs and minimum technical requirements. Special mention goes to the Polytechnic University of Valencia (UPV), a public body fully involved in the Ayuntamiento's project. It collaborates and conducts research, having contributed to various documents such as the feasibility plan and preliminary projects.

Legal regulations: Environmental considerations play a crucial role in the construction of the aforementioned plants, with the preservation of natural habitats and wildlife being fundamental under various applicable regulations. Waste management and health regulations are also important factors to consider. Consequently, for the implementation of the renewable energy project in Aras de los Olmos, multiple directives and regulations at the European, national, and regional levels needed to be considered.

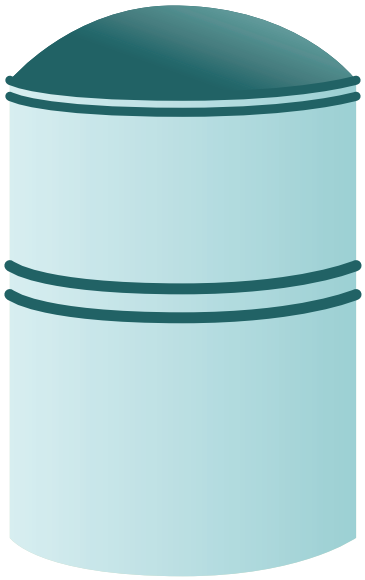
An example at the European level is Directive 2001/42/CE of the European Parliament and of the Council, which focuses on integrating environmental aspects into decision-making processes for public plans and programs. Another example is Directive 2009/28/CE of the European Parliament and of the Council, which promotes the use of energy from renewable sources.

Spanish law is applicable in various areas, such as environmental protection, energy, fauna, flora, and the atmosphere. Compliance with environmental assessments and electricity sector standards is essential. Attention must also be given to the regulations governing the organization and regulation of the electricity generation market. Spain regulates the production of electricity from renewable energy sources through Royal Decree 413/2014, which covers renewable energy, cogeneration, and waste. Aras de los Olmos must align its actions with the established parameters. Additionally, as a local authority, the Law of Public Administrations (Law 39/2015) applies. Given its geographical location, forestry laws must also be considered. The urban planning situation of the land is of great importance throughout the process, and protected natural areas must be respected to avoid harm to the environment. As Aras de los Olmos belongs to the Valencian Community, the regulations of the Autonomous Community apply, which are generally more stringent than those of other regions. The Spatial Planning, Urbanism and Landscape Law (Law 1/2019) holds particular significance in this regard. At the municipal level, the General Urban Development Plan of Aras de los Olmos should be noted. However, there is no difference between the local regulations and those of the Autonomous Community.

In summary, it is crucial for Aras de los Olmos to consider regulations that address the environmental impact concerning territory, soil, environmental conservation, waste management, industry, and the electricity market. Compliance with the provisions established in European, national, Valencian Community, and local regulations is necessary.

References

- All images were taken from the *municipal archive at Aras de los Olmos*. Copyrights belong to its tourism department. All information from the main text were collected from administrative files at Aras de los Olmos, especially from the “Renewable Energy Plan”. As citizens from Aras de los Olmos, the author was also writing based on his own experience.



08

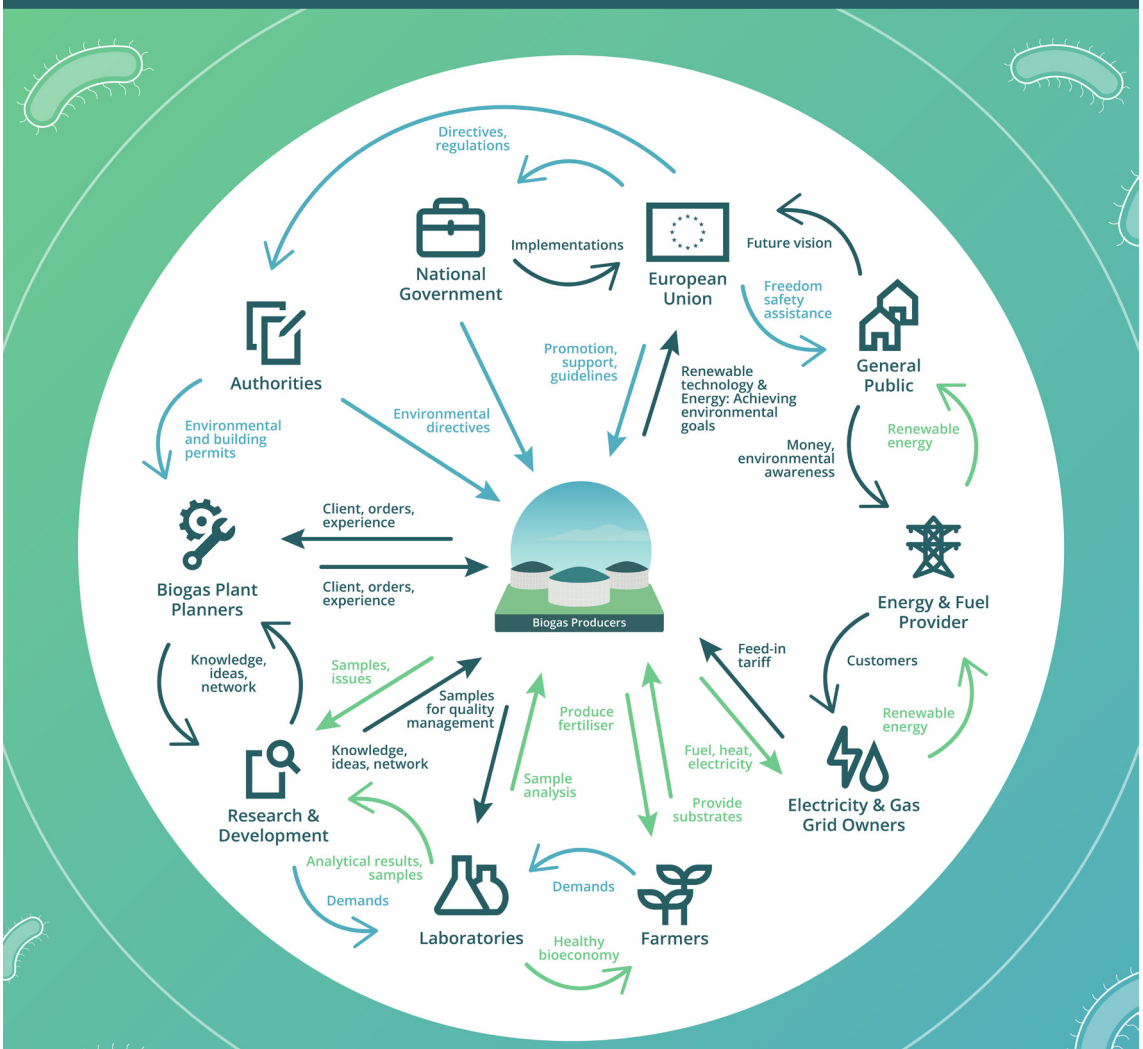
Stakeholders in anaerobic digestion

Author from the Technische Universität Dresden: Pascal Otto

Abstract:

This chapter focuses on stakeholders and the interaction of the biogas sector. The biogas sector is very complex and involves many areas such as energy supply, waste management, agriculture, fertiliser production. The complexity of this sector is illustrated in the **graphical abstract** below. The biogas producers are at the centre of the biogas sector. Around the biogas producers are all the areas that are tangential to them. For the sake of simplicity, only the primary relationships of the actors are shown. This means that farmers, for example, are of course also connected to the European Union, but this connection is of secondary importance for the biogas sector itself. The exact relationships of the actors to each other are dealt with in the respective chapter. The focus is on the actors biogas producers, biogas plant planners, authorities, research and development, laboratories, farmers, electricity and gas grid operators, energy suppliers, the public, the national government and the European Union.

The production of biogas in Europe is directly tied to a series of stakeholders that include the energy industry, agriculture, national and international governments, laboratories, research institutions and the general population. The relations of these stakeholders both between one another and with biogas producers is worked out in this chapter.



Overview of the stakeholders and the relations between them

8.1 Biogas producers

Biogas producers are at the centre of the entire biogas sector and have or have had some form of contact with every stakeholder in the biogas sector. They form the main value chain as they ensure that valuable resources such as biogas, biofertilisers, biomethane, heat, electricity are produced from organic materials such as biowaste, animal excreta, green waste, sewage sludge, energy crops, agricultural waste and food waste. In further explanations, the relationships between the biogas producer and the stakeholders involved are presented.

Biogas producer & Biogas plant planners: Before a biogas or biomethane plant is built, the potential plant operator is in contact with a biogas plant planner that is to take over the construction and planning. A lot of data must be collected in advance so that the biogas planner can take over the conceptual design and dimensioning of the biogas plant. This includes:

- Quantity of the substrate
- Quality of the substrate
- Composition of the substrate
- Location of the potential biogas plant
- Local circumstances
- Potential uses: heat, electricity or biomethane.

Subsequently, the biogas plant planner is an important intermediary between the biogas producer and the local government, the authorities, research and development.

The construction of the biogas plant and the feed-in tariff are discussed with the local government, biogas plant planner and the biogas plant operators. This includes the achievement of climate targets and sustainable development and growth.

With the authorities, the impact on the environment and the general construction of the plant are discussed. Therefore, the biogas plant planner and the biogas plant operator work hand in hand to get all documents and formalities. All necessary environmental and building permits must be obtained for the construction of the plant. These ensure that the construction will not adversely affect the surrounding ecosystems, people, buildings and environment. This can take 6 – 24 month depending on the conditions and the planned concepts.

The biogas plant planner is responsible for ensuring that the construction of the biogas plant begins after all necessary permits and approvals have been obtained. For this purpose, a company is contracted to build the plant under the guidance of the biogas plant planner and the biogas plant operator. This can take 1 to 5 years depending on the conditions, the required building materials and possible problems.

After the plant has been built, the biogas plant planner is available as an advisor to the biogas plant operator. Problems with the technology, the anaerobic digestion or other elements of the process can be discussed and solved. In addition, the biogas plant planner informs the biogas plant operator about potential modernisations. In return, the biogas plant operator provides the biogas plant planner with valuable data about the process, the components and the efficiency of the biogas plant.

As already discussed, the biogas plant operator is usually in contact with the authorities, the national or local government and the European Union via the biogas plant planner. The most important aspect is that the European Union, the national governments and the authorities want to achieve the goals of climate neutrality, increasing biodiversity and promoting the circular economy. The construction of biogas plants is a component that contributes to the realisation of these goals. Through the implementation of directives, regulations and funding, biogas producers are influenced and supported on a European and national level.

The biogas plant operator is also in contact with the operators of the electricity, heat or gas network, as the produced electricity, heat or gas is fed in at interfaces. The biogas plant operator receives money for feeding in the corresponding resource. There are different remuneration models. These depend on the substrate used, the resource fed into the grid, the size of the plant and the year of construction. The subsidy comes from the national or European government. Subsidies can also be paid in different ways:

- A fixed negotiated tariff is paid per kWh.
- The current feed-in tariff to the electricity, gas or heat network is paid
- The current feed-in tariff to the electricity, gas or heat network is paid plus a predefined flat rate

In case the biogas plant operators are not farmers themselves, they are in contact with the farmers. Farmers provide a variety of organic waste such as green waste, silage, animal excrement and energy crops. This makes them essential for the operation of biogas plants in rural areas. In return, the biogas plant delivers concentrated and very good fertiliser, which is cheaper than synthetic fertiliser and is also recycled through the use of raw materials to create new products.

The relationship between biogas producers and laboratories is relatively simple. The biogas producers deliver samples to the laboratory for analysis of various chemical and microbial laboratory parameters. The laboratories then continuously receive orders and analyse these samples. In doing so, they observe the legally prescribed limit values for the recycled usage of the digestate in agriculture. Summarized the laboratories are important for quality management and monitoring of the biogas plants.

The biogas plant operators are also in contact with Research & Development. The interfaces between the two actors are multifaceted and range from optimisation of the microbiome, improvement of the plant operation, general validation, etc. Communication can take place in two different ways:

1. The biogas plant operator comes to the research institutions with a specific problem, and the research institutions respond with analyses, ideas, information or establishing contacts with partners.
2. The research institutions want to investigate a specific issue and ask the biogas plant operator for samples, information and/or technical data.

The research & development department can also work with any other stakeholder to evaluate and address specific issues.

The general public does not interface directly with the biogas plant operator. They usually only purchase electricity, heat, and/or methane from the biogas plants over the energy providers. However, it is extremely important to educate people about the relevance, effectiveness and circularity of biogas plants. This increases the acceptance of biogas plants among the population and they are seen as a positive development in the expansion of renewable energy sources.

8.2 Biogas planners

Besides biogas plant operators, biogas plant planners have one of the most important tasks. Through their work, they help to build, expand and modernise biogas plants and consequently promote the biogas landscape. They are an important communication and planning interface between biogas plant operators and all other stakeholders. In the following, the relationships between biogas plant planners and all other stakeholders are presented.

Before a biogas or biomethane plant is built, the potential biogas plant operator is in contact with a biogas plant planner that is to take over the construction and planning. A lot of data must be collected in advance so that the biogas planner can take over the conceptual design and dimensioning of the biogas plant.

This includes:

- Quantity of the substrate
- Quality of the substrate
- Composition of the substrate
- Location of the potential biogas plant
- Local circumstances
- Potential uses: heat, electricity or biomethane.

With this data, the biogas plant planner drafts an initial concept for a suitable biogas plant for the respective location. The planning takes into account:

- Used substrates
- Available space
- Budget of the plant operator
- Funding systems
- Construction and environmental permits
- Suppliers
- Materials and personnel for the construction of the biogas plant

The construction of the biogas plant and all components involved is organised by the biogas plant planners and carried out in consultation with the biogas plant operator. After the construction of the biogas plant is completed, the biogas plant planner acts as a service provider to the biogas plant operator. The biogas plant planner helps with problems and informs about new subsidy models, modernisation programmes and important renovations that have to be carried out.

In order to ensure the construction of the biogas plant and all other components such as gas storage, combined heat and power plant, secondary fermentation storage, etc., building and environmental permits must be obtained. For this purpose, the biogas plant planners are in contact with the authorities and with companies that are responsible for the certification of all components of a biogas plant. The biogas plant planners present the concept for the construction and operation of the biogas plant in cooperation with the biogas plant operators. Usually, the construction of a biogas plant is not started until the permit has been obtained or at least it is clear how the plant will be approved. After the approval, the biogas plant planner is also given a large number of requirements and handling instructions, including how certain parts of the plant must be built or should function. For this purpose, suitable solutions must be found for individual parts, they must be feasible, meet the technical requirements and not be too costly. Through this dialogue, the result should be an economically feasible concept that more than meets the necessary construction and environmental requirements. This procedure is controversially discussed, as it can inhibit or delay the construction of biogas plants. However, it must be noted that a careful examination should be carried out in order to protect the environment. Especially since faulty concepts and constructions can endanger the groundwater, the climate and the environment through the uncontrolled emission of methane, hydrogen sulphide and fermentation residues.

Biogas plant planners are also in contact with research institutions. The topics are as diverse as those between Research & Development and biogas plant operators.

- New technical components
- Measurement technology
- Concepts for biogas plant operation
- Feed-in models
- Additives for the microbiome

The above-mentioned and many other research topics can be developed in cooperation with research institutions. This is often done within the framework of research projects that promote innovation at biogas plants, create jobs and generally promote the development towards renewable energy sources. Therefore, this relationship between the parties involved is extremely important to drive development and make the resulting products competitive in the market.

In addition, the biogas plant planners are in contact with farmers and regional waste management companies in order to acquire more substrate if necessary and to market the resulting biofertiliser if the biogas plant operator does not use it himself. The feed-in tariffs are negotiated with the network operators for electricity, gas and heat in connection with the subsidy.

8.3 Authorities

The authorities have the function to check the construction of the biogas plant for compliance with the applicable environmental and building laws. For this purpose, they issue the necessary building and environmental permits after a successful inspection. For this purpose, the authorities receive specifications and guidelines from the European Union and the national governments, which must be complied with for the construction, operation and expansion of biogas plants.

In **Germany**, these include:

- Renewable Energy Sources Act **(EEG)**
- German Build Code **(BauGB)** with the regional building codes
- Federal Emission Control Act **(BImSchG)**
- 4th Federal Emission Control Ordinance **(BImSchV)** List of all plants requiring approval) - decides whether a plant has to be approved according to BImSchG or building law
- **12th BImSchV** with regard to the Major Accidents Ordinance (for plants with more than 10 t of biogas)
- **44th BImSchV** for CHP plants with a rated thermal input of more than 1 MW
- Technical Instructions on Air Quality Control 2021 **(TA Luft)**
- Technical Instructions on Noise **(TA Lärm)**
- Technical rules of plant safety **(TRAS 120)** for BImSchG plants
- Safety rules for agricultural biogas plants **T4**
- Ordinance on systems for handling water-polluting substances **(AwSV)**
- Water management law **(WHG)**
- Federal Nature Conservation Act **(BNatSchG)**
- Environmental Impact Assessment Act **(UVPG)**
- Veterinary approval according to **Article 24 VO(EU) 1069**

All these directives and regulations must be discussed in advance with the biogas plant planners and biogas plant operators, and suitable concepts must be found. The end result of this process is a plant that meets these requirements and at the same time jeopardises the economic efficiency of the entire process. The laws and regulations presented refer to Germany and are therefore noted as German federal laws. However, for each of the points: Nature protection, Water protection, Emission protection, Environmental protection, Technical instructions on noise, Air control, Substances hazardous to water and Safety of biogas plants, there are legal texts that were developed by the EU and must also be applied in all other EU countries.

The authorities act as a control instance between the European Union, the national government and the individual stakeholders. On the one hand, they adapt and implement the laws and regulations adopted by the European Union and the respective national government. On the other hand, they ensure compliance with these laws and regulations by biogas plant operators, biogas plant designers, farmers and analytical laboratories.

8.4 Research & Development

Research & Development has the task of dealing with various problems that arise at any point in the process. These can concern, for example, the microbiome of the biogas

plant, chemical and microbial analysis, plant construction, various plant components, the general mode of operation, further utilisation concepts, etc.

These problems are usually dealt with in the form of research projects. With these, cooperations between research institutions, small companies and larger companies can be created, not only to improve the process, but also to create an industry network. In this network, problems can be addressed even more quickly. Research & Development is largely funded and supported by the national government and the European Union. As a result, these political institutions contribute to the research priorities and thus give direction to how science and industry should develop.

The first important link is between Research & Development and between biogas plant operators. Either the biogas plant operators approach Research & Development with concrete problems or samples from the biogas plant. Or Research & Development approaches the biogas plant operators to obtain samples from the biogas plant, information on the operation or opinions of the biogas plant operators. This connection is very important to promote further development and to initiate potential projects.

Research & Development is also in contact with the biogas plant planners. They provide each other with knowledge, ideas and networks. Both stakeholders are close to the chemical, microbial and technical processes taking place. Due to this convergence of knowledge, research projects often arise in cooperation with other stakeholders. The laboratories for chemical and microbial analysis are also among the other actors. They can provide the research institutes with samples, sample handling procedures and analytical results. In turn, they check whether the requirements are sufficient and how valid the results are.

As already mentioned, Research & Development is strongly linked to political actors such as National Governments and the European Union. These decide the budgets and how much money is spent on research programmes. In addition, the relevant committees decide which research programmes are to be funded. In this way, the political stakeholders set the direction of research and industry. In return, the research institutes regenerate valuable data and results on the corresponding research priorities. These result in recommendations for action, improvement strategies and concrete industrial products. This makes it possible to implement current efforts of the national government and the European Union. These include, for example, the Green Deal, the Waste Framework Directive, the Paris Climate Agreement, etc.

8.5 Laboratories

The laboratories carry out chemical and microbial analyses for biogas plant operators, farmers and, in some cases, Research & Development. They receive samples from the aforementioned actors and check compliance with prescribed limit values for, for example, heavy metals, nitrates, phosphates, certain bacteria and fungi. The laboratories are therefore important actors for carrying out quality control and ensuring a healthy bio-economy.

The limit values and analysis procedures are constantly reviewed, revised and adapted in cooperation with research institutions and industry. The National Governments or the European Union then establish the limit values that have been developed as mandatory requirements in environmental law. The analytical procedures are published in the form of standardised protocols and norms to ensure the validation of the data.

8.6 Farmers

The farmers are either the biogas plant operators themselves or the farmers cooperate with the biogas plant operators. In the first case, the biogas plants are operated with the farmers' own substrate and the resulting bi-fertiliser is then brought to the field

and used by the farmer himself. In the other case, the farmer supplies the biogas plant operator with substrates such as animal excrement, plant residues and surplus feed. In return, the farmer receives valuable biofertiliser, which has to be tested by laboratories for various parameters. These are also in contact to ensure a healthy bioeconomy.

Farmers are also in contact with the National Government and the European Union. These have requirements for the use of fertilisers, and pesticides, the cultivation of crops and the keeping of livestock. In addition, the construction of a biogas plant and the use of, for example, manure as a substrate is strongly encouraged in order to minimise emissions of carbon dioxide and methane. In this way, the political actors try to build up an agriculture that does not endanger the environment and protects biodiversity. At the same time, it wants to increase the appreciation of farmers and stabilise remuneration through additional income from biogas plants.

8.7 Energy grid owners & energy providers

The owners of the electricity, heat and gas grids first provide the trading platform to bring the resources generated by the biogas plant operators to the energy suppliers and consumers. Generally, energy suppliers market the energy resources of heat, gas and electricity to consumers. Consumers include the general public, industry, public institutions, etc. The grid owners provide the infrastructure to distribute the produced resources and pay the biogas plant operators the negotiated feed-in tariff. This is supported by government subsidy schemes from the National Governments and/or the European Union, which encourages the use of renewable energy sources. The use of heat is limited, because high losses occur during transport. Therefore, heat should be used locally. For the use of gas and electricity, there are no restrictions due to the well-developed network, but any transport involves losses, which is why local use is always more efficient but not always more effective. Therefore, a centralised solution makes sense for urban areas and a decentralised solution is desirable for rural areas.

8.8 General public

The general public is rather a passive part in this stakeholder analysis. However, the general public is very important, they are the consumers of renewable gas, electricity and heat. They need to express a demand for renewable energy sources to the government and the energy providers. They potentially have to pay a little more initially for the use of renewable energy sources, but they have to know what it is good for. Therefore, it is even more important that the general public shows a high level of acceptance and preference for all renewable energy sources.

The general public requires gas, electricity and heat for heating, cooking, cooling, transportation, lighting, etc. This is provided by the energy and fuel providers. For this, contracts and tariffs are negotiated, how much is paid for each energy resource.

The National Governments and the European Parliament are democratically elected by the people of Europe. In a way, the general public gives their wishes, future visions and ideas to the government to make Europe a better place. With this responsibility, governments make decisions to guide the future in this direction. The people should be given freedom, but also security and support.

8.9 National governments

The National Government of each EU country is democratically elected by the general population with the corresponding program. This government has primarily the task of implementing the promised goals and the

responsibility to comply with the coalition agreement. Besides this task, international and European directives and regulations must be implemented. In particular, the regulations issued by the European Union must be implemented directly and the directives must be implemented indirectly, otherwise penalties will have to be paid to the European Union.

The role of the national government is very individual and depends on the country. Countries adopt different funding methods, research priorities, target agreements and strategies for the expansion of biogas and bio-methane plants. This is why the biogas landscape in Europe is so diverse. Countries like Germany have been using biogas for decades, which is why there are more than 10,000 biogas plants in this country. Other countries, such as France, are only now stepping up the expansion of bio-methane plants. For the further expansion of biogas plants, a Europe-wide strategy that addresses specific biogas would make sense.

8.10 National governments

The European Union is composed of the following institutions: European Council, Council of the European Union, European Parliament, European Commission, European Central Bank, European Court of Justice. The European Parliament is democratically elected by the people of Europe and thus exercises democratic control over all the other institutions. Together with the Council of the European Union, it is also responsible for the legislative process. The European Council sets impulses and directives. The European Commission functions as the executive or executive governmental power and is composed of one commissioner per state. The Court of Justice of the European Union acts as the judiciary.

In summary, the European Union is democratically elected and reflects the wishes, ideas and perceptions of the European people. At the same time, the European Union has the responsibility to realise these ideas on a realistic scale. The instruments of the European Union are directives, regulations, recommendations, decisions and opinions. These legislative texts, which apply to all actors in the Member States and to the Member States themselves, set the future direction of the European Union. The European Union has an influence on all the actors mentioned here, as they have to abide by the rules of the European Union. In particular, the influence of the European Union on the future stakeholders such as renewable energy producers (biogas plants), farmers and research institutions is enormous. The European Union decides through its budget what is subsidised and how much, which has a direct influence on economic efficiency and economic development. In return, the European Union achieves the set targets for e.g. the emission of greenhouse gases, the development of renewable energy sources, etc. In the best case, this relation between target setting and promotion is a win-win strategy.

09 Prospects

Authors from Darwin Bioprospecting Excellence S.L. (Roser Puchol-Royo, Adriel Latorre-Pérez)**, the Brandenburg University of Technology Cottbus-Senftenberg** (Christian Abendroth) **and the LUCRAT GmbH** (Savannah Baptist)

Abstract:

To make bioaugmentation and the programmability of anaerobic microbiomes possible, a considerable amount of research is still required. Nevertheless, the first steps are already being taken aimed at the targeted manipulation of anaerobic microbiomes. For this purpose, outstanding microorganisms with special microbial abilities were presented as examples in the present roadmap. The authors rated some of them as “exotic”, although this reflects a subjective perception. In connection with these outstanding microorganisms, research questions are raised which will continue to occupy us in the coming years or decades. Nevertheless, a commercial benefit for the industry is already becoming apparent. This becomes clear in *chapters 1 to 8*, which are strongly based on the current state of the art and research. To venture a somewhat broader view of the future, all project partners were asked in this chapter to form a picture of the future of the biogas sector and to highlight their respective contribution to it. The results of this survey were compiled in the first section of the perspective chapter. An important step towards realising the desired biogas future is a better understanding of the anaerobic microbiome. An enormous increase in knowledge has been achieved through the innovative technologies of DNA sequencing. For this reason, we have decided to address the topic of DNA sequencing separately in the outlook chapter. The resulting section is a draft for a more comprehensive review article that will be published separately from this roadmap.

Finally, we would like to draw attention to the future role of biochar. Biochar can affect biogas on both an economic, biological, and chemical process level. It is already known that biochar stabilizes and changes the microbiome. Biochar could also act as a potential carrier for mi-croorganisms (bioaugmentation), it can adsorb harmful/inhibitory substances in the reaction sludge and there is the idea of better linking biogas plants with emissions trading via biochar. An expert in this field is the company LUCRAT GmbH from our Advisory Board, which was also kind enough to collaborate with MICRO4BIOGAS on a section about biochar for the pre-sent roadmap. The MICRO4BIOGAS partners are convinced that biochar has an important role for the further development of the biogas industry in the field of microbiome manipulation, which is why the present section on biochar was brought into the prospects chapter.

This chapter describes the efforts and goals of the MICRO4BIOGAS project, focusing on the contributions of each partner to create a comprehensive view of the MICRO4BIOGAS project's impact.

14 EUROPEAN PARTNERS INVOLVED IN 4 KEY AREAS

1. Understanding biogas plant processes
2. Applications and technical challenges
3. Economic, Legal, and Policy Considerations
4. Collaborations and Knowledge Transfer

Our partners answered questions about:

- *The main goal of MICRO4BIOGAS*
- *The most urgent research and industry objectives*
- *The evolution of the biogas market in the long run*
- *Current challenges faced by the biogas industry*

Overview of the project

9.1 A MICRO4BIOGAS opinion poll

Author: Christian Abendroth

The MICRO4BIOGAS project is a big consortium with 14 partners, which are distributed across Europe. Despite regular project meetings, it is difficult to comprehend the full impact of all contributions of everyone. The aim of this section is therefore to create a holistic view based on personal contributions of every partner. The idea here is to highlight basic project tasks, but also short-term and long-term goals that extend beyond the project as well as current hurdles in the biogas market. It should be emphasized that this is not primarily a chapter that is supported by scientific sources. While the roadmap was drawn up almost entirely based on extensive literature research, this section was supposed to open up the possibility for each partner to leave their own personal imprint. This was done with the help of an internal project survey, which asked four important questions: **(1)** What is the main task of the respective partner? **(2)** What near-term development opportunities does the respective partner see for the biogas market? **(3)** Which long-term development goals for the biogas market would be conceivable? **(4)** What specific hurdles will we face in the future?

Question/Task 1: ***"Please describe your main goal in MICRO4BIOGAS":***

Answers to this question can be clustered into three major topics. Multiple partners highlighted the need for a better understanding of processes, which are happening inside of biogas plants. This concerns especially biological questions regarding the anaerobic microbiome. However, many partners stated their role in improving anaerobic digester efficiency. Apart from this, several partners highlighted their involvement into financing, economics, IPR and policy making.

With regard to the first topic, it is clear that we are far from fully understanding the complexity of anaerobic digestion and the underlying microbiome. Although there are already many publications out there on taxonomic profiles in biogas plants, this research is far from complete. Therefore, involved partners seek to sequence DNA from many biogas plants. Many biogas plants in Germany and also in the Netherlands were sampled and the resulting study could become one of the largest published works in this area to date. In the meantime, an extensive culture collection with microbial isolates has already been created, which is currently being investigated. The combination of taxonomic profiling, metagenomics and culturomics could provide new insights into the key organisms involved. However, this research could also provide insights into completely new strains. One of the MICRO4BIOGAS partners highlighted in this regard the MBA03 *(Darwinibacteriales)* strain. There is hardly any information about this strain so far, but it can be found in many taxonomic profiles that have already been published. It should be emphasized at this point that the multi-OMICs approach described here is related to an extensive data set, which is based on chemical process data but also based on plant-specific technical parameters. On the basis of this diverse database, the partners hope to gain a better understanding of the relationships between the microbiome and plant productivity. The expected results are also intended for more than two publications in a peer-reviewed journals. Furthermore, involved partners hope to get more insight into the interrelation between diverse microbial species and environmental, chemical, as well as technical parameters. Some of the strains were even selected for project tasks, which are related to microbial engineering and adaptive evolution. It should be emphasized that the partners do not use genetically manipulated microorganisms (so-called GMOs), as this does not currently seem conceivable or realistic from a legal point of view for biogas plants. In addition, there is a risk that a lot of work is put into the genetic manipulation of a microorganism that ultimately cannot assert itself into the microbiome due to its high complexity and microbial diversity. Interestingly, one of the partners highlight-

ed his interest in the improved digestion of lignocellulose. This is a topic, which can not only be addressed with bioaugmentation, but also with technical pretreatment. In this context, a conflict in the possible treatment of wood scraps and timber by incineration or anaerobic digestion should be mentioned. From the energetic point of view, it makes much more sense to incinerate wood, as only a fraction of the lignocellulose can be converted in methane. The degradation efficiency of lignocellulose can be improved by pre-treatment, e.g. by steam explosion. However, there will be a substantial amount of lignin, which remains in the digestate. The remaining digestate can yield a highly efficient fertilizer. Proponents of the fermentation of wood emphasize the possibility of producing a high-performance fertilizer that would not be possible to produce (at least not in this quality) with combustion. This offers the possibility of material use, which would be preferable to purely energetic use according to the European waste hierarchy (according to the 4R framework: Reduce, Reuse, Recycle, Recover). Due to the material use, the path of fermentation would currently be preferable to incineration. However, it would be interesting to carry out a life cycle assessment (LCA) for a better comparison between combustion and fermentation of wood scraps and timber in the future. Even if this task is not part of MICRO4BIOGAS, LCA itself is also addressed in the project. In this regard, involved partners are interested to assess, whether bioaugmentation could have beneficial aspects from the environmental, social, and techno-economic point of view.

In addition to these very basic questions, there are also more applied questions that are addressed in MICRO4BIOGAS. If promising microorganisms for bioaugmentation approaches are found, the companies involved will ask themselves whether upscaling is possible and economically attractive. The planned LCA should also contribute to answering this question. At this point it should be mentioned that beneficial effects do not depend solely on the efficiency of any microorganisms. The technical boundary conditions are also relevant. In this context, one of the work packages deals with the question of which innovations and technical optimization options are conceivable for biogas plants. Since success in the search for suitable strains for bioaugmentation is lengthy and not foreseeable, the possibility of biostimulation is being considered as an alternative. Compared to bioaugmentation, biostimulation is a promising option to achieve efficiency gains in biogas plants through changes in the underlying microbiome. One of the partners emphasized that bioaugmentation or biostimulation is not just about increasing productivity in relation to substrate yield, but in can also contribute to more stable conditions or to a better removal of pollutants like ammonia, aromatic compounds, pesticides and pathogenic strains. In this context, a first publication has already been published as part of MICRO4BIOGAS. There was an overlapping project with the company Pro-Entec GmbH. With the PEGAKA process, Pro-Entec has developed a technology for optimizing digestion towers in wastewater treatment plants by stimulating them with oxygen. Through the collaboration with MICRO4BIOGAS, the company was able to gain insight into the underlying microbiome for the first time. The work is entitled *"Microbiome Characterization after Aerobic Digestate Reactivation of Anaerobically Digested Sewage Sludge"* and has been published by **Otto *et al.*, (2023)** in the Journal *"Fermentation"* **(https://doi.org/10.3390/fermentation9050471)**. In addition to the cooperation with Pro-Entec GmbH, there is another project-overlapping cooperation.

Two of the MICRO4BIOGAS partners have a side project at a national level in Spain. In this side project, a biogas plant is to be built in region of Aras de los Olmos. The construction of this biogas plant is not part of the MICRO4BIOGAS tasks. Nevertheless, a transfer of knowledge takes place here, from which the Spanish project benefits considerably. The designers of the biogas plant have not yet built a biogas plant themselves, which makes the project a significant challenge. The exchange with plant manufacturers and experts in the biogas industry as part of the MICRO4BIOGAS project, therefore, contributes indirectly to the success of the side project in Aras de los Olmos. Based on this, partners associated with the planned

biogas plant in Aras de los Olmos have some interest in acquiring basic knowledge on the state of the art. In return for the knowledge gained from MICRO4BIOGAS, the actors from Aras de los Olmos have agreed to provide the planned facility as a test platform for the use of knowledge and products from MICRO4BIOGAS in the post-funding period. At the present time, many European biogas plants are stationed in Germany. This fact shows that Europe has reached an uneven level of knowledge about biogas. There are already technically mature solutions here and the next step is to upgrade the level that has been reached (e.g. with the help of a better understanding of the underlying microbiome). In addition, a transfer of knowledge must first take place in other European countries so that these countries can set up biogas plants themselves. To better capture the level of knowledge about biogas and anaerobic microbiomes, a cross-national survey has already taken place in the frame of MICRO4BIOGAS, in which a little less than 200 respondents took part. The results are currently being evaluated.

Finally, it needs to be highlighted that the success of MICRO4BIOGAS and further the succes of the biogas industry in general does not only depend on technological progress but also on financing and policy decision-making. Therefore, several of the MICRO4BIOGAS partners are associated with this topic. Several of the partners hope that the results from MICRO4BIOGAS and the associated public relations work will raise awareness. The partners are looking for the possibility of disseminating these results, especially among stakeholders, to facilitate their decision-making processes. The MICRO4BIOGAS partners hope that the here presented roadmap will also contribute to this. In this context, partners involved use the MICRO4BIOGAS project as a bridge between industry and research. To support this, there is also a partner in the project, who has his main expertise in patent consulting, commercialization, IPR training and IPR strategy. Finally, it should be mentioned that MICRO4BIOGAS is involved in a lively exchange with other scientists from the biogas industry and biogas research. In this regard, the MICRO4BIOGAS project is intended to contribute to better networking of the biogas community and to overall expand the European biogas network.

Question/Task 2: ***"Imagine how the biogas market will change in the short term (in the next 3 years). What is the most urgent objective, that researchers and industrials should follow? Ideally, it should be a realistic scenario":***

The MICRO4BIOGAS partners perceived this question very differently. Some commented more on expected results from the MICRO4BIOGAS project, while others addressed timely expectations for the biogas market in general. Regarding the former, there is generally the expectation that a productivity increase of 5% is realistic through bioaugmentation, although this is of course a very vague statement. The improvement that can be expected depends strongly on the respective system and on the substrates used. For example, no great increase in yield is to be expected with easily digestible substrates, as anaerobes can already degrade these substrates quite completely. However, biogas plants with poorly degradable substrates could benefit significantly from high-performance microbes. Another expectation is that the results of MICRO4BIOGAS will lead to a better understanding of anaerobic microbiomes and that this knowledge will open new financing options for further projects and maybe new products that can be generate out of anaerobic digestion. Regardless of the MICRO4BIOGAS project, the partners involved assume that microbiome research in the biogas sector will progress rapidly in the future through the global research community and that knowledge about the microbial key players involved will now be completed step by step. For example, one of the partners points out that in addition to MICRO4BIOGAS, there are other EU projects that deal with the possibility of improving the biogas industry. As part of the regular MICRO4BIOGAS meetings, there was a direct exchange with other European projects. The partners assume that in the future technical innovations, digitization, and standardization as well as an improved understanding of anaerobic microbiomes will lift the biogas industry to a new level. If one looks at the large

number of biogas plants in Germany and assumes that the rest of Europe will follow up in time, there will be an immense market here soon. Therefore, the partners involved expect a further increase in the number of biogas plants soon. The MICRO4BIOGAS partners have pointed out that several instruments, initiatives, and policies are already available that favor this development. Here, for example, the growing community of a European Biogas Association (EBA) can be mentioned. The "European Green Deal" and the EU plan to further reduce dependence on fossil fuels ("RePowerEU") are also important components for promoting the biogas industry, which will also encourage private investment. Especially public initiatives, e.g., due to the EBA or due to similar projects as MICRO4BIOGAS, will raise awareness from people all over Europe or even worldwide. There are already other European directives/initiatives that support this direction, and this has also been dealt with in detail in the present roadmap. With the growing biogas industry, further guidelines and funding instruments can be expected in the future. In this context, it must be mentioned that in the future it will not only be a matter of building new plants, but also of modernising old ones. As a pioneer in the biogas industry, Germany accounts for 50% of all European biogas plants. Amongst these, there are already some old systems for which there is a prospect that the statutory protection of existing buildings will expire. Upgrading these systems will be a major challenge and it would be great if such activities would be supported on a European level as well. This does not necessarily have to be about funding instruments. The EU sets the course for legislation in the member states. A corresponding directive can encourage member states not only to push for the construction of new biogas plants, but also to support the expansion and maintenance of old plants. In any case it is to be expected that the European Unions efforts towards renewable energies will eventually be enforced on national levels. It is therefore not only to be expected that European countries with an established biogas technology such as Germany, Denmark and Norway will further advance their biogas technology and use. Despite having a shorter biogas technology history, countries such as Poland, the Czech Republic, Hungary, Latvia, and Estonia, are expected to develop their biogas sector further, adding to the overall progress. However, one partner also mentioned somewhat critically that increasing efforts at the political level will also be accompanied by additional regulations (similar to what is currently happening with landfilling). Thus, in the future, stricter rules, and specifications for the implementation of biogas plants should also be expected.

It is also expected that there will be an increasing expansion of biomethane plants. This movement has already started, is being strongly propagated by the EBA and there are already the first biomethane plants in several European countries. The European Union is also promoting the expansion of biomethane plants and their connection to the gas network. The advantage of this technology is that there is no need for combined heat and power plants and biogas can be fed directly into the gas grid. If this technology is successfully expanded, there is in long-term the prospect of a Europe-wide gas network for trading in biogas. With the prospect of a further establishing biogas market, the handling of waste will also continue to change. A separate collection of organic waste does not yet take place across the board in Europe. There is a high potential for residues here, which has so far hardly been exhausted. Ultimately, this will also result in less waste going to landfills. Already in the EU "Directive 1999/31/EG" on landfills, the waste hierarchy (4R framework) is given priority in addition to stricter disposal criteria. It is therefore up to the respective member states of the EU to take approaches for the best possible recycling of waste and strategies for pretreatment into account in their legislation. This does not only include biowaste from households, but also waste, which is difficult to degrade in anaerobic digestion plants for example timber and wood scraps. It needs to be pointed out that timber and wood scraps are allowed for incineration. Incineration will yield electricity as well. However, there is also the possibility of anaerobic wood/timber digestion. Timber and wood scraps can only be digested partially. The digestate can be converted into a high-performance fertilizer due to the lignin that is converte

dinto humic substances. Due to the possibility of providing fertilizer via biogas processes, material use is in prospect here, which is preferable compared to purely energetic use. Therefore, in the case of wood, there is currently a competitive situation. This also raises the question of a stable supply of substrate throughout the year. Substrate supply can be impaired due to competing situations as a result of seasonal changes. A steady substrate availability is necessary, to keep the anaerobic microbiome steadily active. In the case of agriculturally produced substrates in particular, annual fluctuations are to be expected and, last but not least, these substrates are viewed very critically due to the "food vs. fuel" discussion. In this context, it makes sense, of course, to initially strive for the most complete possible use of biowaste from households and animal manure, which are available all year round. However, it needs to be considered that agricultural crops will yield organic waste, even if the crops were not intended as substrates for anaerobic digestions processes. Therefore, intelligent distribution and usage concepts are required, which will ensure an even supply of biogas plants throughout the year in the future. Above all, this is also a logistical and planning problem, where the advancing digitization can certainly contribute.

One of the partners expressed the assumption that improved anaerobic digester technologies will help to reduce energy costs. This needs to be discussed critically. At the present time, energy costs are kept at a low level by using fossil fuels. However, energy production costs are currently rather rising based on an extended use of renewable energy. It should be mentioned that at the present time the environmental damage caused by fossil fuels is hardly considered in the energy price. If one adds indirect costs of dealing with environmental damage, the situation changes. Global efforts are already being made to change this, e.g. as part of the introduction of the CO_2 tax. The United Nations Framework Convention on Climate Change, UNFCCC, already existed in 1992, from which the Kyoto Protocol emerged in 1997. With this, target values for greenhouse gas emissions were set for the first time in 2005, laying the foundation for CO_2 taxation and emissions trading. This direction will tend to strengthen. This not only raises the question of whether electricity can become cheaper in the future, but whether fossil fuels will remain so cheap. Renewable energies such as those based on biogas offer a good alternative to rising prices for fossil fuels.

In addition to biogas, fertilizer and biomethane, a partner has also suggested other utilization concepts. The possibility of using methane as a gaseous and liquefied fuel was discussed here. The possibility of combining it with dark fermentation for hydrogen production was also addressed. Despite years of research, the latter is still in its infancy. Nevertheless, it is to be expected that the constantly improving financing options for alternative energies will also allow research in this area to flourish.

Question/Task 3: ***"Imagine how the biogas market will change in the long term (e.g. in 10 years or even longer)":***

One of the economic experts in the project referred to several studies, according to which a 1.9 - 2.4 fold increase in current biogas production can be expected for 2030. Very likely, public pressure to rescue the climate will increase as well, which will also be reflected in the market for renewable energies. This expected market increase makes one dream about what developments an increasing biogas market will enable in the future. Therefore, we want to look into the future with the third task/question. We encouraged all partners to think outside the box and develop a holistic and futuristic vision of what the biogas industry could look like in the distant future. One partner suggested that production services for biogas plants will be much more elaborated and standardized. Full service companies might evolve that take care of providing solutions through biogas production plants.

Another partner suggested that biogas will globally become a dominant and highly preferred source of renewable energy. Here the opinions were a bit contrasted, as another partner highlighted that competitive situa-

tions to other renewable technologies could lower the interest in anaerobic digester technologies. In this regard, it must be considered that there is already an obligation to dispose of organic waste and that disposal in landfills will not be tolerated in the long term. Composting is an alternative here, although composting does not offer the possibility of energy production. It should also be considered that the biogas industry, in contrast to solar or wind power, allows energy to be stored in the form of biogas. The storage of excess energy via Power-2-Gas biogas plants can also play a role here. In this respect, biogas will play an important role in the long term, even if the production costs for electricity are higher compared to solar or wind energy. A change is also to be expected from a technical point of view. In one of the chapters of the roadmap, the possibility of microbiome manipulation was addressed. Regarding the biogas industry, research has only just begun here. With the help of microbiome manipulation and coupling to dark fermentation, material use via biogas plants could be improved in the long term. In addition to methane and hydrogen, a variety of organic acids could be produced and extracted. These could help replace petroleum in chemical production routes. Possible end products from corresponding synthesis routes are textile fibers, aroma, bioplastics, plasticizers, biopesticides and fungicides, food supplements, oils, and resins and much more. In this regard, the MICRO4BIOGAS partners look forward to a toolbox of microbial solutions to manipulate the process using both biostimulation (i.e., pH changes, nitrogen removal, etc.) and bioaugmentation (i.e., adding microorganisms). With new strategies of biostimulation and/or bioaugmentation, more efficient degradation mechanisms might be in reach. These mechanisms could ultimately help to degrade substrates, which are not fully accessible yet (e. g., lignin, chitin, certain plastics, mono-digestion of chicken manure or mono-digestion of fat). One partner suggested that one day we might even be able to treat waste from the packaging industry in anaerobic digesters.

The partners have the hope that with the above-mentioned developments the overall European dependency of global markets will be reduced. More self-sufficiency will save resources due to reduced efforts in logistics, which will eventually have a positive impact on the environment. To reduce logistic efforts, one partner suggested also small-scale plants. There are already small-scale plants on the market (e. g., for allotment gardener). However, these plants are not efficient enough and have not applied in large amounts yet. Increasing energy prices and advancing technologies might change this. In this context, it must be emphasized that in some regions composting is preferred over biogas production because the substrates in the immediate vicinity are not sufficient to operate a biogas plant. New logistical concepts and new approaches for small biogas plants could create a solution that significantly increases the availability of substrates for the biogas industry. In this context, one of the partners stressed that cutting-edge technologies must be available in a decentralised manner. Municipalities, villages, and small towns currently have little or no experience in microbiology or high-throughput DNA sequencing and probably also cannot find the time to implement complex process control for regularly adjusting/controlling selection pressures for microbiome manipulation. In order to make the relevant technologies usable for these people, further technological advances, automation, standardization and ongoing digitization are required.

Finally, a partner's wish to improve sector coupling should also be mentioned. In the future, biogas technology could be networked with a variety of industries. Not only for the utilization of residues, but also for the provision of material recyclables for agriculture, the chemical industry, for industries with heat requirements and also a cross-sectoral use with solar and wind energy would be conceivable. The biogas industry could thus become an important pillar of the circular economy. In this context, it should be mentioned that more and more research articles from the circular economy address the so-called “meso level”, in which industrial parks are geared towards maximizing cross-company synergies. The cross-sectoral use of biogas plants would fit exactly into this picture.

Question/Task 4: ***"What is in your opinion the biggest obstacle, which the biogas industry is facing today?":***

After the creation of holistic development scenarios for the short and long-term future, the MICRO4BIOGAS partners were further asked which concrete obstacles had to be overcome on the way to the proposed future. A major problem remains that the microbiome of anaerobic digesters is still considered a black box, even after years of research. It is not well understood how operational changes affect the microbiome and it is difficult to predict taxonomic shifts. This brings with it a lack of standardisation.

Apart from lack of knowledge in respect to the anaerobic microbiome, there are also conflicts about regulation. Although the number of regulations is likely to increase in the future, one partner has already presented the current regulations as too complex. In some countries, the regulatory apparatus appears to be a factor preventing the construction of new biogas plants. This is one of the investment risks, which might prevent investors from investing in this technology. The MICRO4BIOGAS partners highlighted further that there is not yet enough awareness amongst the stakeholders and potential investors. Although many of them might have a general idea about biogas, the full potential of biogas has not been fully grasped. To deal with this problem, much more work on public relations is necessary. One partner highlighted that for now the biogas industry is only focused on biogas (partly biomethane) and it needs to be communicated that biogas plants can become much more. In needs to be disseminated that biogas plants have the potential to become multi-product factories with long-term benefits for the economy but also for the environment.

Another problem, which lowers the interest of potential investors are the high construction costs. Compared to the revenue in the end, the production costs are very high. This problem can be solved by national and European funding programs. For example, the European member states could offer transitional financing for investors, so that they are relieved in the short term and repay the grant over a longer period if the implementation is successful. Furthermore, the durability of biogas plants should be integrated into this cost factor. Insurance policies could also be specifically linked to investment projects to minimize the risk.

According to one partner, the current goals in respect to decarbonization regarding biogas plants cannot yet be formulated clearly enough. A bridge is already being established in Germany between the biogas industry and the RED II directive (Renewable Energy Directive) by forcing larger biogas plants to fulfill certain emission criteria. On the one hand, it is a disadvantage, as the relevant regulations can jeopardise a construction project. On the other hand, it can also be an opportunity in the long term to link the biogas market with emissions trading.

9.2 Outline: In search of key players in anaerobic digestion: meta-analysis of biogas microbiomes

Authors: Roser Puchol-Royo and Adriel Latorre-Pérez

One of the aims of MICRO4BIOGAS is to review anaerobic digestion (AD) studies that include microbiome sequencing data to consolidate the knowledge about the microbial communities inhabiting biogas reactors. To achieve this purpose, public data is being used to draw conclusions on the microbiome structure of AD processes and to investigate the effect of the different operational parameters (e.g., temperature, pH, type of digester) on the microbial

communities. A secondary goal derived from this evaluation is to assess the metadata that is generally provided in AD studies and to propose a standardized metadata form to facilitate the reuse of sequencing, technical and chemical data in future meta-analyses.

This section provides an overview of the ongoing study. The next steps will be to systematically compare the results of this meta-analysis with the outcomes obtained from the study of the microbiome of the 80 MICRO4BIOGAS samples.

Bibliographic research: In a first analysis, 38 publications were reviewed to investigate which omic technique is more commonly used to characterise AD microbiomes (**figure 80A**). According to this preliminary analysis, most studies applied metataxonomic or metagenomic approaches, with 16S rRNA gene sequencing being the preferred technique. Moreover, among 16S rRNA samples, the most used technology was Illumina (**figure 80B**). Consequently, the present meta-analysis was focused on this type of data (i.e., 16S rRNA gene sequencing) and this sequencing technology (i.e., Illumina).

To optimize the search, several approaches were followed (**figure 81**). First, the AI tool Elicit **(https://elicit.org)** was used with the search "Anaerobic digestion studies with 16S rRNA sequencing", selecting only articles from 2015 to date. Moreover, a manual search was performed in different search engines: in Google Scholar with the words "anaerobic digestion", "biogas", "microbiology", "metataxonomy" and "sequencing", and in the NCBI database using the taxonomy of "anaerobic digester metagenome" (txid1263854) and "biogas fermenter metagenome" (txid718289).

The 56 scientific articles collected at this point were uploaded to Zotero **(https://www.zotero.org/)**, and filtered manually to collect only those scientific works after 2015 which actually contained 16S rRNA sequencing data and that were sequenced by Illumina technologies. This way, 30 studies were excluded because **(i)** they were reviews or were not related to biogas production by AD, **(ii)** the paper was not publicly available, **(iii)** they did not have 16S rRNA gene data available, or **(iv)** the sequencing platform was different from Illumina.

26 studies were obtained, its references downloaded from Zotero and uploaded to a ML tool: Litmaps **(https://www.litmaps.com/)**. This tool helps looking for similar studies, classifying them according to their relevance and year of publication. This way, 20

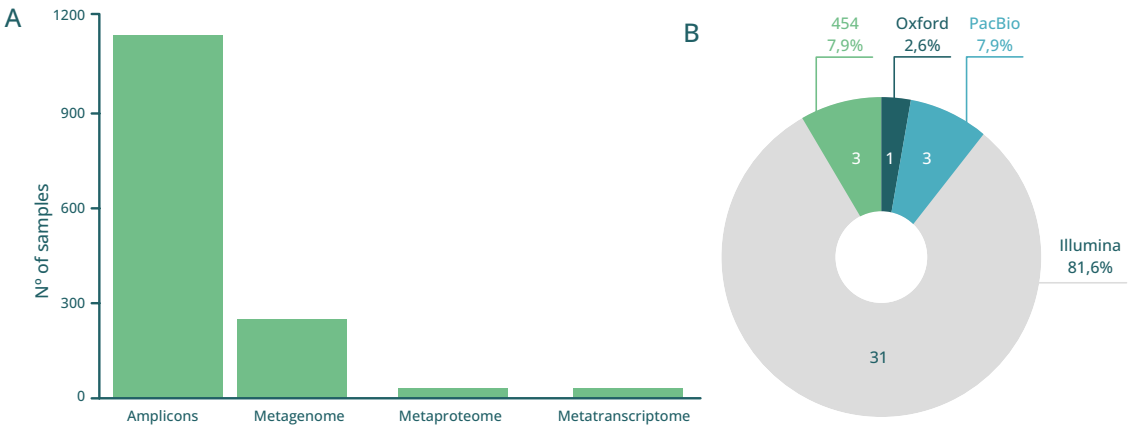


Figure 79: (A) Omic techniques used to study microbiomes in previous studies (n = 38). There is a clear predominance of metataxonomic data ('amplicons'), followed by metagenomics. (B) Type of sequencing technology used for the metataxonomic analyses. Oxford: Oxford Nanopore technologies.

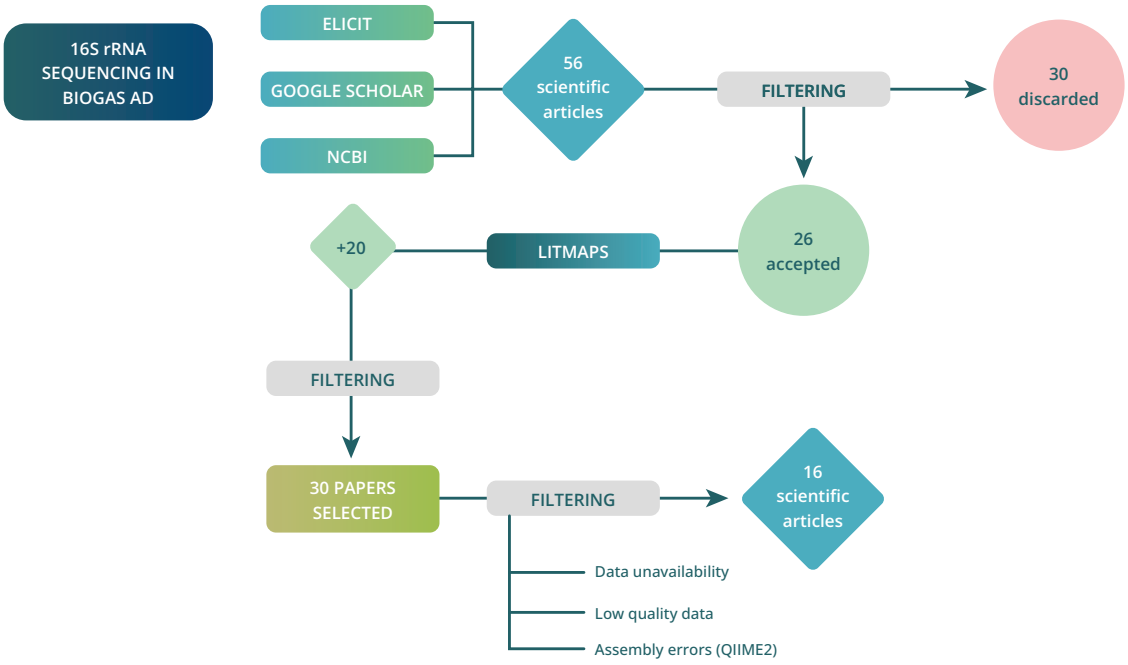


Figure 80: Workflow followed for the bibliographic research.

additional papers were obtained, which were again filtered following the same criteria.

A total of 30 scientific papers were finally selected and the available data was downloaded and prepared for the bioinformatic analysis in qiime2 (v. 2023.2) **(Bolyen *et al.*, 2019)**.

However, due to several problems such as **(i)** failure to download data, **(ii)** bad quality of the raw reads downloaded or **(iii)** errors during the bioinformatic analysis, only 16 datasets could be processed, with a total of 654 samples from 88 different reactors (**figure 81**).

Bioinformatic analisis: The raw reads of all the projects with available data were downloaded and prepared for the analysis. All the raw reads had in common that **(i)** they contained amplification for the hypervariable region of the 16S rRNA ribosomal gene, which varied between V1 to V6 (or combination of two regions), using also a wide variety of primers depending on the procedure, and **(ii)** the amplification products were sequenced by Illumina technologies.

In separate batches for each project, the raw Illumina sequences were loaded into Qiime2 (v. 2021.2.0) **(Bolyen *et al.*, 2019)**. The quality of the sequences was checked using the plugin Demux and the Qiime2-integrated DADA2 pipeline was used for trimming and joining the sequences, removing chimeras and detecting amplicon sequence variants (ASVs) (> 99.9% of similarity). The taxonomy of each sequence variant was determined via the classify-Sklearn module from the feature-classifier plugin, employing SILVA (v. 138) **(Quast *et al.*, 2013)** as reference database for taxonomic assignment. Some samples were removed due to problems during the bioinformatic pipeline (e.g., few sequences were available after preprocessing raw reads, forward and reverse reads could not be properly joined, etc.). The resulting data was analysed with the phyloseq R package **(McMurdie and Holmes, 2013)**. The microbiome package was used to obtain the core microbiome of all the samples.

Average microbial community in anaerobic digestion: Overall, 654 samples from 16 different studies were analysed, and a total of 3617 species, 1688 genera and 68 phyla were detected. The average microbial profile

resulting from this meta-analysis (**figure 82**), was comparable to the results obtained for the 80 AD samples analyzed in MICRO4BIOGAS. The main highlights were:

- Archaeal taxa represented 7% of relative abundance, being *Methanosarcina*, *Methanosaeta* and *Methanoculleus* the most abundant genera.
- Firmicutes was the most abundant bacterial phylum (44%), followed by Bacteroidota (27%). Synergistaceae, Proteobacteria and Chloroflexi were also important members of AD microbiomes.
- At the genus level, *Proteiniphilum* represented the 9% of the average AD microbial community, followed by MBA03 *(Darwinibacteriales)* and DTU014, which were present in ~6% of relative abundance. Beyond them, other important taxa such as *Syntrophomonas*, *Acetomicrobium*, *Christensenellaceae* and *Clostridium sensu* stricto exceeded 1% of average abundance.

Anaerobic digestion core microbiome: Defining a core microbiome is essential to understand which microorganisms are present in the vast majority of AD samples and, therefore, may play a key role in biogas pro-

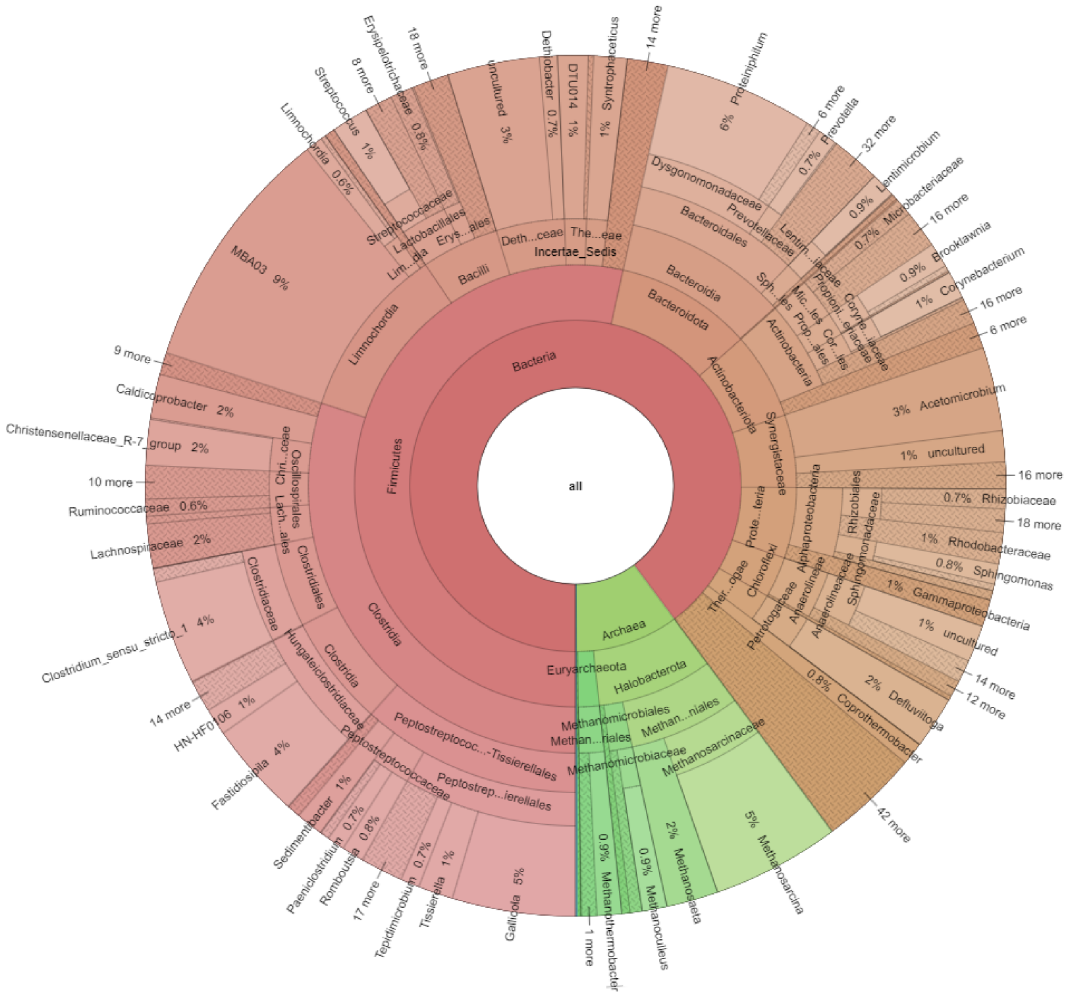


Figure 81: Krona plot with the average microbial profiles of anaerobic digestion samples (n = 16 AD studies).

duction. Due to the high variability among the different studies, the AD core microbiome was calculated using different prevalence thresholds: 70%, 80% and 85%. In other words, only taxa that were present in more than 70% of samples were considered to be core taxa. Moreover, microorganisms were also filtered by relative abundance to reduce the complexity of the dataset. Specifically, only taxa exceeding 0.01% of relative abundance in the sample were considered (Neu *et al.*, 2021). The microorganisms that integrate the core microbiome at a 70% abundance are similar to those found in the MICRO4BIOGAS samples at 99% of prevalence and 0.01% abundance. It is interesting to note that if prevalence threshold is increased to 85%, the only microorganism present is MBA03 *(Darwinibacteriales)* (**figure 83**), which highlights the importance of this uncharacterised bacterial taxa in biogas production. For that reason, MBA03 *(Darwinibacteriales)* is currently being described in the framework of MICRO4BIOGAS.

Analysis of metadata: Beyond microbiomes, metadata provided by each study (i.e., type of reactor, operational or chemical parameters) is also being evaluated. From the metadata available in the 30 papers before the last filtering step, we could conclude that Denmark is the country which accounts for the highest number of studies that investigate AD microbiomes by metataxonomy, followed by Sweden and China (**figure 84A**). Interestingly, Germany and the Netherlands are poorly represented according to our meta-analysis, despite being two of the main leaders in anaerobic digestion in the European Union. MICRO4BIOGAS has helped to close this gap by analyzing 80 different biogas-related samples collected from AD plants across these countries.

Regarding temperature of operation (**figure 84B**), sixteen studies analyzed mesophilic reactors (below 45ºC), five studies used thermophilic temperatures (from 45ºC) and nine publications studied both types of conditions.

From the 30 studies, nineteen were based on lab-scale reactors, while eleven relied on the analysis of full-scale reactors (reactors up to 20L of working volume are often considered as lab-scale reactors, while full-scale reactors normally comprise volumes from 1000L onwards of working volume). However, from the 16 studies that were finally analyzed, only 3 of them studied the microbial population from full-scale digesters. The MICRO4BIOGAS project intends to fill this gap of information, as microbial analyses were carried out over 80 samples from 45 different full-scale reactors.

Important notes on metadata collection: It is crucial to mention that several of the

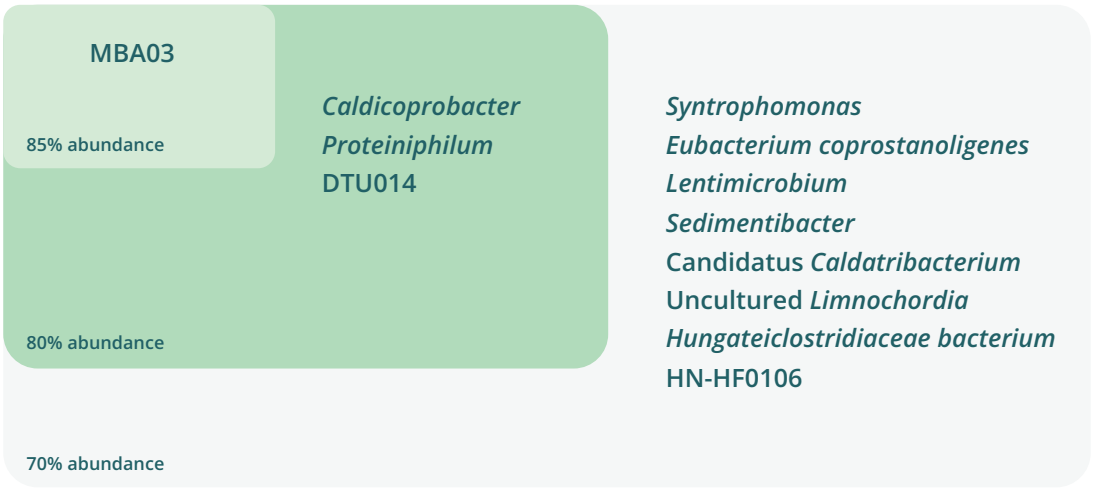


Figure 82: Anaerobic digestion core microbiome at different prevalence thresholds based on 654 public datasets.

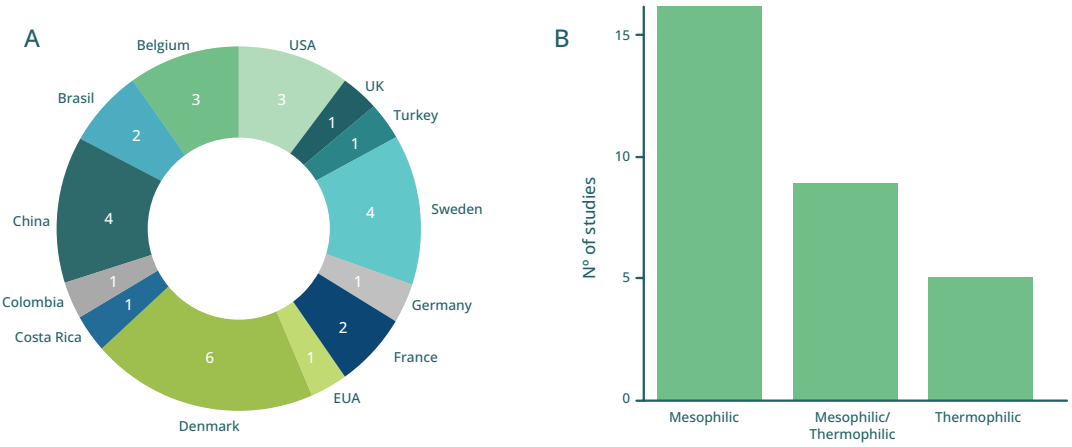


Figure 83: Overview of the studies included in the present meta-analysis. (A) Contribution of each country to AD digestion studies including metataxonomic data. (B) Temperature of operation used in these studies.

studies found during the literature research did not include publicly available microbiome data or the data was only available upon request. Moreover, datasets that were available in public repositories included incomplete metadata, which makes it difficult to reanalyze the data properly. In other cases, quantitative parameter values were given in figures, especially when studying how a parameter varies with time or with the change of another parameter (i.e., changing temperatures, pH, substrate). Moreover, several authors did not assign the different conditions used during the study to the different samples, so this data is virtually non-reusable.

Additionally, serious problems were detected regarding the standardization of parameters. For example, the studies that provided information on biogas production used different parameters (i.e., biogas production, yield or backflow rate and methane content, yield, product and potential), and these were represented using different units (**table 26**). This complicates the integration of data from different sources for a meta-analysis, as some units are not easily comparable. For instance, some studies indicated the production of biogas in m^3/m^3 of digester, but the volume of the digester was not given.

There are also some parameters, which are of high importance for the study of biogas production, that are systematically omitted. For that reason, we consider that, at least, the following information should be included in every AD-related publications in order to improve the reusability of data in future meta-analyses:

- Country of origin of the bioreactor
- Volume of the reactor
- Temperature (ºC)
- Type of feedstock. If possible, specify the percentage of each substrate
- pH
- Hydraulic retention time (h)
- VFA (g/L). If possible, specify the concentration of each VFA separately
- Biogas, methane or electricity production

In addition, in the framework of the MICRO-4BIOGAS project we have designed a standardized form to collect metadata from AD plants. This form was used during the project, and it was modified to include only the parameters that are commonly provided by AD plant operators. We encourage authors to use this form in future studies to ensure the reusability of the data. The final objective

Table 26: Different units used to describe the same parameter in different studies.

Parameters	Different used units
Biogas or methane production	ml (total), m^3/day, m^3/m^3-digester/d, m^3/t, Nm^3/kg-VS
Biogas or methane yield	m^3/kg-VS, ml-CH_4/g-COD, Nm^3/d, Nm^3/kg-VS, l/kg-oDM, percentage of methane
VFA	2 - 7 Vol.-% (20-40 °C)

is to facilitate meta-analyses that will further investigate the effect of operational, chemical and microbiological parameters on the efficiency of the biogas production process.

Conclusions: The ongoing meta-analysis is providing valuable information on the role that different microorganisms play in anaerobic digestion. Preliminary results point out that the conclusions drawn for the samples characterized in the MICRO4BIOGAS project can be generalized to the rest of the samples in the study.

At a metataxonomic level, the abundances of the most relevant groups of microorganisms (i.e., *Firmicutes, Bacteroidetes)* are comparable to the results obtained for the 80 samples analyzed for MICRO4BIOGAS. Moreover, MBA03 *(Darwinibacteriales)* has proved to be present in the vast majority of AD samples, underlining the potentially crucial role that this uncharacterized bacterium may play in biogas production. This bacterial taxa is currently being described by members of the MICRO4BIOGAS consortium.

After analysing the metadata available, Denmark is the country with a higher contribution with number of studies and samples to metataxonomic data, and overall, full-scale digesters are underrepresented among the datasets found and studied, as most of the studies are based on laboratory trials. MICRO4BIOGAS has contributed to closing this gap by studying 80 samples from full-scale reactors that operate under mesophilic and thermophilic conditions.

Although the available information is scarce and not in all cases available, we are working on collecting as much metadata as possible from the studies in order to establish correlations between the metadata, the different operational conditions and the microbial communities of AD processes.

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9.3 Biochar in Biogas Processes

Authors: Savannah Baptist and Christian Abendroth

Introduction: The following chapter is to be a part of a doctoral thesis by Ms. Savannah Baptist. Circular economy refers to an economic system to minimize waste and maximize resource efficiency. Approaches to dispose waste effectively and efficiently are needed. One such approach being tested, is the use of biochar in anaerobic digestion. Biochar can effectively increase the rate of methanation in AD with other beneficial factors of ammonium adsorption, pH control, VFA adsorption, CO_2 adsorption etc. The present article tries to answer, Why the use of biochar in AD is beneficial? It discusses further, what are the underlying mechanisms and contributing factors? And finally, suggestions are made, how do we move forward?

There are currently many articles that link the use of biochar to the phenomenon of direct interspecies electron transfer (DIET). DIET was also discussed in the previous chapters of the roadmap. In AD the phenomenon of direct electron transfer has been identified as an alternative mechanism for syntrophic electron exchange. Exo-electrogenic bacteria can exchange electrons through an external pili or membrane cytochrome **(Wang *et al.*, 2018)**. The addition of conductive material such as carbon cloth, granular activated carbon, minerals (magnetite) have helped further exploit this mechanism. Granular activated carbon (GAC) was found to increase the methane potential through H_2S adsorption and reduce organic load shock **(Luo *et al.*, 2015)**. GAC has also been found to enrich hydrogenotrophic species of methanogenic archaea. Generally, in anaerobic high-performance digester a high organic loading rate is aimed for. The higher the organic loading rate (OLR) the higher the economic efficiency. However, a high OLR can result in an imbalance between acidification and methanation, leading to an accumulation of VFAs and a decrease in pH **(Wang *et al.*, 2018)**. Ultimately, this might lead to acidosis. Due to the adsorptive characteristics of GAC, it could help to tackle this problem. Addition of graphene resulted further in an increase of methane production **(Wang *et al.*, 2018)**. It is therefore conclusive that the use of an adsorptive and conductive supplements can increase the overall methanation velocity. However, it needs to be regarded that production costs of these materials might be high, and the disposal of the respective materials can pose a challenge **(Wang *et al.*, 2018)**. Retaining the material within the reactor may not necessarily be possible, as activated char (AC) is usually added in the form of a powder, and its separation adds to additional cost **(Luo *et al.*, 2015)**. As an alternative and solution to these issues, biochar has been found to be a promising additive.

Biochar is a carbonaceous, porous, bio-stable material produced through anaerobic pyrolysis **(Luo *et al.*, 2015; Chen *et al.*, 2020)**. Biological waste chosen is generally high in lignocellulosic content. Treating this waste through other processes such as anaerobic digestion and composting is time consuming and tedious. Utilizing it in the production of biochar can increase resource efficiency. Biochar has several applications. It has been used as an additive in soil, to increase organic content. Its residues can be directly disposed in the soil without any environmental risk associated **(Luo *et al.*, 2015)**. Production of biochar is significantly cheaper. Biochar behaves similarly to AC and has been tested in biogas plants. The main difference between the two is, biochar is produced at a much lower temperature, and it's not activated **(Luo et *al.*, 2015)**. However, several methods or modifications can be done additionally to activate the biochar and increase its potential.

The physical and chemical properties of biochar are dependent on the feedstock and process used. Several types of raw materials can be used, such as agricultural waste, wood waste, etc. **Hu *et al.* (2021)** suggests three categories of biochar based on its carbon content, which can be high (>75 %), medium (>55% and <75 %) and low (30 – 55%). When biochar is used in AD, characteristics such as pore size, pH, molar ratios of H/C

and O/C are found to play a key role. Though these characteristics are a result of the temperature and atmosphere they may be produced in. Molar ratios of H/C and O/C can be used to estimate the polarity and aromaticity of biochar **(Shen *et al.*, 2021)**.

Numerous articles have been published on the subject of biochar and its application in anaerobic digestion. However, making direct comparisons between these studies can be challenging due to the lack of consistent conditions or missing data on certain parameters.

Literature Selection: The presented section gives an overview on the usage of biochar as an additive in biogas processes. Through this chapter, several questions are raised in respect to the application of biochar. Articles were selected using Clarivate Web of Science ©, Copyright Clarivate 2023 as a search engine. Three searches were performed using all databases to retrieve information. Keywords used in the first search were, biochar AND ”Anaerobic Digestion” OR Biogas AND ”bacteria” OR ”microb*” OR ”arch” OR sequence* OR ”microbiome” OR *DNA* OR *RNA* OR *OMIC*. From a pool of eighteen highly cited articles, only the pertinent papers were selected for inclusion in the database. Based on this search, a few articles related to DIET stood out. Biochar can be conductive in nature, which makes it an interesting additive regarding AD. When added to AD it has found to accelerate the rate of electron transfer. To shed light on the underlying process, biochar AND DIET OR ”Direct electron transfer species” OR ”interspecies electron transfer” AND biogas OR ”anaerobic digestion” were keywords used in the second search. Given the effectiveness of biochar in anaerobic digestion, researchers have explored additional pre-treatment methods to enhance its potential. The third search used the keywords biochar AND ”Anaerobic Digestion” OR Biogas AND modif* OR conver* OR dop* OR magnet*. In the second and third search, only articles published within the last five years were added to a database. In all searches, review articles were excluded.

Effect of Biochar in AD: The influence of biochar depends strongly on its production and dosage. Regarding its production, **Zhang *et al.* (2022),** aimed at evaluating the effects of wood waste biochar in AD (methane production rate, pH, VFA concentration and microbial composition) in relation to pyrolytic time and temperatures. Biochar was used in the concentration of 10 g/L. Results suggested that using biochar produced at 750 °C for 60 min allowed the highest rate of methanation of 2.2 – 21.5 %. Pyrolysis temperature can range from 150 °C to 900 °C **(Chen *et al.*, 2020)**, Characteristics of biochar such as carbon content, ash percentage aromaticity pH surface area and zeta potential tend to increase with the increase in temperature. Biochar has a large specific area due to its porous structure. Depending on the pyrolysis temperature, the degree of graphitization differs. The electrical conductivity increases with the increase in aromatic structures, providing π electrons **(Chen *et al.*, 2020; Shen *et al.*, 2021)**. Due to these electrochemical characteristics, biochar is capable of ion exchange. Further, these factors add to the adsorptive capacity of biochar, which helps in removal of organic and inorganic contaminants **(Chen *et al.*, 2020)**. Other factors such as electrostatic attraction, hydrophobic interaction and pore-filling have been indicated **(Chen *et al.*, 2020)**. Biochar has been found to have surface functional groups such as quinone moieties that can act as electron acceptors and donors, mediating microbial redox reactions **(Shen *et al.*, 2021)**. These surface groups allow the biochar to act as a buffer **(Wang *et al.*, 2018)**. Yet it was found by **Shen *et al.* (2021)**, that the electron donation capacity (EDC) decreased with the increasing pyrolysis temperature whereas it was the opposite for electron accepting. They correlated the methanogenic performance to EDC reason being hydroquinone moieties could play a crucial role in facilitating methanogenesis. It was also seen that biochar produced at 700 °C did not produce high amounts of methane, probably due to less graphenization. Ideally electron exchange capacity determines the biochar's capacity to mediate a redox reaction, however, electron donating capacity is found to play a larger role **(Shen *et al.*, 2021)**. It needs to be considered that depending on the appli-

cation of the biochar, the feedstock for production can produce different types as well. Biochar produced from plant biomass tends to have a higher graphene content due to the high concentration of lignin, whereas biochar that is produced from sewage sludge, animal proteins, containing inorganic salts allow for larger pores and heteroatoms **(Ma *et al.*, 2022)**.

Apart from the ideal production conditions, the ideal dosage of biochar is important as well. The optimal dosage in anaerobic digestion can vary, although multiple authors propose that a range of 8 g/l to 15 g/l can be effective **(Wang *et al.*, 2018; Ma *et al.*, 2020; Pant and Rai, 2021)**. To make results better comparable, **Chen *et al.*, (2023)** introduced a food/microorganism parameter, which they correlated to biochar dosage. Biochar at different dosages promoted different methanation activities. They also related their results to DIET. At low and medium F/M ratios, mass transfer was promoted through electron transfer while at high F/M ratios biochar allowed for syntrophic methanogenesis. **Chen *et al.*, (2023)** recommends at a low F/M ratio (0.5 to 1.0) the addition of 1.0 to 5.0 g/L while at higher F/M ratios 1 to 10 g/L of biochar can be used.

Apart from DIET, the addition of biochar leads to several beneficial effects. A reduction in the lag phase is generally observed. **Wang *et al.*, (2018)** used biochar produced from saw dust while **Ma *et al.*, (2020)** used biochar produced from rice husk. In both cases, they highlighted a reduction in the lag phase, which was 27.5 - 64.4 % and 44% respectively. Apart from the reduced lag phase, biochar also reduces the need for trace elements. **Cai *et al.*, (2023)** used 8 g/L of biochar and found it to decrease the need of adding trace elements by 50%. Biochar acts further as a buffer due to the presence of organic functional groups. The degradation of butyrate to acetate is energetically unfavorable in the presence of H_2. Fermentation of biological waste residues occurs with the help of bacteria that decompose complex organic compounds, producing primary metabolites or volatile fatty acids (VFAs). The VFAs are further degraded to acetate, H_2 and CO_2 with the help of acetogens. These reactions are only thermodynamically feasible when hydrogenotrophic methanogens consume the H_2 formed reducing the partial pressure. The electron transfer through either H_2 or formate is a well-known mechanism for energy exchange **(Shen *et al.*, 2021)**. **Wang *et al.* (2018)** showed that with the addition of biochar butyrate was oxidised to acetate despite high H_2 partial pressure. They also highlighted that biochar enriches electroactive microorganisms. To highlight the potential of biochar to overcome acidosis and H_2-inhibition, **Wang *et al.* (2021)** used biochar to recover an acidified anaerobic system, where syntrophic oxidation occurred despite high VFA and H_2 partial pressure.

An important reason for the effects of biochar described above is its ability to influence anaerobic microbiomes. Porous biochar contributes to biofilm formation and it facilitates the direct electron or hydrogen transfer between syntrophs and methanogens **(Luo *et al.*, 2015)**. Experiments by **Luo *et al.* (2015)** found *Methanobacterium* to be the most abundant methanogen followed by *Methanosaeta* and *Methanosarcina*. *Methanosarcina* was found in the deeper pore channels of the biochar, while Methanosaeta was found on the surface of the biochar. **Shen *et al.* (2021)** found at the genus level *Methanosarcina* was the most abundant methanogen. **Wang *et al.* (2018)** found the growth of *Anaerolineaceae* to be stimulated with the addition of biochar. Bacteria from this family have been shown to be capable of extracellular electron exchange. **Pytlak *et al.* (2020)** found that the addition of biochar increased the found OTUs by 5 to 8% in AD of sugar beet waste pulp. The explained the increase in OTU's due to adhesion of bacteria to the surface of biochar. The hydrophobic nature of biochar could be responsible for the adhesion of bacteria or archaea such as *Methanotrix* as their cell surface may also be hydrophobic in nature.

Luo *et al.* (2015) tested biochar with various particles sizes at different organic feeding levels. As already described above, the addition of biochar shortened the lag phase and the production of biogas increased. It was

observed that, biochar having a pore size of 75 µm, was most effective. The bacterial content was higher in biochar particles with bigger pore size. These results agree with experiments carried out by **Lü *et al.* (2016)**. **Lü *et al.* (2016)** tested the applicability of biochar in mitigating alleviated stressors of high ammonium and acids concentrations in biochar. The pore size of biochar decides the accessibility of microorganisms, and depending on the pore size, different species can colonize the particle. **Lü *et al.* (2016)** conducted anaerobic digestion experiments with high concentrations of ammonium and volatile fatty acids. In the early stages, the fractions containing biochar were found to be populated with *Methanosaeta* however in the later stages the biochar consisted of archaea such as *Methanosarcina, Methanoculleus* and *Methanomassiliicoccus* **(Lü *et al.*, 2016)**. It was found that using medium sized coarse biochar reduced the lag phase for methanisation. In contrast, the fine biochar was found to affect fermentation and acetogenesis **(Lü *et al.*, 2016)**. Additionally, experiments conducted by **Shen *et al.* (2021)** used biochar of particles of size 0.5 mm and lesser, it had a stimulating effect in the acetogenic-methanogenic stages. Biochar can adsorp NH_4^+ through electrostatic attraction as its surface consists of function groups such as OH^-, COO^- **(Ngo *et al.*, 2022; Yin et al., 2019)**.

Since very beneficial effects can be achieved with biochar, similar to GAC, biochar has been found to be a cheaper and sustainable alternative to GAC or other nonmetal catalysts as well. Although significant progress has been achieved in the realm of research, there remains untapped potential within the industry. Many research approaches are currently limited to laboratory- scale investigations, and scaling up to larger volumes for testing purposes can present certain challenges. **Heitkamp *et al.* (2021)** presented results of seven anaerobic digestors that were shock charged with 1.8 kg/ton of biochar for one year. The addition of biochar was able to reduce volatile acids at an industrial level. Regarding microbial data, a decline in methanogenic archaea was observed, although the abundance of the genus *Methanoculleus* was noted, which contrasts with the mentioning's above. The authors expected an enrichment of *Methanosarcina,* which did not took place. It is interesting that the article describes a process improvement despite a reduced number of methanogenic archaea. It is also interesting that the article describes a significantly increased biodiversity and an accumulation of stress-tolerant microorganisms. This again highlights the importance of biochar in terms of biostimulation. A clear connection with DIET could not be established in this article. This could also be due to the type of biochar. However, there is a lack of specific data on the nature of the biochar. A special feature of the article by Heitkamp et al. is also that for the first time the microbiome of an industrial biogas plant is examined after the addition of biochar.

Enhancing Biochar: Pre-treatment, Impregnation and Modification: While biochar possesses several exceptional qualities as discussed in the preceding section, ongoing research is also exploring modifications that can enhance its effectiveness in AD. It can be done through various physical or chemical treatments. **Zhang *et al.* (2021)** activated the biochar through physical and chemical activation processes. To increase the adsorption of volatile organic acids ball milled biochar was modified using NH_4OH. Ball milling enhanced the acidity and polarity of the biochar, specific surface area. Modification with NH_4OH provided the biochar with amine groups on its surface, while modification with H_2O_2 increased available O groups on its surface. These modifications resulted in enhanced adsorption of volatile organic acids **(Zhang *et al.*, 2021)**. Within the realm of chemical treatments, **Jia *et al.* (2021)** prepared biochar with Fe and other metals salts using the sol-gel method. The prepared biochar was much more efficient in removal of Hg^0 in compared to pristine biochar. **Mei *et al.* (2021)** in their experiments modified biochar with $FeCl_3 \cdot 6\ H_2O$ and Urea, to form an Fe-N doped biochar. Doping with Fe made the carbon layer within the biochar thin and regularized the pore structure. The Fe-N modification reduces the aromaticity and weakens the π − π interaction between pollutants present. Additionally, the surface charge was also affected. Due to this treatment, carbon atoms present in the structure

were replaced with nitrogen. This helped to create pyridinic and pyrrolic structures with unpaired electrons, increasing the electron donating capacity. The presence of earth alkali metals can increase the pore size of the biochar by reacting to form larger polyaromatic structures and O-functional groups as cited by **Ma *et al.* (2022)**.

Choudhury and Lansing (2020) showed biochar impregnated with Fe was able adsorb > 90% of H_2S produced. During their experiments, biochar was pyrolyzed in an anaerobic environment. **Lee *et al.* (2017)** experimented with preparing biochar in an environment of N_2 and CO_2. Biochar prepared in a CO_2 environment was found to be relatively hydrophilic, polar, and aromatic in comparison to biochar in an environment of N_2. Biochar prepared in this manner could be used for better bacterial adhesion. **Ngo *et al.* (2022)** used acid-alkali to treat biochar in digestion of chicken manure. The treated biochar exhibited an 86.3% efficiency in removing total ammonia nitrogen **(Ngo *et al.*, 2022)**. **Altamirano-Corona *et al.* (2021)** tested the potential of biochar and magnetite in AD. Both materials exhibited the capability to decrease digestion time and enhance the quality of the produced biogas.

Drawing from the evidence presented, it is reasonable to assert that employing biochar in anaerobic digestion holds significant advantages. It is worth noting that much of the research highlighted in this context has been conducted and tested at a laboratory scale. Anaerobic digestion can be conducted using diverse substrates and reactor systems. Similarly, biochar can be derived from various organic waste sources, each with its distinct properties. An industrial facility must make prudent decisions regarding the most viable options from both economic and resource perspectives. To establish the validity of these findings, further investigation into larger, scaled-up facilities is necessary.

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GLOSSARY

- **Acetotrophic:** a methanogenic archaea that ferments acetate into methane and carbon dioxide.
- **Activated carbon for desulphurisation:** activated carbon with a large surface area specially produced for desulfurization. By impregnation with potassium iodite the carbon gets active centers which can absorb sulfur compound.
- **Anammox:** anammox is a relatively new technology that is currently still in the industrial implementation phase. The first methods are already available on the market. In these, ammonium can be oxidized anaerobically, which was not possible in previous systems.
- **Anaerobic digestion:** AD is a process consisting of four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Combined, these four phases allow to produce methane from organic biomass using complex microbiomes.
- **Anaerobic fermentation:** biochemical conversion/degradation process that takes place under oxygen exclusion.
- **Archaea:** microorganisms with a key role in anaerobic digestion. Anaerobic methanogenic archaea are converting acetate, hydrogen, carbon dioxide and other substrates to methane and carbon dioxide as end products.
- **ABF:** an anaerobic baffled reactor is a reactor with a series of baffles. Wastewater passes through each baffle in turn. The reactor allows increased contact time with the active biomass, which in turn improves the process performance.
- **Bacteria:** microorganisms with a key role in anaerobic digestion. Anaerobic and facultative bacteria hydrolyze and ferment the input substances to acetate, hydrogen, carbon dioxide and other products prior to methane production.
- **Bioaugmentation:** the addition of specific microorganisms to a biological system such as a bioreactor or to the environment. Bioaugmentation in anaerobic digestion improves the efficiency and stability of the anaerobic digestion process. The added microorganisms can enhance the degradation of complex organic compounds, increase biogas production, and reduce process inhibition caused by toxic substances or fluctuations in operating conditions.
- **Biodegradation:** natural degradation process caused by microorganisms.
- **Bioeconomy:** bioeconomy refers to the transformation from a market-based petroleum-based economy to a market economy in which fossil resources are replaced by various renewable raw materials. This form of economy aims to establish a climate-neutral, bio-based and resource-efficient economy in which the exploitation of nature and pollution of the environment is kept to a minimum.
- **Biomethane:** biomethane is the purified form of raw biogas. It can be used as a natural gas substitute, making it one of the most important renewable gases of the future.
- **Biostimulation:** promoting the growth of specific microorganisms in a biological system through applying selective process conditions.
- **COD:** there are various chemical process parameters that are highly relevant for biogas processes. This includes the chemical oxygen demand (COD). This describes the amount of oxygen that would be necessary to completely oxidize a substrate. It is therefore a good measure of the energy content of substrates for biogas plants.
- **Cultivation:** refers to the process of growing and multiplying microorganisms such as bacteria and archaea in a controlled environment. The process of microbial culti-

vation typically involves selecting a specific microorganism of interest and providing it with the necessary nutrients, temperature, pH, and oxygen levels to support its growth in laboratory.

- **Culture-independent analysis:** refers to a set of techniques to study microorganisms without relying on traditional cultivation methods. This is because only a small fraction of microorganisms can be grown in culture. Culture-independent involve extracting DNA or other molecules directly from environmental samples. Therefore, it is possible to identify and characterize the genetic material of all microorganisms present in a sample, including those that cannot be cultured.
- **Combustion engines:** technical equipment for the generation of power and heat by combustion of chemical energy sources.
- **Combined heat and power plant (CHP):** combustion engines in which the mechanical work generated is used to produce electricity, and at the same time the heat produced is dissipated for use. This combination enables these engines to achieve overall efficiencies of over 90%.
- **Condensate:** water that is formed by condensation in biogas pipes. After formation, the biogas has a relative humidity of 100% and, depending on the temperature in the fermenter, a temperature of over 30°C. In the pipes, the biogas cools down and gaseous water condenses. Condensate must be removed from the pipes, otherwise it fills them and blocks the gas flow.
- **Decisions of the European Union:** alongside regulations and directives, decisions are the third form of EU legal acts that have legally binding effects on all individuals. Decisions are binding in their entirety and may be addressed to specific addressees such as member states, companies, individuals or the general public.
- **Desulphurisation:** removal of gaseous sulphur compounds from biogas.
- **Direct interspecies electron transfer (DIET):** DIET is a microbial interaction mechanism in which two or more different types of microorganisms exchange electrons directly between them. DIET allows microorganisms to share energy and other resources, leading to a more efficient use of available resources and a better survival strategy. The electrons are transferred through specialized structures called conductive pili, nanowires, or extracellular electron shuttles. The electron transfer enables one microbe to donate electrons to another, facilitating metabolic processes that would otherwise not be possible.
- **Digestate:** the residue that remains after biodegradation. Compared to the substrate, this has a lower solid content, is usually easier to stir and pump, has a small proportion of organic compounds, and the nutrient content (phosphorus, potassium, nitrogen, and sulphur) is largely retained.
- **Directives of the European Union:** a directive is a form of a legal act of the European Union. This legal act is binding and not directly applicable, which means that it must first be transposed into national law by the Member States.
- **Dry matter (DM)/ Total solids (TS):** dry Matter, describes the content of water-insoluble solids. In addition, there is the oDM/ VS (organic dry matter/ volatile solids), a value that describes the proportion of organic compounds in the DM.
- **Energy crops:** partial or whole plants that are suitable as substrate for biogas production due to their high energy content. Examples include Silages of corn or grass, corn grains or cereals, residues of sugar-containing plants such as sugar beets or fruits.
- **Eukaryotes:** Eukaryotic organisms such as anaerobic fungi, ciliates, amoebae and flagellates might have a significant role in anaerobic digestion due to their ability to

hydrolyze a wide range of substrates and their intracellular methanogenic archaea. Unicellular eukaryotes implement many functions in ecosystems, but are understudied in anaerobic digestion.

- **European Green Deal:** the European Green Deal is a central component of the European Union's climate policy. It addresses the anthropological climate crisis at its core and provides concrete guidance and action to address the climate crisis. In summary, net greenhouse gas emissions in the European Union should be reduced to zero by 2050 at the latest.
- **Ex-zones:** zones in which the formation of an explosive atmosphere due to the release of biogas or the ingress of air cannot be reliably always prevented. In these zones, special requirements apply to avoid ignition sources.
- **Feed-in tariff:** feed-in tariff, is a state-fixed payment for electricity that is used to promote certain types of electricity generation. This is used in particular to promote renewable energies such as wind power, solar power, hydropower, geothermal power and biomass plants.
- **Fermentation tank/digester:** container in which the biochemical conversion of substrate to biogas takes place. The most important component of any biogas plant.
- **Floating ceilings/ floating blankets/ floating layer:** floating layer that forms on the surface of the substrate or digestate in a container when it is insufficiently mixed. It is formed by aggregation of the solid components in the substrate. Depending on where it occurs, it can be troublesome or useful.
- **FOS/TAC ratio:** the FOS/TAC determination is a titration test for determining the quotients of acid concentration and buffer capacity in the fermentation residue. FOS stands for Volatile Organic Acids, unit [mg/l acetic acid equivalents] and TAC for Total Inorganic Carbonate (alkaline buffer capacity, unit (mg CaCO3/l).
- **HIT:** hydrogen interspecies transfer (HIT) is a form of mediated interspecies electron transfer (MIET) and an alternative to direct interspecies electron transfer (DIET). For a long time only, the MIET was known. Since 2005, the DIET has come into the focus of AD research as it can contribute to increasing process performance in biogas plants.
- **Hydrogenotrophic:** a methanogenic archaea that produces methane via the reduction of carbon dioxide with hydrogen.
- **Hydrolysis:** hydrolysis usually describes dissolving in water. In the biogas sector, however, it is also one of the key steps in biogas production and describes the primary dissolving and enzymatic crushing of complex biomolecules to make them accessible for further degradation.
- **Hydrogen partial pressure:** the measure of thermodynamic activity (i.e. pressure) of Hydrogen molecules in a given system.
- **Inhibitors:** Substances that inhibit or stop a process. Examples of inhibitors by producing Biogas are that are antibiotics, disinfectants or solvents, herbicides, salts or heavy metals.
- **KEGG:** the KEGG (Kyoto Encyclopedia of Genes and Genomes) is a freely accessible database of information about biomolecules. It allows insight into numerous metabolic pathways and describes the genes involved.
- **Keystone species:** an organism with a critical role contributing to the stability or productivity of its ecological community.
- **Lotka-Volterra:** a model that predicts predator-prey interactions and is generally known in respect to the “rabbit-wolf graph”. It is now known that this model can also

be used to evaluate microbial interactions based on DNA data. This is of great interest for AD research.

- **MAP:** precipitation relates to technology, which allows for the removal of nitrogen due to precipitation with magnesium and phosphor salts.
- **MEC:** "Microbial Electrolysis Cells"
- **Mesophilic:** an organism that thrives at relatively moderate temperatures between 25 and 40°C.
- **Methanisation/methanation:** in the context of power-to-gas processes, the term describes synthesis using carbon dioxide as a feedstock.
- **Methanogenesis:** is the biological process by which microorganisms, called methanogens, produce methane gas as a byproduct of their metabolism.
- **Microbiome:** entire community of microorganisms (including bacteria, archaea, fungi, and viruses) existing together in a particular environment.
- **NMDS:** non-metric distance scaling relates to a non-parametric multivariate statistical test.
- **N-Stripping:** N-Stripping relates to technology, which allows for the removal of nitrogen.
- **Obligate anaerobe:** microorganism with very low oxygen tolerance that can only live in anaerobic conditions.
- **OMICs:** the analysis of a large population with similar sub-elements. These can be, for example, genes (genomics), proteins (proteomics), or metabolites (metabolomics).
- **Paris Agreement:** the Paris Agreement is a treaty under international law concluded by 195 Parties to the United Nations Framework Convention on Climate Change (UNFCCC) with the aim of protecting the climate as a successor to the Kyoto Protocol. In it, the states agreed to limit anthropogenic global warming to well below 2 °C above pre-industrial levels.
- **PE-HD:** polyethylene high-density, thermoplastic material suitable for use as piping material. Connections of pipeline parts are made by welding.
- **PERMANOVA:** a non-parametric multivariate statistical test.
- **Power-to-gas:** aeneral term for processes in which electrical energy is used for the synthesis of energy-rich gaseous chemical compounds.
- **Psychrophilic:** psychrophilia is the characteristic of living organisms that prefer low temperatures. The temperature here is usually between -5 and +20 °C.
- **PVC-U:** polyvinyl chloride unplasticized, thermoplastic material suitable for use as piping material. Connections of pipeline parts are made by gluing.
- **RNA:** Ribonucleic acid is a nucleic acid composed of a chain of many nucleotides. It is a DNA-like transcript of short DNA fragments. These can be used structurally by organisms or to transmit information (translation).
- **Receiving feeder:** technical device for continuous feeding of solid substrates into the fermenter.
- Regulations of the European Union: a regulation is a form of a legal act of the European Union. This legal act is immediately and simultaneously applicable as direct law in all Member States.
- **Renewable energies:** renewable energy sources are characterised by the fact that they are practically inexhaustible or renew themselves relatively quickly. These include energy sources from wind power, solar, hydropower, geothermal and biomass plants.

GLOSSARY

- **Renewable Energy Directive:** the Renewable Energy Directive provides the legal framework for the development of renewable energy in all sectors of the EU economy and supports cooperation between EU countries. It aims to give the EU states concrete targets for the expansion of renewable energies and limits for the emission of greenhouse gases. This Directive has been adapted and tightened over time in its designs and requirements.
- **Renewable raw materials:** plant raw materials with varying degrees of degradability that are available cheaply and sustainably due to their natural growth.
- **Retention time:** average time it takes a particle to traverse a given volume of space.
- **Separator:** technical device for separating digestate or substrate into a solid and a liquid component.
- **Silage leachate/seeping juices:** juice emerging from the plant material with a high content of degradable organic matter and low pH. The water content comes partly from precipitation and partly from biodegradation.
- **Silage camps:** building equipment for receiving and storing substrate with the aim of preserving it by means of lactic acid fermentation.
- **Space load:** the space load indicates the amount of organic DM (oDM) in kilograms that can be fed to the digester per working volume per unit of time.
- **Stakeholders:** stakeholders are directly or indirectly involved in a plan, project, company or sector and have expectations or perceptions of how the area should develop in the future. They pursue individual interests and try to influence decisions according to their own ideas.
- **Sustainable Development Goals (SDGs):** these include 17 Sustainable Development Goals (SDGs), which are set and implemented in a political framework by the United Nations. They aim to establish the fundamentals of sustainable development at the economic, social and environmental levels.
- **Symbiosis:** an interaction between two different organisms. Includes mutualism, commensalism, and parasitism.
- **Syntrophy:** syntrophy is in its meaning close to a symbiosis, but with an metabolic interdependence. It refers to a dependent interaction between two or more organisms where one partner provides a metabolic product that the other partner utilizes.
- **Thermophilic:** an organism that thrives at high temperatures usually higher than 45 °C. Often archaea but also includes some fungi and bacteria.
- **Volatile fatty acids/volatile organic acids (VFA):** in chemistry, this term describes organic acids that already change into the gaseous state at room temperature. In the context of biogas production, this term is used to describe the shorter fatty acids produced during the hydrolysis of fats.
- **16S-rRNA:** the 16S rRNA gene is an essential gene for the biosynthesis of ribosomes. Due to its vital function, its DNA sequence is highly conserved and is often used as a basis for the taxonomic classification of microorganisms.

MICRO4 BIOGAS

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