Biotechnological approaches and strategies for intensification of metanogenesis

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Keywords:

Biogas
Biomethane
Digester
Design
Intensifiction
Methanogenesis
Stability

Article history:

Received 20.04.2025
Received in revised form 25.08.2025
Accepted 31.12.2025

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DOI: 10.24263/2310-1008-2025-13-2-7

Abstract

Introduction. Biomethane production contributes to the development of a circular economy by enabling the generation of renewable energy through the efficient utilization of organic waste as a feedstock for the anaerobic conversion of organic matter into methane.

Materials and methods. A comprehensive review of scientific publications was conducted to analyze the main trends in digester design optimization, factors influencing microbial communities during biogas production, and methods for biogas upgrading to biomethane. The review was based on sources indexed in major scientific databases (Scopus, Web of Science, Google Scholar), as well as professional publications of the Higher Attestation Commission of Ukraine and official statistical data from the UN and the EU.

Results and discussion. By 2040, Europe aims to replace up to 10% of its natural gas consumption with biomethane. Biomethane is considered carbon-neutral, and its integration with CO_2 utilization technologies, such as biological methanation and Power-to-Gas systems, enables the potential achievement of "negative emissions," contributing to decarbonization of the energy sector. The core equipment for methanogenesis is the bioreactor (digester), whose design and operating parameters are critical for biogas optimization and long-term process stability. Advanced configurations, including Anaerobic Baffled Reactors (ABR) and Upflow Anaerobic Sludge Blanket (UASB) reactors, are widely used for treating municipal and industrial wastewater, as well as manure and agricultural residues.

Due to the high variability in substrate composition and properties, direct comparison of digester designs in terms of efficiency is limited, and the optimal configuration depends on process goals, economic feasibility, and local conditions. Methanogenesis is a multistage biotechnological process (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) performed by syntrophically interacting bacterial and archaeal communities, requiring a holistic research approach that accounts for microbial structure and functionality. Emerging technologies, such as microbial electrolysis cells, system integration (e.g., with hydroponic farms), metal nanoparticles, and advanced monitoring combined with "omics" tools, aim to enhance process stability, productivity, and methane yield while reducing operational costs.

Conclusions. Future research should focus on molecular-biological modeling of anaerobic microbial communities to enable more precise adjustment of operating parameters (temperature, pH, nutrient dosing) based on microbiome state data, as well as on the development of advanced digester designs and intelligent control systems.

ISSN: 2409-4951 (Online), 2310-1008(Print)

Introduction

Traditional microbial decomposition of organic matter is an ancient biotechnology for biogas production, which can be upgraded to biomethane. Methanogenesis produces biogas consisting mainly of methane (~60%) and carbon dioxide (~40%), with minor impurities (Li et al., 2017). However, conventional archaeal-driven bioconversion faces challenges, including low biodegradability of some agricultural feedstocks (e.g., animal manure and straw), relatively low methane content, long process times, and incomplete utilization of energy stored in lignin and cellulose (Ning et al., 2021).

Anaerobic digesters vary widely in complexity, ranging from simple cylindrical structures without moving parts to highly automated industrial installations. They are commonly classified according to their design and key operating parameters, including temperature regime, solids content, and substrate loading characteristics, such as batch substrate concentration (BSC) and continuous substrate concentration (CSC).

The most common digester configurations include cylindrical reactors; cylindrical reactors with internal partitions; inclined cylindrical reactors; egg-shaped digesters; and trench-type digesters constructed in the ground and covered with a rigid lid or gas-impermeable membrane. A fundamental requirement for all digester designs is structural tightness, as methane-producing microorganisms are obligate anaerobes (DelaVega-Quintero et al., 2025).

Anaerobic cultivation can be operated in either continuous or intermittent (batch) modes. In continuous systems, raw materials are supplied at regular intervals or continuously, while an equivalent volume of digestate is simultaneously withdrawn. The majority of modern industrial digesters operate under continuous feeding regimes due to their higher stability and process control (Yankovska et al., 2018). In contrast, intermittent systems are filled with substrate and operated for a fixed digestion period, after which the reactor is opened and completely emptied. Such systems are generally simpler and less expensive; however, they suffer from design limitations, lower technological efficiency, and reduced process stability, and are therefore considered functionally outdated. Based on the total solids content of the feedstock, anaerobic digestion systems are classified as wet or dry. Wet digestion systems process substrates with a dry solids (DS) content of 16% or less, whereas semi-dry and dry systems typically operate with substrates containing 22–40% DS.

Anaerobic conversion of organic matter into methane is a complex biotechnological process that occurs in the absence of oxygen and is mediated by a highly interdependent microbial consortium comprising diverse bacteria and archaea (DelaVega-Quintero et al., 2025). The efficiency of methanogenesis depends on the metabolic activity and balanced interactions among these microorganisms. Through the decomposition and mineralization of organic matter, microbial communities play a fundamental role in both natural and engineered ecosystems. In terms of chemical composition and energy value, biomethane is comparable to fossil natural gas, enabling its integration into existing gas distribution networks and its use for electricity and heat generation, as well as transportation fuel. To obtain biomethane, biogas produced via anaerobic conversion of organic matter must be purified. Biogas upgrading is performed using various technologies, including cryogenic separation and membrane-based processes. The large number of scientific studies currently being conducted, together with the wide diversity of equipment, feedstock pretreatment methods, and biogas purification technologies aimed at improving the efficiency of anaerobic conversion of organic matter to methane, indicates that an optimal and universally applicable biomethane production technology has not yet been established.

ISSN: 2409-4951 (Online), 2310-1008(Print)

Materials and methods

A comprehensive review of scientific publications was conducted to analyze the main trends in digester design optimization, factors influencing microbial communities during biogas production, and methods for biogas upgrading to biomethane. The review was based on sources indexed in major scientific databases (Scopus, Web of Science, Google Scholar), as well as official statistical data from the UN and the EU.

Results and discussion

Improving the design of biogas plants (digesters) is one of the most important areas of innovation. Digesters exist in single-, two- and multi-stage. The need to use two- and multi-stage systems arises due to the specific sequence of biochemical reactions of the methanogenesis process, which have different optimal conditions for cultivating the corresponding microbial consortia and for some raw materials are difficult to regulate in one-stage conditions. Two- and multi-stage systems provide higher energy efficiency and stability in the fermentation of raw materials of plant origin (Wang et al., 2005a, b). However, single-stage systems are simple to design, assemble and operate and are cheaper. Therefore, they are currently the most common and are used both for industrial biogas production and for small decentralized production. A comparative analysis of single-stage and two-stage systems demonstrates the advantages of the latter, especially when processing complex substrates. Modern bioreactors are significantly different from previous models and contribute to increasing biogas yield, improving process stability and expanding the types of raw materials used. The complexity of the biogas production process, which occurs in several stages, and the diversity of microflora necessitate the development of complex bioreactor designs. The design features of anaerobic bioreactors are determined by the type and volume of waste being processed, the required degree of degradation, optimization of energy yields and economic factors. The most commonly accepted classification of anaerobic reactors is based on the shape of the macrostructures of biomass methanogens in them. According to this principle, all designs can be divided into reactors with suspended-sedimented activated sludge, attached biomass (biofilm) and with a fluidized bed on a synthetic or natural carrier (Koniuszewska et al., 2020). The first type of digesters include a traditional methane tank, an anaerobic lagoon, an upflow reactor through an anaerobic sludge bed (UASB), and an aerated concrete reactor (ABR) (Mojiri et al., 2012).The second type includes downflow reactors (DSFF-reactor), an aerated fluidized bed reactor (AFB), biological filters. A number of designs - an upflow anaerobic biofilter (AF) and a hybrid reactor (AF+UASB) - combine elements of both types of reactors (van Lier, 2006).

Type 1 digesters are the simplest anaerobic bioreactors. The substrate is fermented at a low rate when it comes into contact with biomass pellets that settle at the bottom of the bioreactor. The design is simple, suitable for processing various types of waste with a load of 0.1 to 2 kg of COD/(m^3/day). However, the geometric parameters of such bioreactors require large areas for placement, the process is practically impossible to intensify and control (Douglas, 2017). The use of this technology is justified when wastewater contains solid particles that are difficult to digest and easily precipitate.

Traditional methane tanks are cylindrical tanks with a hermetic overlap and a conical or flat bottom. There are methane tanks with a floating overlap, methane tanks-gas holders and methane tanks-compacting. The best technological indicators are achieved in ovoid-shaped methane tanks, which have minimal heat loss, and less sand accumulation and cork formation (Alengebawy et al., 2024).

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The contact reactor is built together with a settling tank (Figure 1).

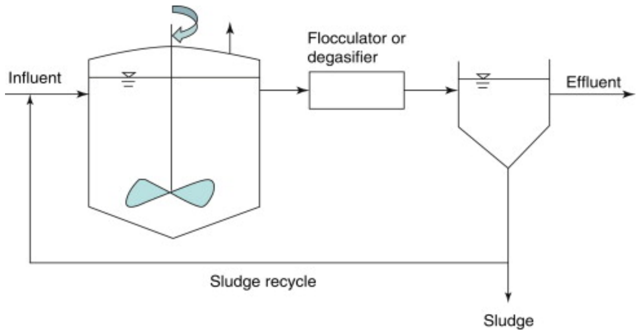

Figure 1. Anaerobic contact reactor

The contact reactor is built together with a settling tank. Contact reactors are characterized by a high concentration of activated sludge, and, accordingly, a relatively short residence time of biomass in the reactor. The advantages are a sufficiently high quality of processing, the ability to control the process. The disadvantages include a relatively low load, from 4 to 30 g/l of COD/m^3/day.

The anaerobic baffled reactor (ABR) is characterized by its simplicity of design and wide possibilities for improvement: the introduction of various types of loading, heat exchange devices and elements for biogas removal (Foxon et al., 2025). It consists of a series of vertical compartments or chambers, which forces wastewater to flow upward through a series of sludge layers, which is essentially a serial connection of UASB reactors. In modern models, the flow between the chambers occurs through small-diameter pipes, which provides a longer hydraulic retention time (HRT) in neighboring elements and a high flow rate (Figure 2).

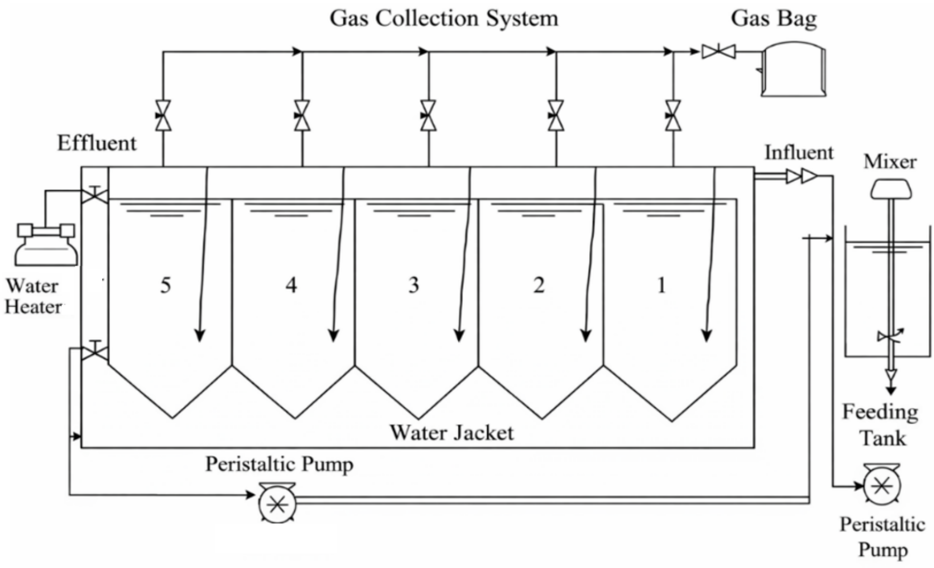

Figure 2. Anaerobic bafflet reactor

ISSN: 2409-4951 (Online), 2310-1008(Print)

The ABR design provides high purification efficiency, stability to organic and hydraulic shock loads. The principle of operation of the ABR is to separate the processes of acid formation and methanation by dividing the working volume into sections. The desirability of separating acid formation and methanation is based on different optimal conditions for these phases. By physically separating them in different compartments, the ABR creates conditions for more efficient methanogenesis and potentially higher biogas yields.

The disadvantages include insufficient mixing, leaching of biomass by the biogas flow and accumulation of volatile fatty acids (VFA). A number of modifications have been proposed to improve the characteristics of the reactor, including batch ABR (PABR), carrier ABR (CABR), separate-feed ABR (SABR), modified ABR (MABR), anaerobic-aerobic ABR (AABR) and granular sludge ABR (GRABBR). The list of ABR modifications suggests that the original design, while having advantages, has the potential for improvement.

Type 2 digesters use the principle of immobilizing microflora on natural (gravel) or synthetic (PVC rings) media. The media can be loaded either in an ordered or bulk manner. Structures with immobilized biomass have better resistance to toxic effluents and quickly adapt to changes in the composition of the raw material. The main difference between a biofilter and a biofilter is the loading of an inert media without the possibility of its removal by the flowing liquid flow. A biofilm is formed on the media, with which the substrate comes into contact. Anaerobic biofilters are classified according to the direction of substrate flow, loading, and media material.

A typical representative of type 2 digesters is downflow stationary fixed film reactor (Figure 3).

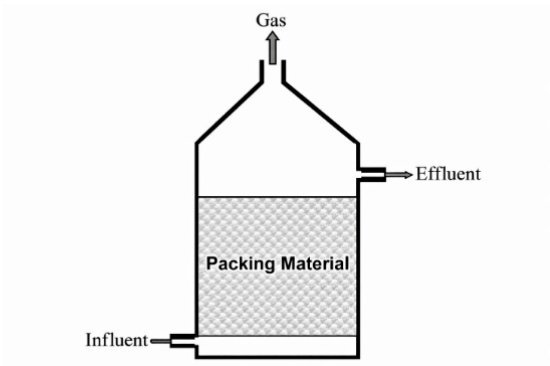

Figure 3. Downflow stationary fixed film reactor (DSFF)

Wastewater is fed into the upper part of the reactor and, flowing through the loading layer, is removed from the bottom. The design is simple, does not require much pumping equipment, but the biofilm requires meticulous selection of a carrier with the necessary surface properties. Soft materials with high internal porosity (not less than 200 m² per 1 m^3) are most often used. The flow of wastewater from top to bottom and biogas bubbles rising upwards will contribute to the mixing of biomass in the reactor. The biogas yield and its quality may be somewhat lower compared to other anaerobic bioreactors, since the removal of gas is complicated by the shape of the loading and the counterflow of liquid. The loading may become clogged with suspended particles.

In anaerobic filters with upward flow AF (Anaerobic Filter). wastewater is fed into the lower part of the reactor and rises upwards, biogas is removed from the top, and the surface

ISSN: 2409-4951 (Online), 2310-1008(Print)

of the carrier is effectively used. Biomass is present not only in the form of biofilm, but also in flocs and granules (Comparetti et al., 2013). The flow prevents the bottom of the anaerobic filter from growing and clogging, the occurrence of stagnant zones and ensures uniform distribution of biomass. Such bioreactors are suitable for working with sharp fluctuations in the concentration of pollutants in wastewater and are most often used in the treatment of wastewater from meat processing, dairy and textile industries (Rodrigues et al., 2024). To prevent the accumulation of sludge inside the digester, a tubular reactor with a fixed film is used, which is a biofilter with a planar loading. The reactor can be with an upward or downward flow. The carrier is pipes or plates arranged in such a way as to create vertical channels. The working load is up to 30 kg COD/m^3·day. This form of loading, in addition, will contribute to a more complete removal of biogas from the volume of the anaerobic filter. The principle of operation of another reactor of the second type is based on the self-immobilization of biomass in the form of granules (granulated sludge). Such devices are characterized by good decantation characteristics and high methanogenic activity. An example is devices with an upward flow of liquid with a suspended granular layer of sludge (Figure 4).

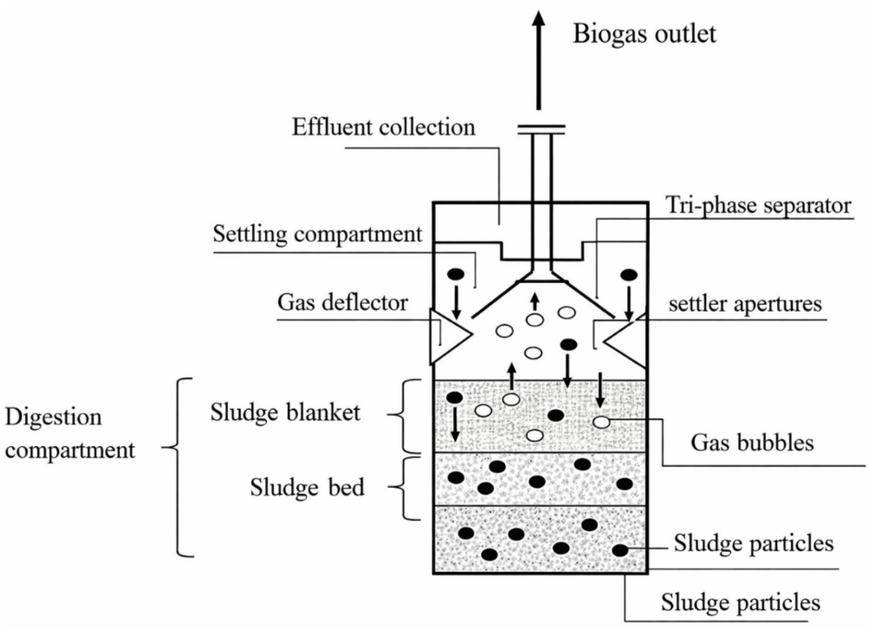

Figure 4. Upflow anaerobic sludge blancet reactor (UASB)

Since the reactor does not require an internal filling to fix the biomass, it is economically attractive, but its performance characteristics depend on the granulation of the biomass. In addition, the UASB reactor has a gas-liquid-solid separation system in its upper part, which prevents or limits the removal of suspended particles and promotes gas removal and sludge decantation. The separation of gas, treated water and granular biomass is carried out using a special sludge-gas separation device.

The retention of granular biomass is also carried out due to its high sedimentation capacity. UASB reactors are highly sensitive to the composition of the wastewater, especially

ISSN: 2409-4951 (Online), 2310-1008(Print)

the presence of solids and inhibitors in it, however, they are relatively compact and simple, providing good mixing conditions. UASB reactors are characterized by the ability to withstand high organic loads of up to 40 kg COD/m^3·day, short hydraulic retention time (HRT), low energy requirements, simple design and maintenance, and high solids retention time (SRT) due to the granular sludge. Similar to anaerobic filters, UASB reactors have been widely used in industry at the agro-food wastewater treatment level (Mainardis et al., 2020).

The phenomenon of sludge granulation is not fully understood, and makes it difficult to start up UASB reactors when the production does not have a suitable source of seed culture, such as sludge from another operating UASB reactor. There is currently no general theory of granulation, and the available data do not always correlate well with each other. Disadvantages include a long start-up period for the formation of granular sludge, the need for further processing, sensitivity to toxic compounds, potential leaching of sludge, sensitivity to pH and temperature, and problems with granulation. On an industrial scale, UASB reactors are widely used in large treatment plants, especially in tropical countries (China, India, BrADil).

Hybrid designs have also been developed that combine the advantages of a UASB reactor and an anaerobic filter: the UBF (Upflow Bed-Filter) reactor (Figure 5).

The lower part contains a layer of granular sludge, and the upper part, usually 25-30% of the volume, is filled with an inert carrier, fixed or floating. This allows the use of biomass of a higher concentration, provides high productivity and optimal mixing modes. Thus, it is possible to avoid the clogging of the lower layers of the carrier, which is typical for an anaerobic filter, and to reduce its amount.

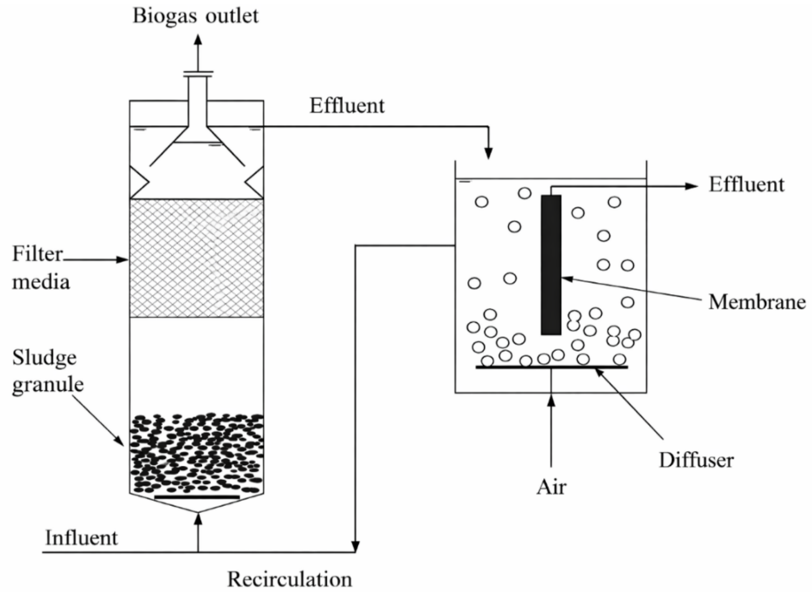

Figure 5. Upflow Bed-Filter reactor (UBF)

Type 3 digesters are characterized by the presence of a fluidized microbial layer on a synthetic or natural carrier. The contact area of the carrier is 200 m^2/m^3 and more, active mixing occurs during methanogenesis. Fluidized bed reactors have a degree of fluidization

ISSN: 2409-4951 (Online), 2310-1008(Print)

of more than 50%, and expanded sludge bed reactors - about 20%. The organic load can exceed 40 kg of COD/m^3·day. The hydraulic residence time is less than 12 hours. However, these reactors consume more energy, have a complex design, and are accordingly more expensive to build and operate compared to Type 1 and Type 2 reactors (Figure 6).

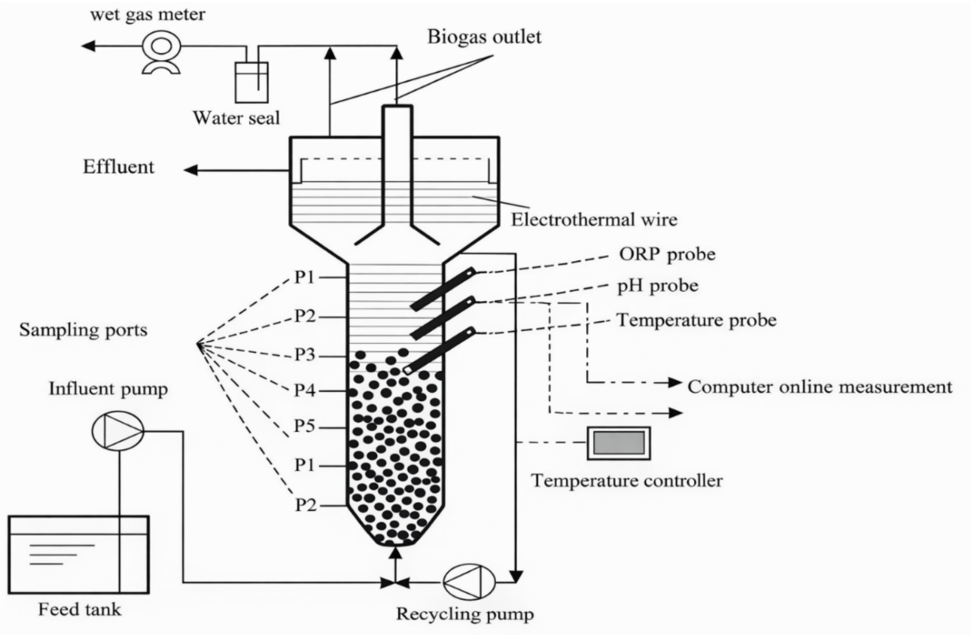

Figure 6. Bioreactor with expanded and suspended activated sludge bed (EGSB)

The fundamental difference of this type of reactor from UASB is the higher velocity of the upward flow of liquid due to recirculation and intensification of mass transfer between sludge granules and wastewater. Such reactors are suitable for processing low-concentration wastewater. A fluidized bed reactor is one of the most productive bioreactors with fixed microflora due to more complete contact of contaminants with biomass. Fluidization occurs due to the upward flow of liquid and the released biogas bubbles. At the same time, the contact surface between the active biomass and untreated waste increases significantly. In such bioreactors, low-concentration wastewater with dissolved or finely dispersed contaminants is treated. However, maintaining the fluidized bed requires significant energy consumption.

Digester with membrane filtration system **(**AnMBR). AnMBR combines anaerobic digestion with membrane filtration, which allows the hydraulic retention time (HRT) to be controlled independently of the solids retention time (SRT). The membrane retains biomass inside the reactor, while the treated liquid is removed as permeate. This enables operation at short HRT and long SRT, reduces reactor volume, and ensures high effluent quality.

The combination of a classic anaerobic digester with a membrane ultrafiltration (microfiltration) system allows to retain microbial biomass in the reactor, achieve a significant extension of SRT (solid retention time), which increases the stability of the process and contributes to a higher biogas yield (Liao et al., 2006). Membrane fouling and high energy costs for pumping remain the main disadvantages of the technology (Figure 7) (Benyahia et al.,2024; Stuckey, 2012).

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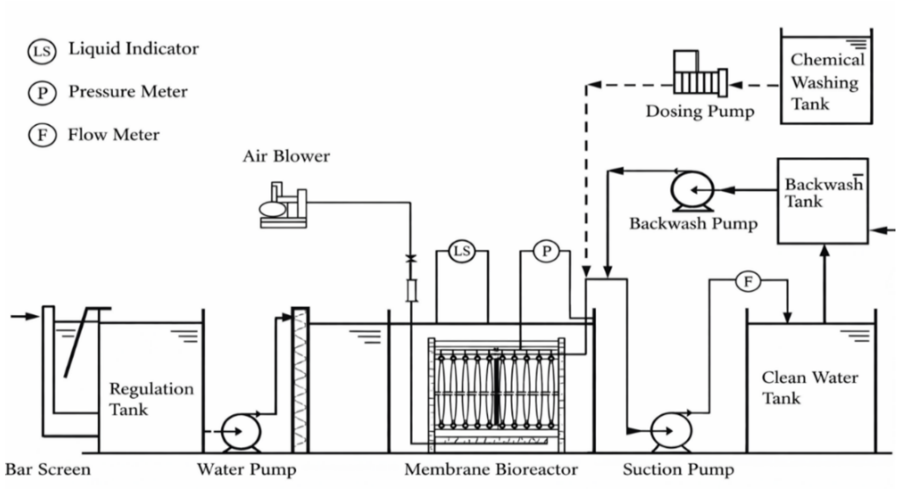

Figure 7. Digester with membrane filtration system (AnMBR)

However, the use of new module configurations (external/internal, electric) demonstrate encouraging results at the stage of laboratory experiments (Li et al., 2023).The use of various membrane modules (cross-flow, submerged, fluidized) reduces the degree of membrane surface contamination due to the turbulence of the substrate flow. Algorithms have been developed that allow predicting biokinetic parameters during membrane operation in different load regimes and biomass concentrations.

Two-phase systems. The classical anaerobic process consists of four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. In a traditional reactor, all these processes occur simultaneously in one container, but the stages require different environmental conditions. Hydrolysis and acidogenesis are characterized by relatively rapid bacterial growth (several hours/day), resistance to load changes. Optimal pH 5.5–6.5 (phase 1). During methanogenesis, very slow growth of archaea (up to 15-30 days) is observed, and it is highly sensitive to the accumulation of volatile fatty acids (VFA). Optimal pH 7.0–8.0 (phase 2). Dividing the process into two consecutive reactors allows to optimize the hydrolysis of complex substrates and increase the methane yield due to stabilization of the methanogenic phase, ensuring better processability (Wang et al., 2005; Stabnikova et al., 2008a). In the acid reactor (phase 1), complex organic substances are converted into simple acids (acetic, propionic, etc.), and an optimally prepared substrate enters the methanation tank, where only CH_4 and CO_2 generation occurs (phase 2) (Tang et al., 2023). However, the stability of two-phase systems depends on the hydraulic retention time (HRT), C/N ratio in the substrate, pretreatment of substrate, temperature regime, and composition of the inoculum (Kabaivanova et al., 2024; Liu et al., 2008; Stabnikova et al., 2008b).

Combined systems: Two-phase + AnMBR. The combination of these two technologies creates a system where the first reactor acts as a buffer and hydrolyzer, and the second (AnMBR) is a highly efficient methane generator. In the first reactor, solid organic waste is solubilized (dissolved). Having greater resistance to changes in cultivation conditions, acetogens eliminate the negative consequences of a sharp drop in pH. This reduces the load of solid particles on the membrane in the second reactor, reduces the rate of formation of a gel layer on the membrane, and stabilizes methanogenesis. This combination ensures the stability of the load on the methanogenic phase, high permeate quality, and the possibility of

ISSN: 2409-4951 (Online), 2310-1008(Print)

nutrient recovery. Optimization of conditions for each group of microorganisms increases organic conversion (Gao et al., 2010). However, capital and operating costs for membrane modules and control systems are higher compared to other types of digesters, and the membranes still require periodic chemical washing and gas scouring for cleaning (Bueno et al., 2024; van Lier et al., 2015). Particularly promising are the systems with AnMBR + phototrophic MBR, which allow for additional CO_2 absorption, improve the quality of treated wastewater and increase the biogas yield (Fischer et al., 2024).

The main types of digesters used for anaerobic conversion of organic matter to methane:

– for liquids with a low solids content (<3%) - upflow reactors through anaerobic sludge (UASB);
– with a high solids content (6-12%) - induced bed reactors (IBR);
– for raw materials of a wide range of consistencies, from dilute to highly concentrated, - suspended bed reactors.

The variety of composition and properties of substrates does not allow for a clear comparison of different digester designs in terms of efficiency and manufacturability. Depending on the characteristics of the effluent, desired results, economic feasibility and local climatic conditions, any design of the above systems may be optimal.

Microbial communities and stages of methanogenic bioconversion of organic matter

Anaerobic digestion (AD) is a complex biotechnological process carried out by a diverse community of interdependent microorganisms, including bacteria and archaea, in the absence of oxygen. The efficiency of this process directly depends on the metabolic activity and complex interactions of these microorganisms (Alipoursarbani et al., 2025). The AD process includes several sequential stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Each phase is provided by microbial consortia that are characterized by high specialization and interdependence (Pal et al., 2021).

Hydrolysis. Hydrolysis is the initial stage of AD, in which complex organic polymers (lipids, polysaccharides and proteins) are broken down into simpler, soluble ones, including sugars, amino acids and fatty acids. This process is carried out by hydrolytic bacteria that produce extracellular enzymes, including amylases, proteases, cellulases and lipases. Microbial groups involved in hydrolysis include the families *Clostridiaceae, Pseudomonadaceae and Syntrophomonadaceae*, as well as the genera *Streptococcus, Enterobacter, Clostridia, Micrococci, Bacteroides, Butyrivibrio, Fusobacterium and Selenomonas*. Hydrolysis is often rate-limiting for the digestion of complex organic wastes, especially for lignocellulosic biomass (Das and Mondal, 2016). The key enzymes involved in hydrolysis belong to the class of hydrolases, including esterases, glycosidases and peptidases. Glycosidases, for example, include cellulases (endo-1,4-beta-D-glucanase, cellulose 1,4-beta-cellobiosidase, beta-glucosidase) required for cellulose degradation, as well as xylanases, xylosidases, mannanases, mannosidases, alpha-galactosidases, and alpha-glucuronidase that break down hemicellulose (Pal et al., 2021).

Acidogenesis (Acid formation). In the acidogenesis stage, microorganisms convert sugars, amino acids, and fatty acids obtained during the hydrolysis step into short-chain organic acids such as formic, acetic, propionic, butyric, and pentanoic acids. In addition, alcohols (methanol, ethanol), aldehydes, carbon dioxide (CO_2), and hydrogen (H_2) are produced as intermediates. This step is carried out by acidogenic bacteria (acidogens), including members of the genera *Clostridium*, *Bacillus*, *Lactobacillus*, *Pseudomonas*, *Micrococcus*, and *Flavobacterium*. Acidogenic bacteria play an important role in creating

ISSN: 2409-4951 (Online), 2310-1008(Print)

favorable anaerobic conditions for the growth of obligate anaerobes involved in the later stages of the process. Approximately 20-30% of the total CO_2 is produced at this stage. Key enzymes in acidogenesis include hexokinase, phosphoglucose isomerase, phosphofructokinase, aldolase, triose phosphatase isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, mutase, enolase, pyruvate kinase (for glycolysis); pyruvate:ferredoxin oxidoreductase (PFOR), NADH:ferredoxin oxidoreductase (NFOR) and ferredoxin hydrogenase (for H_2 production); as well as lactate dehydrogenase, phosphotransacetylase, acetate kinase, ethanol dehydrogenase, acetaldehyde dehydrogenase, acetyl-CoA acetyltransferase, and butyryl-CoA dehydrogenase (Pal et al., 2021).

Acetogenesis. In the acetogenesis stage, acetogenic bacteria (*Syntrophomonas* and *Syntrophobacter*) convert organic acids and alcohols formed during acidogenesis into acetate, H_2, and CO_2 (Quin et al., 2025). Homologous acetogenic bacteria can also reduce CO_2 and H_2 to produce acetate. The products of acetogenesis, acetate and hydrogen, are directly used by methanogenic microbes in the final stage of AD. Approximately 70% of methane production is associated with the reduction of acetate in this stage (Pal et al., 2021). Key enzymes include those involved in the oxidation of acetate (citrate synthase, aconitase, isocitrate dehydrogenase), butyrate (CoA transferase, butyryl-CoA dehydrogenase), propionate (pyruvate carboxylase, malate dehydrogenase, fumarate hydratase, fumarate reductase, succinate dehydrogenase, succinyl-CoA synthetase, methylmalonyl CoA mutase/epimerase/decarboxylase, propionate-CoA transferase), and ethanol (acetaldehyde dehydrogenase).

Methanogenesis. Methanogenesis is the final stage of anaerobic fermentation, in which methanogenic archaea (*Methanobacterium*) produce methane (CH_4). They use substrates formed in previous phases, in particular acetic acid, H_2, CO_2, methanol, methylamine or dimethyl sulfide. Thus, *Methanosarcina* is a universal methanogen capable of using acetoclastic, hydrogenotrophic and methylotrophic pathways (Mesquita et al., 2023). It should be noted that *Methanothrix* is an acetoclastic methanogen that is sensitive to high concentrations of ammonia. Among the hydrogenotrophic methanogens, *Methanoculleus, Methanobacterium, Methanoregula* and *Methanomassiliicoccus* are distinguished (Piercy et al., 2025). The main metabolic pathways of methanogenesis are the acetoclastic pathway, which involves the breakdown of acetate and is responsible for approximately 70-72% of CH_4 production, and the hydrogenotrophic pathway, which consists in the reduction of CO_2 with H_2 and accounts for about 28-30% of CH_4 production. There is also a methylotrophic pathway that uses methylated compounds (Siroka et al., 2018). Methanogens actively consume hydrogen, which helps maintain a low partial pressure of H_2. This thermodynamically enhances the fermentation stages, in particular the conversion of sugars to short-chain fatty acids, and prevents their excessive accumulation. Maintaining low hydrogen concentrations creates favorable conditions for the development of acidogenic bacteria and prevents the accumulation of short-chain organic acids during the acidogenesis stage. The vital activity of methanogens leads to a decrease in the content of H_2, which enhances fermentation in the previous stages, ensuring a sufficient level of volatile fatty acids (VFA).

The concept of "microbial consortia" is central to the efficiency of AD, which is driven by synergistic interactions between different microbial groups. This means that research should focus on optimizing the entire community, rather than simply introducing a single "supermicrobe". However, not all methanogens are equally beneficial. Significant increases in the abundance of *Methanomassiliicoccus* resulted in reduced biogas production at high butyric acid concentrations. Thus, the type of methanogen and its specific metabolic interactions in the consortium matter, and an increase in the abundance of certain

ISSN: 2409-4951 (Online), 2310-1008(Print)

methanogens may be an indicator of process instability rather than efficiency (Alipoursarbani et al., 2025). The main stages of anaerobic conversion of organic matter to methane are given in Table 1.

Table 1

The main stages of anaerobic conversion of organic matter to methane

Stage	Microbial groups	Main functions	Products
Hydrolysis	Hydrolytic bacteria	Breakdown of organic polymers	Monomers (sugars, amino acids)
Acidogenesis	Acidogenic bacteria	Conversion to organic acids, alcohols, H_2, CO_2	VFA, alcohols, H_2, CO_2
Acetogenesis	Acetogenic bacteria	Oxidation of VFA and alcohols to acetate, CO_2	Acetate, H_2, CO_2
Methanogenesis	Methanogenic archaea	Conversion of acetate, H_2, CO_2 to CH_4	CH_4, CO_2

Syntrophic relationships and interspecies electron transfer. Microbial consortia involved in anaerobic digestion function in synergistic and syntrophic relationships, where the metabolic products of one group of microorganisms serve as substrates for the next. This interaction is particularly critical between hydrogen-producing acetogenic bacteria and hydrogenotrophic methanogens. The close coupling and syntrophic association between acetogenesis and methanogenesis is a thermodynamic necessity. Many acetogenic reactions that produce H_2 are thermodynamically unfavorable unless H_2 is continuously consumed by methanogens. This explains why maintaining a healthy methanogenic population is so critical to the entire process (Siroka et al., 2018).

Factors affecting microbial communities in the biomethane production process. Operational parameters play a significant role in the formation of microbial communities and their metabolic activity in anaerobic digesters, which affects the biomethane yield.

Temperature. Depending on the operating temperature, mesophilic (30–40 °C) and thermophilic (45–60 °C) regimes are distinguished. Lower temperatures are not used, since the process duration becomes inadequately long. Mesophilic systems are considered more stable and require less energy than thermophilic ones. However, the higher the fermentation temperature, the higher the reaction rate and gas yield. In developing countries, including Ukraine, systems with lower heating costs (mesophilic temperature range) are mainly used in order to increase the profitability of production (Zhadan et al., 2023). Mesophilic conditions (30–40 °C) usually support a more diverse and tightly connected microbiome, ensuring greater process stability. In contrast, thermophilic conditions (50-65 °C) result in a limitation of microbial diversity, making the systems more sensitive to changes in operational parameters. With temperature changes, specific changes are observed in the dominant microbial genera, such as *Firmicutes, Bacteroidetes, Thermotogae, Methanobacteriales* and *Methanosarcinales*. An increase in temperature promotes higher metabolic activity, accelerating substrate degradation and leading to more intensive methane production. However, thermophilic systems tend to be less stable. A small increase in temperature (1-2 °C) can cause a significant decrease in methane production due to the accumulation of volatile fatty acids (VFA) and acidification of the reactor. Mesophilic systems, on the other hand, provide greater stability and are able to process a wider range of biomass, making them less vulnerable. This requires a trade-off in the design and operation of the AD: the higher

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productivity potential of thermophilic systems requires a careful weighing of the desired outcomes, e.g. high biogas yield, against stability (Costopolou et al., 2023).

pH plays a significant role in regulating the composition and activity of the microbial community. Acetoclastic methanogens, in particular, are extremely sensitive to pH, and their productivity will be reduced at pH values below 6.2. Changes in pH lead to changes in the relative abundance of various microorganisms, such as *Proteobacteria, Firmicutes* and *Bacteroidetes*, as well as the genera *Lactobacillus, Methanocorpusulum, Petrimonas, Proteiniphilum* and *Methanosarcina.* Excessive accumulation of VFA can lead to acidification of the reactor and a drop in pH, which limits the growth of methanogenic communities and potentially causes the AD to shut down. High protein content in substrates contributes to their enrichment with ammonium (NH_4+), preventing a sharp drop in pH (Costopolou et al., 2023).

Hydraulic retention time (HRT) and organic load (OL) significantly influence microbial communities and digester performance. Short HRT can reduce microbial diversity and acetogenic bacteria, while fluctuations in OL affect bacterial and archaeal community structure. High or unstable OL may decrease diversity further, causing shifts between Methanobacterium and Methanosarcina. HRT below a critical threshold and excessively high OL can lead to methanogen loss, VFA and hydrogen accumulation, and pH drop. Nevertheless, combining high OL with moderately reduced HRT can enhance methane production per unit reactor volume (Costopolou et al., 2023).

Substrate composition. The composition of the substrate is a key factor shaping microbial communities and determining biomethane production. Complex feedstocks favor more diverse microbial populations. Main bacterial phyla, such as Chloroflexi, Bacteroidetes, Firmicutes, and Proteobacteria, are prevalent regardless of substrate type, but specific feedstocks enrich particular populations. For example, Anaerolineaceae and Rikenellaceae dominate in food waste, while *Candidatus Methanoculleus thermohydrogenotrophicum* is more abundant in lipid-rich substrates. Substrate characteristics, including polysaccharide, protein, and lipid content, directly affect conversion and biogas yield, with lipids having the highest methane potential (94.8%), followed by proteins (71%) and carbohydrates (50.4%). Low C/N ratios can reduce AD efficiency and biogas production (Ivanov et al., 2002; Costopolou et al., 2023).

Inhibitory compounds. *Ammonia* concentration is a significant inhibitor in anaerobic digestion. Levels above 5 g/L, mainly as free ammonia nitrogen, passively diffuse into cells, disrupting metabolism, pH, and proton motive force. Acetoclastic methanogens are most sensitive, while microbial adaptation often involves syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis. *Methanothrix* is generally sensitive to NH_4^+, whereas *Methanosarcina, Methanoculleus*, and *Methanobacterium* are more resistant. Elevated ammonia leads to accumulation of VFAs, especially propionate and acetate, causing instability in AD. The addition of trace elements (Fe, Co, Se, Ni) or zeolite can alleviate inhibition and improve biomethanization. In a hybrid anaerobic solid–liquid (HASL) system enhanced with a submerged biofilter, food waste digestion was improved: COD and VFA generation increased in the acidogenic reactor, while total COD removal and methane production increased in the methanogenic reactor, reaching 99% COD removal and 26% higher methane production compared to conventional HASL (Wang et al., 2003).

Lipids are a major problem in municipal and food-industry wastewater because they are present in high amounts and are difficult to remove completely. Although most fats can be eliminated by physico-chemical methods, the remaining emulsified lipids must be treated biologically. During anaerobic digestion, fats are converted into long-chain fatty acids, which inhibit microbial activity and methane production. The addition of iron salts reduces this

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inhibition by binding long-chain fatty acids into insoluble compounds, thereby improving their degradation and overall anaerobic digestion performance (Ivanov et al., 2002, 2014; Tay et al., 2006; 2008).

Sulfate concentrations of 1–3 g/L in anaerobic systems decrease methane production due to sulfate reduction to sulfide by sulfate-reducing bacteria, which inhibit methanogens through toxicity, substrate competition, and trace element precipitation (Chelliapan and Sallis, 2015). Elevated sulfide levels can severely suppress biogas formation and cause corrosion, odor, and health hazards. The addition of ferric hydroxide during anaerobic digestion of sulfate-rich wastewater enhances treatment by stimulating iron-reducing bacteria, completely eliminating hydrogen sulfide from both biogas and liquid phases and reducing the sulfate-reducing bacteria-to-methanogen ratio sixfold (Stabnikov and Ivanov, 2006).

Volatile fatty acids (VFAs) accumulated in excessive amounts also has an inhibitory effect. This occurs due to high organic load, increased carbohydrate content or high concentrations of other obstructive compounds such as Na^+, total ammonia nitrogen (TAN) and lipids. Microorganisms responsible for the degradation of propionate and butyrate (*Syntrophobacter, Pelotomaculum, Smithella, Syntrophomonas*) can decrease in number with increasing organic load, leading to the accumulation of VFAs. *Methanothrix* concentrations may decrease, while *Methanoculleus* and acetate-oxidizing bacteria (*Tepidanaerobacter acetatoxydans*) may increase, indicating a shift in acetate consumption from acetoclastic methanogenesis to SAO. *Methanosarcina* abundance may increase with the increase in VFA in mesophilic reactors, as their growth promotes acetate accumulation.

VFA are positive for methane generation when their concentration remains within a certain range. *Proteiniphilum* plays a key role in the conversion of VFA to CO_2. The interplay of operational parameters and inhibitory compounds is critical. High organic loading can lead to the accumulation of VFA, which lowers pH, inhibits methanogens, and this in turn enhances VFA accumulation due to the lack of hydrogen consumption. This complex network of interactions highlights the need for holistic system management to prevent cascading failures. Microbial communities exhibit adaptability and resilience to stress. Understanding these adaptive mechanisms is key to developing more robust AD systems (Mills et al., 2024). The influence of the parameters of the anaerobic conversion of organic substances on the biomethane yield is analyzed in Table 2.

To increase the efficiency and stability of the anaerobic digestion process, various methods of influencing microbial populations are used.

Bioaugmentation is the introduction of specific stress-resistant or highly efficient microorganisms into AD systems. The main goal of this is to enhance the system's ability to produce biomethane and mitigate the effects of stressful conditions. The advantages of bioaugmentation: high efficiency, resistance to stress loads, overall enhancement of the AD process, reduction of toxic inhibition (e. g., ammonia) and overcoming the limitations of the psychrophilic regime. The metabolic activity of the microbial community is also improved, the adaptability of microbes to cultivation changes is increased, and the overall biogas production process is enhanced. Representatives of the *Clostridiaceae, Pseudomonadaceae* and *Syntrophomonadaceae* families are mainly used for bioaugmentation. However, there are difficulties associated with compatibility with the local microbiota, dosage and frequency of inoculum introduction (Alipoursarbani et al., 2025).

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Table 2

Influence of operational parameters and substrate composition on microbial communities and biomethane yield

Parameter	Impact on microbial community	Biomethane production
Operational parameters		
Temperature	Mesophilic (30-40 °C): diverse microbiome, stability. Thermophilic (50-65 °C): limited diversity, sensitivity to change, specific dominants (*Firmicutes, Bacteroidetes, Thermotogae, Methanobacteriales, Methanosarcinales*).	Increasing temperature accelerates biosynthesis CH_4.
pH	Critical for structure and activity. Acetoclastic methanogens are sensitive (<6.2). Changes in phyla (*Proteobacteria, Firmicutes, Bacteroidetes*) and genera (*Lactobacillus, Methanocorpusulum, Methanosarcina*).	Optimal pH is important for VFA degradation. A drop in pH limits methanogenesis.
HRT and OL	Short HRT reduces acetogenic diversity. Fluctuations in OL shape microbial community structure, with high OL decreasing diversity and causing shifts between *Methanobacterium* and *Methanosarcina*.	Short HRT leads to VFA and H_2 accumulation, pH decrease, and methanogenesis inhibition.
Substrate composition		
Substrate composition	Complex substrate: more complex microbiome, higher metabolic diversity. Major phyla (*Chloroflexi, Bacteroidetes, Firmicutes, Proteobacteria*). Specific substrates enrich different populations.	Low C/N ratio reduces methanogenesis efficiency.
Ammonia concentration	NH_4+>5 g/L inhibits AD (FAN-NH3). Acetoclastic methanogens are most sensitive. Adaptation: transition to SAO + hydrogenotrophic methanogenesis. *Methanothrix* is sensitive, *Methanosarcina* is resistant.	Micronutrients and zeolite can mitigate the negative effects of methanogenesis inhibition by excessive NH_4^+.
Lipids	Alters the microbial community structure by inhibiting acidogenic and methanogenic microorganisms due to the accumulation of long-chain fatty acids.	Reduce biomethane production by suppressing methanogenic activity.
Sulfate	Shifts the microbial community by promoting sulfate-reducing bacteria, which compete with methanogens for substrates and inhibit their activity through sulfide production.	High sulfate concentrations reduce biomethane production by inhibiting methanogens through sulfide formation.
Volatile fatty acids	Excessive accumulation inhibits AD. Propionate/butyrate decomposers may decrease. *Methanothrix* may decrease, *Methanoculleus* and SAOB increase. *Methanosarcina* may increase also.	VFA are precursors, but excessive accumulation causes acidification, pH drop and methanogen limitation.

Co-digestion involves mixing different organic substrates in different ratios. This approach is often preferable to mono-digestion due to numerous advantages. It allows for improved carbon to nitrogen ratio (C/N, optimum 25-30:1), increased biodegradability,

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efficient removal of solids (VS) and contributes to the sustainability of production. Co-digestion helps to achieve a balance of nutrient components, dilutes inhibitory compounds and provides buffer capacity of the system. As a result, an increase in methane yield and more efficient operation of the digester is observed. In addition, co-digestion contributes to increased microbial diversity and stability of the system. Examples of successful co-digestion are combinations of fruit/vegetable and food waste, as well as cow manure, chicken manure and whey. Lignocellulosic agricultural residues are also a priority feedstock for co-digestion (Quin et al., 2025).

Inoculation *i*s the introduction of microorganisms into the digester to initiate anaerobic fermentation. Traditional sources include cow manure from a manure pit or waste activated sludge from an operating digester. Marine sediment has also been found to be a promising microbial source for inoculation. Optimizing the process requires determining the optimal inoculum to substrate ratio. Typically, it is added in the range of 10–20%. Studies have shown that the initial inoculum adapts to the digester conditions, making further continuous addition of manure unnecessary once microbial adaptation is achieved. Proper inoculation enhances the bacterial community, leading to increased specific biogas production and methane content, as well as reduced hydraulic retention time. Timely and appropriate inoculations can also reduce the risks of acidification by maintaining a balance between acidogens and methanogens (Abouelenien et al., 2021).

Application BioIronTech process that is a microbial iron-based treatment in which Fe(III) is biologically reduced to Fe(II), transforming iron particles and forming ferrous/ferric compounds that adsorb and precipitate pollutants such as sulfides, lipids, and recalcitrant organics. By removing these inhibitory compounds, the process stabilizes anaerobic digestion and enhances biomethane production (Ivanov et al., 2014).

New and promising technologies. New, promising technologies are constantly emerging in the field of biogas production. One of them is the use of microbial electrolysis cells (MEC), which, in combination with anaerobic digestion, can increase the efficiency of biomethane production by using electricity to stimulate microbial processes. Another innovative direction is the integration of biogas plants with other systems, for example, with hydroponic farms, which allows the reuse of nutrient-rich by-products of anaerobic digestion for plant cultivation. The use of nanotechnology, in particular metal nanoparticles, is also considered as a way to increase the efficiency of biogas production by stimulating the activity of microorganisms. New approaches to biogas generation itself are also being developed, aimed at optimizing the process and increasing the yield of the target product. The effectiveness of implementing promising technologies for archaic bioconversion of organic matter into biogas is shown in Table 3.

Microbial electrolysis cells (MECs) are advanced bioelectrochemical systems that use an external electrical potential or current to produce hydrogen or biomethane from spent biomass or wastewater. This technology involves an anode that is functionally similar to microbial fuel cells (MFCs), but the cathode receives additional current from a DC source. Specially tuned for "electromethanogenesis," electroactive microorganisms known as electrotrophs attach to the anode. Here, they oxidize organic substrates to carbon dioxide using an electrode material, typically graphite-based, as the final electron acceptor.

The electrons generated by this anodic oxidation reaction flow through an external circuit. These electrons are then used to biocatalyze reduced target molecules such as hydrogen (H_2), acetate (CH_3COOH), or methane (CH_4). In particular, the oxidation of chemical oxygen demand (COD) in wastewater at the anode can be effectively combined with biogas upgrading to biomethane, creating an integrated and highly efficient process (Mers et al., 2023).

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Table 3

Development of promising technologies for archaic bioconversion of organic matter into biogas

Technology	Main advantage	Result	Key mechanism
Microbial electrolysis cells	Increased methane yield, process stability	Improved methanogenesis	Stimulation of microbial processes
Integrating bioconversion with hydroponics	Substrate optimization, environmental friendliness	Improvement of methanogenesis	Better nutrient balance
Nanotechnology	Stimulation of microorganisms	Improving biogas quality	Increasing metabolic activity
Process optimization	Reduction of operating costs, process stability	Increase of biogas yield	Control of parameters (temperature, pH)

The integration of MEC into anaerobic digestion (MEC-AD) systems significantly improves the overall efficiency of the digestion, which is manifested in an increase in methane content and its production rate. Studies have shown that the methane production rate and stabilization time in MEC-AD systems were on average 1.7 and 4.0 times higher than in traditional AD reactors. The increase in methane yield (up to 200%) and process stability (4 times faster stabilization) indicate that MEC is not just an additional technology, but a transformational one. They fundamentally change the microbial environment, providing more reliable and efficient anaerobic digestion, especially for complex, highly concentrated feedstocks. Demonstrate increased tolerance to adverse environmental factors such as high organic loads, the presence of inhibitors. Current research focuses on the optimization of cathode materials. Platinum is a highly efficient material for proton recovery, however, activated carbon is being studied as a suitable and cheaper alternative. Integration with carbon nanotubes (CNTs) for biocathodes is also a promising direction. The role of specific microbial populations such as *Geobacter* and *Shewanella* in electron transfer is crucial for optimizing both electricity and hydrogen/methane production in MEC (Noémi et al., 2023).

Integration of biogas plants with hydroponic farms. The integration of anaerobic digestion (AD) with aquaponics or hydroponics creates an innovative closed system that effectively manages waste and simultaneously generates energy, promoting circularity in food production. In such systems, the waste generated in aquaponic systems - uneaten fish feed, fish feces and dead fish - are sent to an anaerobic biodigester after a post-filtration process. This allows, together with the production of biogas, to avoid environmental pollution.

Co-digestion of aquaponic effluent with cattle manure has shown better biogas production results compared to mono-digestion. This integration leads to powerful synergistic effects, such as increased overall biogas production, improved organic load conversion and achieving a better nutrient balance, which contributes to the overall stability of the process. The mineral richness of tilapia sludge, which is a component of aquaponic effluent, can enhance the enzymatic activity of microorganisms in the manure, optimizing the fermentation process (Paes et al., 2025). The concept of a "closed system" goes beyond simple waste treatment, creating a self-sustaining cycle where waste from one process becomes a valuable input for another, reducing the need for primary resources and minimizing emissions to the environment.

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Nanotechnology. Nanoparticles (NPs) have unique properties such as large specific surface area and high reactivity, which makes them attractive for many applications, including bioenergy. In anaerobic digestion (AD), metal nanoparticles can significantly affect the activity of anaerobic microorganisms, stimulating both hydrogen and methane production. The mechanisms of this positive effect depend on the chemical formula of the nanoparticles. Nanoparticles based on trace elements such as iron, cobalt and nickel promote the synthesis of essential enzymes and coenzymes that are crucial for the metabolic activity of anaerobic microorganisms, thereby directly improving their productivity. For example, the optimal concentration of nickel nanoparticles (NiNPs) can increase the volume of biogas and methane by 1.7 - 2.0 times. The addition of magnetic nanoparticles (MNPs) up to 100 mg/L can increase biogas production by 1.9-2.1 times compared to the control. Nanoadditives without trace elements can absorb inhibitory factors, reducing their harmful effects on microbial activity. Studies have shown that methane production phases are more sensitive to the addition of these nanoparticles than hydrogen production phases. In addition, nanoparticles can improve biogas quality by significantly reducing or completely removing hydrogen sulfide concentrations (Zhu et al., 2021). The prospects for the application of nanotechnology in bioenergy are promising. Current trends include the development of specialized multifunctional nanomaterials that are stable and biodegradable. Nanomaterials are expected to lead to significant efficiency improvements, cost reductions in the bioenergy sector, and be integrated with other technologies, such as artificial intelligence, to further optimize bioenergy production.

Advanced monitoring methods and “omics” approaches

Advanced monitoring methods and “omics” approaches are being used to gain a deep understanding of microbial diversity, physiology, interactions, and metabolic networks in AD systems. This is crucial for optimizing the productivity and sustainability of bioprocesses. Key methods include high-throughput sequencing (e.g., 16S rRNA) for microbial community analysis. More comprehensive “omics” approaches, such as metagenomics, metatranscriptomics, metaproteomics, and metabolomics, provide unprecedented insights into microbial functionality (Ana et al., 2021). The advantages of these methods are to identify the functional importance of specific microorganisms and to elucidate complex microbial interactions, such as metabolic cross-feeding. They also allow the identification of key microorganisms and regulated functional proteins that influence the process. In addition, these approaches allow the evolution of the microbial community to be tracked in response to changing operating conditions. The application of this knowledge involves the integration of detailed chemical and biological fingerprinting with machine learning models. This creates a new approach to understanding the microbial ecology of the AD. Bioaugmentation, co-fermentation and microbial pretreatment - all these directions explore the principles of synergy in microbial communities. It is not just about adding more microbes or substrates, but about creating conditions under which different microbial groups complement each other's functions, leading to more reliable and effective cooperation. The increasing use of "omics" technologies and machine learning indicates a shift from an empirical trial-and-error approach to a more guided, predictive and mechanistic understanding of the AD. This is a significant trend in the field, moving towards the development of "smart" digesters (Song et al., 2021).

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Biogas purification and enrichment

Biogas, a product of anaerobic digestion of organic matter, is an important renewable energy source. However, for its effective use, in particular as biomethane - an analogue of natural gas, it is necessary to purify it from impurities and increase the concentration of methane. The main undesirable components of biogas are carbon dioxide (CO_2), hydrogen sulfide (H_2S), water (H_2O), as well as trace amounts of nitrogen (N_2), oxygen (O_2), ammonia (NH_3) and siloxanes (Figure 8).

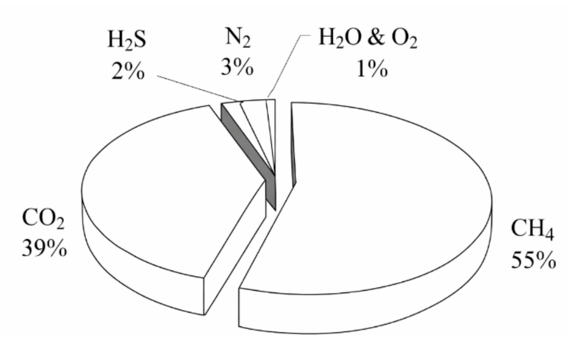

Figure 8. Chemical composition of biogas

The main technologies for cleaning and enriching biogas are: membrane (removal of carbon dioxide, hydrogen sulfide and other impurities); adsorption (purification using activated carbon, zeolites and other adsorbents); cryogenic (separation into methane and carbon dioxide using the method of using low temperatures).

Membrane technologies are one of the most promising and widely used for removing carbon dioxide, hydrogen sulfide and other impurities from biogas. The principle of operation is based on the different permeability of the components of the gas mixture through a selective membrane. Biogas is supplied under pressure to one side of the membrane. Components with higher permeability (for example, CO_2, H_2S, H_2O) pass through the membrane faster than methane (CH_4), which has a lower permeability. Thus, two streams are obtained at the exit of the membrane module: a methane-enriched retentate (product) and a permeate containing separated impurities. Polymer membranes are most commonly used, including polyimide, polysulfone, cellulose acetate, and polydimethylsiloxane. Each type of material has its own advantages and disadvantages in terms of selectivity, permeability, resistance to biogas components, and cost. In recent years, membranes based on inorganic materials (ceramic, metal) and mixed matrix membranes (Mixed Matrix Membranes, MMMs) have been actively developed and researched. Equipment for membrane biogas separation is compact, modular, easy to operate, and easily automated. For most processes, there is no need for chemical reagents (Kramer, 2023).

Disadvantages include the sensitivity of membranes to some impurities (H_2S, siloxanes, heavy hydrocarbons), which may require preliminary purification of biogas; methane losses with permeate (2-15 %); the need for periodic replacement of membrane modules (Aguilloso et al., 2024).

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Adsorption technologies are based on the ability of solid porous materials (adsorbents) to selectively absorb certain components of a gas mixture on their surface. Pressure swing adsorption (PSA) or vacuum pressure swing adsorption (VSA) technology is most often used for biogas purification. The PSA process includes four stages: adsorption, desorption (pressure reduction), purging and pressure equalization. Biogas under pressure is passed through a layer of adsorbent, which selectively absorbs CO_2, H_2S, N_2 and water vapor, while methane passes further. After saturation of the adsorbent, the pressure in the column is reduced (or a vacuum is created in the VSA), which leads to the desorption of the absorbed impurities. For continuous operation, several adsorption columns operating in parallel are used.

Types of adsorbents: (a) activated carbon: used to remove H_2S, siloxanes and volatile organic compounds. It has high porosity and a developed surface. Can be treated with special substances to increase selectivity to certain impurities; (b) zeolites (molecular sieves): these are synthetic or natural aluminosilicates with a regular microporous structure. Effective for the removal of CO_2, H_2S, N_2 due to the molecular sieve effect and different strengths of adsorption interaction. The type of zeolite is chosen depending on the size of the molecules to be removed; (c) other adsorbents: metal oxides (e.g. iron oxide for chemisorption of H_2S), silica gels (for desiccation), aluminogels and new materials such as metal-organic frameworks (MOFs), which demonstrate high selectivity and adsorption capacity (Awe at al., 2017).

The use of adsorption technologies ensures the production of high-purity methane (up to 99%), the possibility of simultaneous removal of several impurities and, with proper regeneration, a long service life of the adsorbents. However, their implementation requires relatively high capital costs, regeneration consumes a lot of energy, and some sorbents are sensitive to moisture and heavy hydrocarbons (Awe et al., 2017).

Cryogenic biogas separation technologies are based on the difference in boiling/condensation temperatures of its components. When gradually cooled to low temperatures, components with higher boiling points condense and are separated first. The biogas is first compressed and cleaned of water, H_2S and other impurities that can freeze and block equipment at low temperatures. The gas is then gradually cooled. Carbon dioxide (CO_2) is condensed and separated from methane (CH_4) at temperatures from -35 °C to -85 °C and elevated pressure (depending on the specific process scheme). High purity methane (over 99%) remains in the gaseous phase and can be liquefied (if necessary) by further cooling to approximately -162 °C. In addition, there is the possibility of obtaining a by-product - liquid CO_2, which has commercial value. (Tan et al., 2017). However, high capital and operating costs and significant energy consumption make these technologies cost-effective only for large-scale production.

The choice of the optimal technology for biogas purification and enrichment depends on many factors, including the required degree of methane purity, production volumes, economic indicators and environmental requirements. Often, hybrid systems are used to achieve optimal results, combining the advantages of different technologies. For example, H_2S removal by adsorption before feeding biogas to a membrane or cryogenic plant. Further research and development is aimed at creating more efficient, selective and cost-effective materials and processes for biogas purification.

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Conclusions

Methanogenesis is a complex, multifactorial process that can be intensified through the combined optimization of microbial consortia and digester design. Advanced reactor configurations, such as UASB, EGSB, AnMBR, and hybrid biofilm–membrane systems, enhance substrate solids concentration, mass transfer, and biomass retention, leading to shorter hydraulic retention times and higher biogas yields.

The efficiency and stability of methane production are largely determined by the composition and balance of microbial consortia, including methanogenic archaea and syntrophic bacteria. Targeted manipulation of the microbiota—through inoculation strategies, stimulation of direct interspecies electron transfer, and fine-tuning of operational parameters—can significantly improve process resilience and methane yield.

An integrated microbiological–engineering approach has been shown to increase methanogenesis productivity by 20–70%, depending on the type of feedstock. For small-scale biogas systems, simple and robust digester designs that maintain favorable conditions for microbial activity remain particularly important. Future research should focus on molecular-level modeling of microbial communities and the development of intelligent digester control systems to enable precise and adaptive process management.

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Cite:

Ukr J Food Sci Style

Vorontsov O., Stabnikov V. (2025), Biotechnological approaches and strategies for intensification of metanogenesis, *Ukrainian Journal of Food Science*, 13(2), pp. 186–210, https://doi.org/10.24263/2310-1008-2025-13-2-7

APA Style

Vorontsov, O., & Stabnikov, V. (2025). Biotechnological approaches and strategies for intensification of metanogenesis. *Ukrainian Journal of Food Science, 13*(2), 186–210. https://doi.org/10.24263/2310-1008-2025-13-2-7