BIOLOGICAL BIOMETHANATION: IN-SITU, EX-SITU AND HYBRID METHODS FOR UPGRADING OF BIOGAS OR SYNGAS

Lappeenranta–Lahti University of Technology LUT

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Sakibur Rahat

Examiners:

Jouni Havukainen; Associate Professor, School of Energy Systems, LUT.

Mika Horttanainen; Professor, School of Energy Systems, LUT.
ABSTRACT

Sakibur Rahat

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Examiner(s): Associate Professor Jouni Havukainen; Professor Mika Horttanainen

Keywords: Anaerobic Digestor (AD), Biomethanation, In-situ, Ex-situ, Trickle Bed Reactor, Syngas, Carrier materials, Methanogenesis, Microbes, Biotic and Abiotic factors

Biomethanation is a process that converts biomass, organic waste or syngas into biogas which can be further upgraded into natural gas grid biomethane via either conventional upgrading techniques such as pressure swing adsorption (PSA), chemical solvent scrubbing (using amines), and pressurized water scrubbing or biological methods such as in-situ, ex-situ or hybrid Biomethanation process. Biological process of biomethanation has gained popularity due to less environmental impacts and more economic benefits than the conventional techniques. Enriched biomethane produced from biological biomethanation contributes as renewable fuel. Several pilot scale and full-scale ex-situ biomethanation techniques have been implemented in recent years. However, In-situ and syngas biomethanation need more attention as these processes can bring more benefits using wide range of biomass and less equipment. This review will provide comprehensive overview of the current state, challenges, and prospects of biological biomethanation. This thesis presents the most recent case studies and the intra and inter comparative analysis of in-situ, ex-situ and syngas biomethanation to figure out the most suitable reactor type for scaling up with highest methane content. However, reviewing biotic and abiotic factors address the prospects and potentiality of using Trickle bed reactors, utilizing direct electron transfer (DIET) and carrier materials to maximize the output. Compared to in-situ biomethanation, ex-situ biomethanation has made more progress in scaling up successfully, and it can achieve high methane purity (around 95-99%) with Trickle bed reactors. In-situ biomethanation has difficulties due to poor gas-liquid transfer rate, process instability and microbial inhibition by high hydrogen partial pressure. These can be overcome by using carrier materials, applying DIET, optimizing operational conditions by controlling biotic and abiotic factors. Methanobacter was found to be more common in biomethanation and sometimes they show better performance with synergistic culture with other bacteria.
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SYMBOLS AND ABBREVIATIONS

\( p \) pressure \([\text{bar, Pa}]\)

\( qm \) mass flow rate \([\text{kg/s}]\)

\( R \) gas constant \([\text{J/kg K}]\)

\( T \) temperature \([\text{ºC, K}]\)

\( V \) volume \([\text{m}^3]\)

\( \nu \) specific volume \([\text{m}^3/\text{kg}]\)

Abbreviations

DIET Direct Electron Transfer

CSTR Continuous-Stirred Tank Reactors

TBR Trickle Bed Reactors

BC Bubble Column Reactors
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(Acknowledgements)

(Symbols and abbreviations)

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1 Introduction

One of the most urgent problems our world is currently dealing with is climate change, which is brought on by the rising amounts of greenhouse gases in the atmosphere. The main cause of these emissions is the burning of fossil fuels including coal, oil, and natural gas. With the current emission rates, global warming, which is currently about 1.0 °C above pre-industrial levels, is likely to rise by 1.5 °C between 2030 and 2052 as a result of human activity. There is an increasing demand for renewable energy sources in order to lessen the effects of climate change and lessen our reliance on fossil fuels. Therefore, technological means of storing this energy, such as chemical energy carriers, are needed. The power-to-gas (P2G) technology transforms extra electricity into a gas that may be stored (Lecker et al., 2017a). Even if the technology for producing H\textsubscript{2} is highly sophisticated, it has several limitations in terms of H\textsubscript{2}’s long-term storage, safety, and low energy density, as well as the need to modify the natural gas system technically. Contrarily, CH\textsubscript{4} is particularly appealing since the infrastructure for storage and delivery is already in place in many regions. The volumetric energy content of CH\textsubscript{4} is 36 MJ m\textsuperscript{-3} which is more than three times larger than that of H\textsubscript{2}, and it may be easily injected into the gas grid (Luo et al., 2012).

Biogas is produced from a well-known commercial process - anaerobic digestion (AD). Biogas often converted to biomethane by removing CO\textsubscript{2} because it mostly contains CH\textsubscript{4} (40–75%) and CO\textsubscript{2} (25–60%). There are several methods of Biogas upgrading (Villadsen et al., 2019) Through the CO\textsubscript{2}-reductive route of hydrogenotrophic methanogens, biological biogas upgrading (biomethanation) uses external H\textsubscript{2} to convert the CO\textsubscript{2} portion of the biogas into extra CH\textsubscript{4} (Villadsen et al., 2019). Anaerobic digestion creates biogas, primarily made of methane, through this process by converting organic waste. A variety of organic wastes, including sewage, food waste, and agricultural waste, can be transformed into useable energy sources in different proportions using this procedure. The generated biogas can be converted into higher-quality methane and utilized as a fuel for transportation, or it can be used to produce power and heat. Biomethane production and upgrading technologies have gained prominence in Europe since the passage of the EU landfill directive in 1999. Based on various in-situ or ex-situ biomethanation plants, past research have concentrated on the production potential of biomethane and process development. The majority of research do
not take into account the ecological factors that influence the system and microbial processes. The biological biomethanation process is quite susceptible to various environmental conditions from a microbiological point of view. Both biotic and abiotic elements may be present. According to biotic factors, the methanogenic archaea (Methanobacterium, Methanothermobacter, and Methanoculleus) were shown to be more stable than the bacterial community (Lutispora) (Logroño et al., 2020; Jiang et al., 2021). The microorganisms that play the primary role in the biological biomethanation process can be affected by the optimal ranges and limiting variables of the raw materials utilized in the entire biomethanation process as well as the products and by-products produced in the various processes. For instance, the process's applied temperature can dramatically change the yield. Thermophilic processes, whether ex-situ (Kozak et al., 2022) or in-situ (Jiang et al., 2021), produce higher levels of er methane than the mesophilic process. Even, Methanogenesis has consistently been demonstrated to take place in low-temperature lake sediments and is reliant on the presence of organic materials, which is a psychrophilic process (Dhaked, Singh and Singh, 2010). The changing of composition of the media and reducing agent decrease acetate formation which results in >97% methane generation at obtaining grid quality (Logroño et al., 2020). Also, the feeding ratio of Hydrogen and Carbon dioxide impacts methane production (Jiang et al., 2021). The rate of stirring also influence the process, more than 1000 rpm found inhibitory (Logroño et al., 2020). The ex-situ hydrogen required to convert excess CO₂ and CO can fluctuate the metabolites. When hydrogen is injected into reactors with mixed cultures, volatile fatty acids (VFA) such acetate, propionate, butyrate, or even longer-chain or branched C4 and C5 organic acids frequently accumulate (Logroño et al., 2022).

The aim of the thesis is reviewing the biological biomethanation cases (in situ, ex-situ, and hybrid methods) to find the recent progress of research and technologies. This review also addresses the biotic and abiotic factors that affect the biomethanation for optimizing the operational conditions and effective methane production. In addition, what are the input material demands for biomethanation to work properly and what are the performance levels that different biomethanation processes can achieve are also reviewed here.
2 Biomethanation: Starting Point

Biomethanation is the process of producing high purity content methane from biogas or syngas. This review excludes the main pathways of AD and Thermal gasification process, except the product gas. For biomethanation, the starting point might be anaerobic digestion or thermal gasification. Anaerobic digestion produces biogas including methane and carbon dioxide in a microbial process without the presence of oxygen. Various factors influence this biological process of methane generation range from the organic feedstock composition, the design and types of the reactor, and the operational condition. On the other hand, thermal gasification is a source to obtain syngas which also can be used in biomethanation. Syngas provides CO, H₂ and other trace gases useful for biomethane production. However, roughly 35–45% of the volume of the biogas produced by anaerobic digestion is typically made up of CO₂. If biogas is intended for use as vehicle fuel, it must undergo an upgrading process to eliminate CO₂ and reach a purity level of over 95% biomethane (Li et al., 2017).

2.1 Anaerobic Digestion

Anaerobic bacteria degrade complex organic materials during anaerobic digestion, a biochemical process that takes place in the absence of oxygen. Anaerobic digestion is a potential technique for stabilizing sewage waste activated sludge. It has advantages such as eliminating the need for aeration equipment and its associated expenses, while also generating methane that can be used as a sustainable energy source in wastewater treatment plants (Mata-Alvarez, Macé and Llabrés, 2000; Lin et al., 2012; Wang et al., 2013; Feng et al., 2014). The four main mechanisms involved in the anaerobic digestion of organic matter are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. This process also depends on temperature. In The mesophilic process microorganisms can produce biogas by anaerobic digestion at a temperature of 37°C (Li et al., 2017). The process also can occur at different temperature ranges, including psychrophilic conditions (12-16°C) in landfills, swamps, or sediments, or thermophilic conditions (55-60°C) in anaerobic digesters or geothermally heated ecosystems (Winter and Temper, 1987). After production of biogas, it undergoes a conditioning step to eliminate unwanted impurities such as H₂S. Finally, when the gas is
used in vehicle fuel purpose, the gas is upgraded to meet the purity standards necessary for use as vehicle fuel, with a required biomethane purity of over 95 mol% (Li et al., 2017).

![Figure 1: Typical Anaerobic digester (Savills, 2021)](image)

The details of all four stages will not be discussed in detail, except the third step - methanogenesis, as it is most relevant to methane production. Methane-producing bacteria, also referred to as methanogens, produce methane during the third of the process' four stages. Methane can be created in two different ways on AD, either breaking the molecules of acetic acid to create methane and carbon dioxide, or by decreasing carbon dioxide with hydrogen. Hydrogen-based carbon dioxide reduction increases the production of methane but is constrained by the hydrogen concentration in the digesters. As a result, the acetate reaction is the main source of methane. (Omstead et al., 1980). Methane and Carbon dioxide are the major elements of biogas, but it also contains Hydrogen sulphide, Nitrogen, Hydrogen and oxygen. The biogas produced through anaerobic digestion contains several substances, including methane, carbon dioxide, hydrogen, hydrogen sulfide, ammonia, siloxanes, and other compounds. These substances have the potential to either hinder the anaerobic digestion process or cause issues with corrosion in pipelines and distribution networks of plants. To increase methane content, from the remaining CO₂ additional hydrogen injection
is needed, but excessive hydrogen doesn’t increase methane content when it exceeds a particular limit (Jiang et al., 2021). Furthermore, Hydrogen injection rate also depends on the reaction temperature, for example thermophilic condition can utilize higher volume of hydrogen resulting in 4-5 times increased methane content than the mesophilic condition (Chakraborty et al., 2018; Grimalt-Alemany et al., 2020; Oliveira et al., 2020; Jiang et al., 2021; Ghofrani-Isfahani et al., 2022). In addition, thermal destruction of pathogenic bacteria at higher temperatures is considered a major advantage. But some of the articles show that, although thermophilic conditions have been found to result in slightly faster rates of hydrolysis and fermentation, this has not resulted in higher methane yield. Research has shown that there is no significant increase in the total methane yield from organic matter across a range of fermentation temperatures, from 30°C to 60°C (Hashimoto, Varel and Chen, 1981; Mursec et al., 2006). However, thermophilic anaerobic fermentation has some disadvantages, such as reduced process stability and poorer dewatering properties of the fermented sludge. Additionally, it requires significant energy for heating (Winter and Temper, 1987).

2.2 Thermal Gasification

In biomass gasification process, biomass is transformed into syngas which is composed of CO, CO₂, H₂, CH₄ and water vapor. The main reactions of thermochemical gasification are Biomass decomposition, partial oxidation, steam reforming, water-gas shift and methanation. The temperature should be between 600 and 1000 °C, depending on the type, quality, and gasifying agent of the biomass feedstock. To prevent total burning of the biomass and to keep the reactor's reducing environment, the oxygen consumption should be kept under control. The main supply materials should be different biomasses with low moisture content and high heating value, such as food waste, animal waste, municipal waste, plant material, sewage, green waste, and wastewater (Nanou, 2013; Sapariya et al., 2021). The syngas is then upgraded to biomethane by removing or utilizing CO₂ (Li et al., 2017). The use of air, oxygen, and carbon dioxide will have an adverse effect on the methane yield and the H₂/CO ratio, according to the thermodynamic analysis of biomass gasification for the generation of biomethane. However, the addition of steam will improve carbon conversion and reduce the carbon deposit sector. (Wang, Bi and Wang, 2015). Biomethane
can be produced from syngas through a process called syngas biomethanation. This process involves the use of microorganisms to convert syngas into biomethane.

The syngas is first cleaned and conditioned before being fed into a bioreactor where it is converted into biomethane by microorganisms (Paniagua, Lebrero and Muñoz, 2022). The cleaning phase includes removing fine particles, mercury, ammonia, sulfur, chlorides, and other heavy metals from synthetic gas mixtures. The conditioning phase involves adjusting the ratio of $\text{H}_2$ to CO to satisfy the needs of the biomethanation procedure. Conditioning also includes extracting carbon dioxide for carbon sequestration and transforming carbonyl sulfide (COS) to hydrogen sulfide for sulfur cleanup (Paniagua, Lebrero and Muñoz, 2022).
The process has been shown to be effective in producing biomethane with high methane content (Paniagua, Lebrero and Muñoz, 2022). A study showed that the use of a two-stage process consisting of thermophilic anaerobic digestion of sewage sludge combined with an injection of syngas can produce biomethane (Yellezuome et al., 2022). The study also showed that the produced biogas was connected to the second stage consisting of a bioreactor where it was converted into biomethane by microorganisms (Yellezuome et al., 2022). Thermal processes, such as gasification and pyrolysis, can be used to convert digestate into solid, liquid, and gaseous forms, which can then be utilized in anaerobic digesters for increased efficiency (Giwa et al., 2019; Cheng et al., 2020). Biochar and bio-oil have been shown to enhance methane production when added to the digestate (Qiu et al., 2019; Yu et al., 2020). Syngas and pyrogas contain various gases, including CO, H₂, CO₂, CH₄, and N₂, which can be used for chemical production or as an energy source for thermal processes (Bridgwater, 1995; Kan, Strezov and Evans, 2016). Biological conversion of syngas to methane can be achieved under mild conditions using a robust anaerobic consortium. The preference of biological conversion pathways depends on environmental conditions such as pH and temperature, with acetic acid production dominating under mesophilic conditions and H₂ production preferred under thermophilic conditions (Luo et al., 2012; Liu et al., 2018). The combination of chemical approach and anaerobic digestion can successfully transform various waste feedstocks into value-added products (Andreides, Pokorna and Zabranska, 2022).

3 Biological Biomethanation

Depending on where the process takes place, there are three different forms of biomethanation: in-situ, ex-situ, and hybrid. In order to decrease carbon dioxide and improve the methane content of the biogas, hydrogen is delivered into the primary anaerobic digester or post-digester of a biogas plant. The injected hydrogen and the endogenous carbon dioxide are used in this process by the hydrogenotrophic methanogens, which are naturally present in the anaerobic digestion process, to create more methane. Ex-situ biomethanation, a procedure different from anaerobic digestion, involves the reaction of biogas or carbon dioxide with hydrogen in a bioreactor. This procedure involves growing and preserving a
particular microbial population of hydrogenotrophic methanogens in the bioreactor in order to convert external carbon dioxide and hydrogen sources into methane. The efficacy of biomethanation depends on a number of factors, including hydrogenotrophic methanogens, temperature, and methane gas supply. Investigating the features of in-situ hydrogen biomethanation under various circumstances is crucial. This study conducted two experiments (lasting 91 and 105 days) to examine how the performances of reactors and microorganisms were affected by the feeding gas and operating conditions. The pH was stable and the gas–liquid mass transfer was not limited throughout the experiment. The results indicated that the hydrogenotrophic methanogenesis was more efficient at thermophilic condition, and the predominant archaea genera at mesophilic and thermophilic temperature were *Methanobacterium* and *Methanothermobacter*, respectively. The CH$_4$ content was highest (over 90%) when the H$_2$ and CO$_2$ ratio was 4:1 and *Methanothermobacter* was dominant. These results might help to encourage the production of hydrogen biomethane. (Jiang *et al.*, 2021). H$_2$ is directly injected into a reactor where organic wastes are degraded anaerobically, and CO$_2$ is produced as a by-product. The injected H$_2$ and the endogenous CO$_2$ are then converted into CH$_4$ by the indigenous methanogens in the reactor. Ex-situ biomethanation is when H$_2$ and CO$_2$ are supplied from external sources to a separate reactor that contains only hydrogenotrophic methanogens and nutrients. The ex-situ reactor produces CH$_4$ with a high purity and can be used to upgrade the biogas from other processes. Hybrid biomethanation is a combination of in-situ and ex-situ biomethanation, where some of the organic matter is digested at its source and the rest is transported to a separate facility.
Biological and chemical methanation are two different processes to produce biomethane. Chemical methanation uses catalysts to create biomethane through the reaction of carbon dioxide and hydrogen, whereas biological methanation uses microorganisms to create it through anaerobic digestion. The chemical process uses the Sabatier reaction, a thermochemical process that creates methane and water from carbon dioxide and hydrogen by using metal catalysts like nickel or ruthenium. (Gahleitner, 2013). Both processes have potential applications in biogas upgrading, power-to-gas, and renewable energy storage. However, they differ in terms of reaction conditions, efficiency, selectivity, and environmental impact. Based on the review article by Stefan Rönsch (2016) and editorial article by Claire Dumus (2020), comparison between biological and chemical biomethanation is presented in Table 1.
Table 1: Comparison between biological and chemical biomethanation (Rönsch et al., 2016, Dumas et al., 2020)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Biological biomethanation</th>
<th>Chemical biomethanation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reaction temperature</strong></td>
<td>25-65°C</td>
<td>300-500°C</td>
</tr>
<tr>
<td><strong>Reaction pressure</strong></td>
<td>Atmospheric to 10 bar</td>
<td>1-30 bar</td>
</tr>
<tr>
<td><strong>Catalyst</strong></td>
<td>Microbial consortia or pure cultures</td>
<td>Metal catalysts (e.g., Ni, Ru)</td>
</tr>
<tr>
<td><strong>Methane yield</strong></td>
<td>Up to 100%</td>
<td>Up to 80%</td>
</tr>
<tr>
<td><strong>Methane purity</strong></td>
<td>Up to 99%</td>
<td>Up to 97%</td>
</tr>
<tr>
<td><strong>By-products</strong></td>
<td>None or minimal</td>
<td>Water and trace amounts of CO</td>
</tr>
<tr>
<td><strong>Energy consumption</strong></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td><strong>Environmental impact</strong></td>
<td>Low carbon footprint</td>
<td>High carbon footprint</td>
</tr>
</tbody>
</table>

3.1 In-situ Biomethanation

In this process, H₂ is injected straight into the AD, has received the most attention in recent years. This strategy is thought to be low-cost because it uses the AD reactor as the upgrading unit instead of building out extra infrastructure for biogas treatment (Wahid et al., 2019). With this method, continuous-stirred tank reactors (CSTR) could produce CH₄ concentrations of around 99%. (Wang et al., 2013). However, in-situ Biomethanation operation necessitates strict monitoring and management of operational parameters. The direct injection of H₂ into the AD results in the loss of CO₂'s buffer capacity, which may cause the pH to rise above 8.5, disrupting the process and inhibiting methanogenesis (Luo and Angelidaki, 2012). H₂ in the liquid phase should be used widely because exogenous H₂ supply causes an increase in H₂ partial pressure, which negatively affects specific anaerobic bacteria involved in AD and may result in a process imbalance like the accumulation of VFAs (Rusmanis et al., 2019). However, prior research revealed that H₂ was poorly soluble during in-situ BM, calling for the use of sophisticated diffusion devices or intensive reactor stirring to produce tiny gas bubbles that can expand the contact area between gas and liquid (Bassani et al., 2017; Voelklein, Rusmanis and Murphy, 2019). However, a recent study
proposes the use of in situ biomethanation directly in the main reactor during regular operation with high organic loading rates. The argument is that using in situ biomethanation would be more cost-effective in terms of capital expenditure (CAPEX) than ex situ technologies, since retrofitting existing anaerobic digesters with the necessary infrastructure would allow for their continued use.

Figure 4: In-situ Biomethanation

It appears that once the \( \text{CH}_4 \) concentration reaches a certain level, excessive \( \text{H}_2 \) injections are ineffective at promoting it. Research articles by Yun and Zhu compares the influence of thermophilic and mesophilic temperatures on in-situ hydrogen biomethanation. It reports that thermophilic temperature resulted in lower \( \text{CH}_4 \) content in the first experiment, but higher \( \text{H}_2 \) consumption rate and \( \text{CH}_4 \) content in the later experiments. It also suggests that there is a limit to how much \( \text{H}_2 \) injection can increase \( \text{CH}_4 \) content. It shows when \( \text{H}_2 \) consumption hit 100% for both temperatures, the rate for \( \text{H}_2 \) at thermophilic temperature observed to be higher than that at mesophilic condition (Jiang et al., 2021). This outcome is consistent with earlier research showing that hydrogenotrophic methanogens benefit from a
thermophilic environment for H$_2$ methanation (Yun et al., 2017; Zhu et al., 2019). How quickly a gas can shift from one phase to another, such as from gas bubbles to liquid in a reactor, is determined by its gas transfer coefficient. It depends on elements like liquid viscosity, surface tension, bubble size, and gas velocity. Gas transfer coefficient influences how effectively microbes convert hydrogen and carbon dioxide to methane during in-situ biomethanation (Rusmanis et al., 2019). Gas transfer coefficient can be improved by taking some approaches. These are increased and uniformed stirring, addition of packing materials which may decrease the bubble size of gas, biogas recirculation and creating a high pressure environmental inside the reactors. (Lindeboom et al., 2011; Luo et al., 2012; Zhao et al., 2020). The conversion of CO$_2$ to CH$_4$ in its original location is a potential method for improving biogas. This approach has several benefits, such as being straightforward and effortless to use, capturing and utilizing carbon, serving as a storable renewable electricity source, and being both cost-effective and environmentally sound (Fu, Angelidaki and Zhang, 2021).

3.1.1 Some Recent Case Studies on In-situ Biomethanation

Almost all of the research regarding In-situ biomethanation are limited to laboratory or bench scale, with working volume of roughly 1-2 L. The current progress of In-situ biomethanation are presented in table 2. The table shows 3 full scale in-situ biomethanation, conducted by the same research group in between 2018 and 2021. Full-scale in situ biomethanation with Venturi-type injection system of H$_2$ shows only 63% methane content. On the other hand, integrating full scale H$_2$ injection and reactor mixing showed a high methane content at about 95%. However, these scaling up from Jensen’s study have many limitations as well. In all full-scale tests, the synthesis of CH$_4$ rose at the complete utilization of CO$_2$, although the conversion of H$_2$ to CH$_4$ was indirect and incomplete. Sometimes the applied conditions showed even negative changes in methane concentrations even after providing full scale hydrogen supply (Jensen, Jensen, et al., 2021).

A pilot scale in-situ biomethanation process with a 30 L up-flow anaerobic sludge blanket (UASB) was established by Derakhshesh et al (2022) showed a high methane percentage at 98%. In that research the combination of the electrocoagulation (EC) process with anaerobic
digestion was evaluated for the first time, as a unique in-situ biomethanation strategy. Some other laboratory scale researches are shown in table 2.

Overall, a full scale In-situ biomethanation plant usually consists of biogas digester, an electrolyzer, a gas compressor and a gas diffuser. The electrolyzer, which separates water into $\text{H}_2$ and $\text{O}_2$ using renewable electricity, provides the hydrogen. A gas diffuser, which can be a membrane diffuser, sparger diffuser, or jet nozzle diffuser, is then used to compress the hydrogen before injecting it into the digester. For effective biomethanation, the diffuser should offer adequate gas-liquid mass transfer, mixing, and bubble size distribution. After purification, the generated biomethane can be drawn from the digester headspace or separated from the liquid phase. This procedure requires more equipment and energy for hydrogen synthesis and injection compared to anaerobic digestion without in-situ biomethanation, but it also allows for more effectively production of methane and carbon dioxide utilization (Rafrafi, Laguillaumie and Dumas, 2021a).

<table>
<thead>
<tr>
<th></th>
<th>Type of Plants</th>
<th>Reactor volume or set-up scale</th>
<th>Purity of produced methane content (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Full-scale in situ biomethanation</td>
<td>1200m³ thermophilic (52 °C) manure-based biogas reactor</td>
<td>95</td>
<td>(Jensen, Jensen, et al., 2021)</td>
</tr>
<tr>
<td>2</td>
<td>full-scale in situ biomethanation with Venturi-type injection system of $\text{H}_2$</td>
<td>Full scale Digester’s inner diameter 10.6 m, and a liquid height 12.5 m. Headspace volume $\sim$90 m³</td>
<td>63</td>
<td>(Jensen et al., 2018)</td>
</tr>
<tr>
<td>3</td>
<td>Integrating $\text{H}_2$ injection and reactor mixing for low-cost $\text{H}_2$ gas-</td>
<td>full-scale reactor</td>
<td>95</td>
<td>(Jensen et al., 2018)</td>
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<tr>
<td>4</td>
<td></td>
<td>Hydrogenotrophic methanogenesis via exogenous H$_2$ input</td>
<td>99</td>
<td>(Luo and Angelidaki, 2013a) (Luo et al., 2012) (Bassani et al., 2015) (Luo and Angelidaki, 2013b) (Martin et al., 2013) (Wang et al., 2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Laboratory scale</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td></td>
<td>Electro-methanogenesis</td>
<td>95</td>
<td>(Hagos, Liu and Lu, 2018)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5 L for the anaerobic digestion reactor and 0.5 for the microbial electrolysis cell reactor, and the purity of produced methane (%) was 75.8.</td>
<td></td>
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</tr>
<tr>
<td>6</td>
<td></td>
<td>electrocoagulation process with up-flow anaerobic sludge blanket (EC-UASB)</td>
<td>98</td>
<td>(Derakhshesh et al., 2022)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plexiglas pilot-scale UASB bioreactor with a total volume of 30 L and a working volume of 28 L.</td>
<td></td>
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<tr>
<td>7</td>
<td></td>
<td>In-situ biogas upgradation by 2 recirculation of gases and supply of</td>
<td>99</td>
<td>(Khan et al., 2022)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lab scale reactor with 2.5 L working volume</td>
<td></td>
<td></td>
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<tr>
<td>Page</td>
<td>Description</td>
<td>Details</td>
<td>Note</td>
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<tr>
<td>8</td>
<td>In-situ Biomethanation</td>
<td>laboratory scale with 1.7 L working volume</td>
<td>hydrogen conversion (94 and 87% for SS and FW, respectively) and methane content in biogas (79 and 68% for SS and FW, respectively), *FW-foodwaste, SS-Sewage sludge (Poggio <em>et al.</em>, 2023)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>In-situ biomethanation with two-stage anaerobic digestion process (without additive and co-digestion)</td>
<td>2L for the hydrolysis reactor and 5L for the methanogenic reactor</td>
<td>Initial purity was 75.8% Methane production recovered (70.4 %) after \textit{temperature shock within 30 days.} (Yang <em>et al.</em>, 2023)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>In-situ biogas upgrading assisted by bioaugmentation</td>
<td>two lab-scale CSTR of 1.5 L working volume (2 L total volume)</td>
<td>Methane production rate increased 11% after mesophilic (Palù <em>et al.</em>, 2022)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>In-situ biogas upgrading with $H_2$ addition in an anaerobic membrane bioreactor (AnMBR)</td>
<td>AnMBR experimental apparatus, jar fermenter working volume of 2.37 L</td>
<td>Compared with the CSTR mode of operation, the AnMBR mode of operation without added $H_2$ obtained higher CH$_4$ contents. CH$_4$ content reached 92% with addition of 11 equivalents of $H_2$ relative to CO$_2$</td>
<td>(Hafuka et al., 2022)</td>
</tr>
<tr>
<td>12</td>
<td>In-situ Biomethanation with starvation and feeding</td>
<td>Lab scale reactors' working volume with 200 mL</td>
<td>CH$_4$ content 83.5% (Fed with glucose) 89.7% (Fed with acetate)</td>
<td>(Nan et al., 2022)</td>
</tr>
</tbody>
</table>

3.1.2 Challenges for In-situ Biomethanation

While the main microorganisms and pathways involved in CO$_2$-to- CH$_4$ bioconversion are known, there is still much to learn about the molecular mechanisms, metabolic processes,
and microbial responses under various operating conditions, particularly during the electromethanogenesis phase. Advanced techniques such as genome-centric metatranscriptomics, isotope tracing, and high-throughput sequencing will be necessary to reveal the phylogenetic and metabolic characteristics of CO₂-to-CH₄ bioconversion. Currently, investigations into CO₂-to-CH₄ bioconversion have been limited to laboratory experiments. It is unclear how the process will perform and maintain stability over the long-term in situ, and at an industrial scale. Conducting further research in this area will help to identify potential challenges and necessary solutions for successful implementation. Additionally, to evaluate the sustainability of the process, crucial parameters can be determined through a life-cycle assessment (LCA). Genetic engineering techniques may enhance the efficiency of CO₂ bioconversion (Fu, Angelidaki and Zhang, 2021).

Firstly, it is still need to find the main factors limiting industrial application and what preparations should be made before implementation. Secondly, potentiality of membrane technology to address gas-to-liquid transfer difficulties and practical difficulties that may arise. The real challenges from the full-scale plants are - achieving efficient hydrogen and biogas mixing and gas-liquid mass transfer in the digester (Agneessens et al., 2017; Voelklein, Rusmanis and Murphy, 2019), maintaining the injection rate and hydrogen partial pressure to prevent acetogenic and methanogenic microbes from growing (Braga Nan et al., 2020), avoiding the formation of volatile fatty acids (VFA), particularly acetate, which can cause the pH to drop and compromise the integrity of the process (Voelklein, Rusmanis and Murphy, 2019), lowering the price of producing and storing hydrogen, which can be costly and energy intensive. It is not clear, whether DIET (direct interspecies electron transfer) could help overcome metabolic inhibition occurred by raised partial pressure of hydrogen, and whether existing strategies for improving DIET in typical AD processes could be applied to in situ CO₂-to-CH₄ bioconversion. DIET is a process where direct electron transfer occurs between living cells without the requirements of redecide molecules such as hydrogen or formate. Different methods, such as conductive pili, cytochromes, nanowires, or conductive materials, might cause DIET. By encouraging the syntrophic interaction between acetogenic bacteria and hydrogenotrophic methanogens, which convert acetate and carbon dioxide to methane, DIET can improve in-situ biomethanation. Additionally, diet can boost methane production, enhance carbon dioxide uptake, and limit the formation of volatile fatty acids and hydrogen, which can stifle the anaerobic digestion process (Wang and Lee, 2021). From
the research we can say that DIET can assist to overcome the energy barriers and the microbial inhibition resulted from high partial hydrogen pressure (Wang and Lee, 2021; He et al., 2022). For DIET Carbon-based conductive materials (CMs) such as granular activated carbon (GAC), biochar, and carbon cloth (CC) can be used which will facilitate direct interspecies electron transfer (DIET) as solid-state electron donor/acceptor between fermentative bacteria and methanogens (He et al., 2022; Kutlar, Tunca and Yilmazel, 2022). Exogenous hydrogen (EH₂) is injected into the anaerobic reactor to give the methanogens an extra supply of electrons. The interspecies hydrogen transfer (IHT) pathway, which competes with DIET for electrons, can similarly be inhibited by EH₂. The synthesis of methane from lipid-rich waste/wastewaters can be boosted and the DIET process improved by combining CMs with EH₂. Also, the microbiome and interspecies interactions involved in CO₂-to- CH₄ bioconversion is not fully understood. To tackle the challenges, it is important to understand the problems regarding electron transfer mechanisms between the cathode and methanogens in bioelectrochemical systems, and knowing how to improve the efficiency of biogas upgrading for large-scale application using genomic, metagenomic, transcriptomic, proteomic, and metabolomic analysis to advance the development of in situ biogas upgrading.

3.2 Ex-situ Biomethanation

Recently, ex-situ Biomethanation has gained attention in securing the stability of reactor. In this process, H₂ and biogas CO₂ are supplied as gaseous substrates in a separate unit tailored for hydrogenotrophic methanogens, providing flexibility to source waste CO₂ from other processes. Ex-situ Biomethanation usually has an increased volumetric CH₄ production rate with shorter gas retention time compared to in-situ Biomethanation (Voelklein, Rusmanis and Murphy, 2019; Wahid et al., 2019). The ex-situ process of biomethanation has traditionally been suggested due to its high volumetric hydrogen consumption rates in comparison to in situ technologies (Lecker et al., 2017b). Furthermore, separation from the breakdown of solid organic matter is preferred in order to avoid inhibiting the degradation of volatile fatty acids, which requires low liquid hydrogen partial pressure to be thermodynamically favorable (Rachbauer et al., 2016; Lecker et al., 2017b) (Schmidt and Ahring, 1993; Dolfing et al., 2008)
Ex-situ Biomethanation

*upgradation process includes any of the following processes: CSTR, fixed bed, bubble column, film reactor (FR), and up-flow anaerobic sludge blanket reactor, and trickle bed reactor (TBR) etc. Each of the techniques are described below.

3.2.1 Continuous-Stirred Tank Reactors (CSTR)

CSTR (Continuous-Stirred Tank Reactors) is a continuous flow system where microorganisms convert CO$_2$ into methane in a cylindrical vessel with a stirrer that mixes the contents of the reactor and maintains a uniform concentration of microorganisms and nutrients throughout the reactor. The reactor is operated at a constant temperature and pH, and the effluent is continuously removed from the reactor (Jiang et al., 2022). The effluent includes the mixture of substrate and microbes which undergo anaerobic digestion process in the CSTR reactor. This liquid comes from the inlet flow which feeds the organic waste and water. The liquid needs to be removed on a continuous basis at the same rate as the inlet flow to ensure the constant volume and residence time under the reactor. Over 95% of the bioreactors currently in use are of the CSTR type, and the CSTR is a well-established
technology. It can provide efficient mixing to achieve effective mass transfer of gas and liquid (Rittmann, Seifert and Herwig, 2015).

The CSTR's drawbacks include the requirement to keep the bacteria needed to catalyse the process inside the reactor (Boe, 2006), and short-circuit loss of non-degraded volatile particle matter (Jha et al., 2013). Short circuit loss in a CSTR reactor means the loss of non-degraded volatile particle matter that bypasses the reactor without being fully digested due to imperfect mixing or flow distribution. There are numerous benefits of CSTR. With CSTRs, the required chemical can be constantly generated without needing to periodically empty and re-fill the tank. CSTRs are capable of managing heterogeneous reactions involving catalysts or solid particles as well as complex reactions involving numerous reactants and stages (Team, 2021). CSTRs can use numerous reactors in series or extend the residence time to obtain high conversion rates (Rafrafi, Laguillaumie and Dumas, 2021a; Jiang et al., 2022; Nan et al., 2022).

On the other hand, CSTR has several negatives. Compared to plug flow reactors (PFRs), CSTRs have lower total throughput per unit volume, which necessitates the use of larger reactors or more expensive running costs. Temperature gradients are common in CSTRs, which can have an impact on the reaction rate and selectivity and raise safety concerns (Jiang et al., 2022; Nan et al., 2022).

3.2.2 Fixed Bed Reactors

In fixed bed reactors (FBR), microorganisms are allowed to be immobilised using some kinds of containing or support materials. These can be silica gel or activated carbon. Microbes grow in fixed beds where they get the water and nutrients. Microbes transform CO₂ into CH₄ in an immobilised condition, which provides increased stability and higher transformation rate (Dumas et al., 2020).

3.2.3 Bubble Column Reactors

Microorganisms suspended in liquid are used in vertical bubble column reactors to convert CO₂ to CH₄. These reactors are filled with a mix of liquid and gas bubbles, allowing for the
provision of necessary oxygen and nutrients. Uniformity of microorganism concentration throughout the reactor is maintained with the aid of the bubbles, which also assist in consistent mixing (Rafrafi, Laguillaumie and Dumas, 2021b; Wu et al., 2021; Kamravamanesh et al., 2023).

3.2.4 Trickle Bed Reactors

Using immobilized microorganisms, CO₂ can be transformed into CH₄ with the use of trickle bed reactors. These microorganisms are immobilized on a stable support material like silica gel or activated carbon. The idea is to immobilize the microbial biomass by packing a column with a carrier material that has an extensive surface area. Through this process, the stability and activity of the microorganisms are heightened, leading to greater conversion rates. The trickling liquid is often a nutrient solution that offers the best conditions for the microorganisms to develop and metabolize the CO₂ and H₂. Recent studies have shown that trickle bed reactors can achieve methane concentrations of up to 98% CH₄ (Sposob, Wahid and Fischer, 2021) or even pilot plants can achieve 97.4% Methane (Jønson et al., 2022). Analyzing the review paper by Sposob (2021) we can mention some advantages and disadvantages of using TBR in Ex-situ biomethanation.

The advantages are TBR facilitates biomethanation with high gas flow and liquid flow rates, high mass transfer capacity and minimum pressure drop. Methanogenic archaea get optimal conditions in TBR reactors because its optimal control of pH, moisture content and partial pressure of H₂. Besides optimal operational conditions, TBR also enhance the growth and diversity of microbes by utilizing different types of biocatalysts, pure or mixed microbial culture, granular sludge, biofilms or suspended and immobilized cells (Sposob, Wahid and Fischer, 2021). On the other hand, Trickle bed reactor has several disadvantages. It is sensitive to clogging, channeling, and flooding phenomena, which can affect the gas-liquid-solid contact and reduce the reactor performance. It also requires careful selection and pretreatment of the packing material, which can influence the biocatalyst attachment, biofilm formation, mass transfer, and pressure drop. It has challenges in scaling up and integration with existing biogas plants, such as maintaining optimal operational conditions, ensuring process stability and safety, and complying with gas quality standards. Trickle bed reactor needs further research and development to optimize the process parameters, improve the
biocatalyst performance, evaluate the techno-economic feasibility, and demonstrate the long-term operation (Jønson et al., 2022; Kamravamanesh et al., 2023).

3.2.5 Comparison Between Different Ex-Situ Reactors

Overall, ex-situ BM has been studied in different reactor configurations, such as CSTR, fixed bed, bubble column, and trickle bed reactor (TBR). While up-flow configurations with submerged filters or CSTR frequently showed high effluent CH₄ concentrations, the construction and maintenance of additional ex-situ units incur additional costs, and CSTR systems are limited in upscaling due to high energy demands for mixing (Luo and Angelidakis, 2012; Bassani et al., 2017) (Strübing et al., 2017). TBR, on the other hand, is the most promising configuration to overcome process scale-up constraints as it provides a large contact area between methanogenic archaea and gas phase, resulting in a four orders of magnitude higher diffusion coefficient without the need for additional energy for mixing (Aryal et al., 2021). In addition, TBR has high methane production capability comparing to other mentioned reactors, peaking up to 15.4 m³/m³d (Sposob, Wahid and Fischer, 2021) where CSTR has 3.7m³/m³d and up-flow reactor shows 0.25m³/m³d (Bassani et al., 2017; Strübing et al., 2017; Voelklein, Rusmanis and Murphy, 2019). In comparison to other reactors, FFR and CSTR generally have a number of advantages, including the ability to operate at substantially shorter hydraulic retention periods, which enables more rapid treatment at a lower cost of storage. Some advantages and disadvantages are described in the table 3 below.

Table 3: Advantages and disadvantages of different reactors in Ex-situ Biomethanation

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed bed reactor (FBR)</td>
<td>High gas-liquid mass transfer coefficient, better biomass retention and stability, less</td>
<td>Potential clogging and channeling issues, Limited flexibility and scalability, sensitive to hydrogen</td>
</tr>
<tr>
<td>Reactor Type</td>
<td>Advantages</td>
<td>Disadvantages</td>
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<tr>
<td>--------------------------------</td>
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<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Bubble column reactor (BCR)</td>
<td>Reactor design is simple, High efficiency gas holdup and mixing, High volumetric productivity</td>
<td>Biomass retention and stability is low, gas recirculation requires high energy needs, risk of foam formation and gas leakage, controlling the temperature is not easy.</td>
</tr>
<tr>
<td>Trickle bed reactor (TBR)</td>
<td>Large gas-liquid-solid contact area, High biomass retention and good stability, Moderate energy needs and operational cost</td>
<td>Potential clogging and channeling issues, Limited scalability and flexibility, Low tolerance to hydrogen sulfide and oxygen, Complex design and control</td>
</tr>
<tr>
<td>Continuously stirred tank reactor (CSTR)</td>
<td>Simple design and operation, good mixing and temperature control, Moderate methane yield and purity</td>
<td>Low gas-liquid mass transfer coefficient, Low biomass retention and stability, High energy consumption for stirring, Potential washout of microorganisms</td>
</tr>
</tbody>
</table>

### 3.2.6 Some Recent Case Studies on Ex-Situ Biomethanation Plants

Unlike in-situ Biomethanation Ex-situ biomethanation has achieved more pilot scale and full scale advances. 9 most recent pilot scale cases are presented in table 3. The highest methane content can be found in a pilot-scale biomethanation of cattle manure in 100 L pilot scale digester using dense membranes. (Lebranchu et al., 2019). which is 99.9%. In this paper, authors evaluated the techno-economic feasibility of installing biomethanation-based power-to-gas systems in an operational wastewater treatment plant (WWTP). They created and assessed five scenarios that include both in-situ and ex-situ biomethanation, as well as on-site renewable electricity production and grid electricity used in a temporary mode.
Depending on the situation, they discovered that the levelized cost of energy (LCOE) of biomethane ranges from 109 to 156 £/MWh. In addition, they took into account the by-product income and current policy frameworks, which decreased the LCOEs by 57% to 75%. This membrane is used to inject hydrogen into the digester. Other pilot scale reactors show different methane content percentages, for example a full scale TBR plant by Jønson et al (2022) yield 95.7% in parallel operation and 97.4% in serial operation. Bubble column reactor was also used in 2 pilot plants established by Laguillaumie et al., (2022) producing 94% and 95% methane respectively. CSTR, three phase biocatalytic reactor, fixed bed reactor and some lab-scale research are also shown in table 3.

Table 4: Different Ex-situ Biomethanation plants

<table>
<thead>
<tr>
<th>Plants</th>
<th>Working volume (L)</th>
<th>H2 – CO2 ratio</th>
<th>Output Methane content (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Ex-situ biogas upgrading in thermophilic trickle bed reactors packed with micro-porous packing materials</td>
<td>0.8 L working volume</td>
<td>H2 utilization efficiency &gt;99% (Volume Not given)</td>
<td>&gt;95</td>
<td>(Ghofrani-Isfahani et al., 2022)</td>
</tr>
<tr>
<td>2 Ex-situ biological methanation of H2/CO2 with a mixed microbial culture in a pilot scale bubble column reactor</td>
<td>Pilot scale 22 L bubble column reactor (BCR)</td>
<td>Inflow H2/CO2 ratio is 4-6</td>
<td>94</td>
<td>(Laguillaumie et al., 2022)</td>
</tr>
<tr>
<td>No.</td>
<td>Study Description</td>
<td>Reactor Type</td>
<td>Inflow H₂/CO₂ ratio</td>
<td>Conversion Efficiency</td>
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<tr>
<td>3</td>
<td>Pilot-scale study of biomethanation in biological trickle bed reactors converting impure CO₂ from a Full-scale biogas plant</td>
<td>Pilot scale biomass reactor on full-scale biogas plant</td>
<td>3.8–3.9:1</td>
<td>95.7 (parallel operation) 97.4 (Serial operation)</td>
</tr>
<tr>
<td>4</td>
<td>Ex Situ Biomethanation of Hydrogen at Alkaline pH</td>
<td>Laboratory-scale biogas reactor (CSTR)</td>
<td>≥97</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Biological methanation of H₂ and CO₂ in a continuous stirred tank reactor</td>
<td>Laboratory scale with working volume of 5 L</td>
<td>99.5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>KSTAR facility</td>
<td>1.5 L</td>
<td>4:1</td>
<td>99.5</td>
</tr>
<tr>
<td>7</td>
<td>Trickle bed reactor</td>
<td>0.5 L</td>
<td>4:1</td>
<td>98.2</td>
</tr>
<tr>
<td></td>
<td>Bioelectrochemical reactor</td>
<td>0.8 L</td>
<td>4:1</td>
<td>97.6</td>
</tr>
<tr>
<td>8</td>
<td>Ex-situ with bubble column reactor</td>
<td>22 L bubble column reactor (BCR)</td>
<td>4-5.4:1</td>
<td>&gt;95</td>
</tr>
<tr>
<td>9</td>
<td>Ex-situ Biomethanation with graphene or pyrochar</td>
<td>Lab-scale thermophilic reactor</td>
<td>4:1</td>
<td>Not given</td>
</tr>
<tr>
<td>10</td>
<td>Pilot-scale biomethanation in</td>
<td>68 L TBR Pilot scale reactor</td>
<td>4:1</td>
<td>&gt;98.5</td>
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<tr>
<td>11</td>
<td>Pilot-scale biomethanation of cattle manure using dense membranes</td>
<td>100 L Pilot scale digester.</td>
<td>4:1</td>
<td>99.9</td>
</tr>
<tr>
<td>12</td>
<td>power-to-gas systems based on biomethanation in an operating wastewater treatment plant</td>
<td>8000 m$^3$</td>
<td>4:1, 3:1</td>
<td>&gt;99</td>
</tr>
<tr>
<td>13</td>
<td>carrier material used biomethanation of industrial biogas-\text{CO}_2 in a trickle-bed reactor</td>
<td>0.5 L</td>
<td>4:1</td>
<td>99.8</td>
</tr>
<tr>
<td>14</td>
<td>Thermophilic Biogas Upgrading via ex Situ Addition</td>
<td>3 L</td>
<td>4:1</td>
<td>92-97</td>
</tr>
<tr>
<td>15</td>
<td>Ex-situ with CSTR and pure culture</td>
<td>10 L</td>
<td>4:1</td>
<td>85</td>
</tr>
<tr>
<td>16</td>
<td>Biocatalytic methanation in an anaerobic three-phase system</td>
<td>88 L</td>
<td>4:1</td>
<td>98</td>
</tr>
</tbody>
</table>
3.2.7 Challenges in Ex-situ Biomethanation

Ex-situ Biomethanation has been vigorously developed in recent years from bench scale to pilot and full scale. It has solved many problems associated with conventional energy intensive process for upgrading biogas to pure biomethane. However, there are still many challenges are present. Firstly, the low gas-liquid mass transfer rates which impacts the conversion of CO₂ to methane. Due to inconsistent hydrogen supply, electricity fluctuations and low gas-liquid transfer rate, the structure and dynamics of microbial community may change (Braga Nan et al., 2020; Liu et al., 2020; Rafrafi, Laguillaumie and Dumas, 2021a).

Utilizing carbonaceous substances like graphene or pyrochar as abiotic additives to improve system performance and boost microbial robustness is one approach to overcoming these difficulties. Another is optimizing operational conditions like temperature, pH, gas flow rate, and reactor configuration (Wang et al., 2020; EUScienceInnov, 2021).

Selecting the suitable reactor is also a challenge during scaling up of the process. Because most of the reactors have limitations comprising energy intensive steps.

3.3 Comparative Analysis of In-Situ, Ex-Situ, and Hybrid Biomethanation System

In-situ and ex-situ methods have different aspects for consideration and are not straightforward to compare with each other. Both of the types have many inclusiveness regarding the reactor type, hydrogen supply or involved microbial process. However, regarding reactor scaling up, process efficiency, input flexibility, microbial stability, operational location and cost of maintenances both type of biomethanation shows some differences that are described and summarised in table 4.
Table 5: Comparison between In-situ and Ex-situ Biomethanation (Voelklein et al., 2019; Rafrafi et al., 2020; Nan et al., 2020; Browne et al., 2016; Liu et al., 2020; Sposob et al., 2021, Thapa, Park and Jun 2022)

<table>
<thead>
<tr>
<th>In-situ biomethanation</th>
<th>Ex-situ biomethanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>The reaction that produces methane happens directly in the digester without oxygen</td>
<td>The methanation reaction is performed in a separate unit</td>
</tr>
<tr>
<td>The process could be altered by H₂ injection by influencing the microbial population of the anaerobic reactor</td>
<td>The microbial communities are selected and adapted to high H₂ partial pressures in a trickle bed reactor or any ex-situ reactor</td>
</tr>
<tr>
<td>The highest methane production was achieved when hydrogenotrophic methanogens and Methanosarcina sp. were prevalent in the whole operation.</td>
<td>The dominant methanogens were Methanobacterium sp. and Methanothermobacter sp., which are known to be highly efficient for CO₂ reduction</td>
</tr>
<tr>
<td>The main challenges are process stability, H₂ mass transfer and microbial community adaptation</td>
<td>The main challenges are technical and economic feasibility, reactor design and scaling-up</td>
</tr>
<tr>
<td>The original location of the process is preserved</td>
<td>The process is performed off-site</td>
</tr>
<tr>
<td>The methods are less expensive and less manageable</td>
<td>The methods are expensive and manageable</td>
</tr>
<tr>
<td>The process can cause inhibition or toxicity to some microorganisms due to high H₂ concentrations</td>
<td>The process can avoid inhibition or toxicity by selecting tolerant microorganisms in a separate reactor</td>
</tr>
<tr>
<td>It only depends on the existing CO₂ source of Anaerobic digester. If the source is limited, feedstocks can be provided in AD.</td>
<td>It is more flexible where any source of carbon dioxide can be found. For example, biogas, syngas, and flue gas are capable of injection in upgrading biogas.</td>
</tr>
</tbody>
</table>
3.4 Syngas Biomethanation

Syngas is a gas mixture containing H₂, CO, CO₂, CH₄, CₓHy, and N₂ in various percentages and different compositions, influenced by several factors including thermochemical reactions and the materials employed (Bridgwater, 1995). The parameters governing thermochemical reactions and the materials employed determine the syngas composition. In syngas biomethanation, syngas is converted into carbon dioxide (CO₂), carbon monoxide (CO) and hydrogen (H₂), to methane (CH₄). The biological transformation of endogenous CO₂ to biomethane quality requires a sufficient amount of reducing equivalents, which the syngas cannot make up. So, this process can be incorporated with existing Anaerobic digester, where In-situ biomethanation occurs, or in an ex-situ system where purified biogas from syngas can be further converted into highly purified methane (>94.7%). The two-stage procedure makes it possible to produce biomethane without hindering the digestion of anaerobic sludge (Andreides et al., 2021).

Figure 6: A novel two-stage process for biological conversion of syngas to biomethane (Andreides et al., 2021)

In this Process, both non-biodegradable waste materials or hardly-biodegradable biomass can be utilized to produce biomethane (Feng and Lin, 2017; Pecchi and Baratieri, 2019).
Syngas contains some impurities such as sulfur, ammonia, and tar which may cause problems in biomethanation (Takors et al., 2018). Using of metal catalyst also gaining popularity in syngas biomethanation (Molino, Chianese and Musmarra, 2016; Ghaib and Ben-Fares, 2018; Watson et al., 2018).

3.4.1 Some Recent Syngas Biomethanation Studies

Current studies of syngas biomethanation shows that syngas biomethanation has several benefits, for example- various types of biogas catalyst can be utilized ranges from pure culture to co-cultures, mixed cultures (mixed microbial consortia -MMC) and biofilms. Based on different reactor types such as CSTR, TBR, and BC methane yield also changes. In some cases, using TBR shows the highest methane content which is around 96% (Ghofrani-Isfahani et al., 2022) while CSTR reactors yield lower content roughly around 60%. Some of the current studies are presented in table 1.

Table 6: Summary of the most recent syngas biomethanation studies (Paniagua, Lebrero and Muñoz, 2022)

<table>
<thead>
<tr>
<th>Reactor Type</th>
<th>Volume (L)</th>
<th>Final CH$_4$ composition (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSTR</td>
<td>1.50</td>
<td>70</td>
<td>(Diender et al., 2018)</td>
</tr>
<tr>
<td>CSTR</td>
<td>9.50</td>
<td>49</td>
<td>(Voelklein, Rusmanis and Murphy, 2019)</td>
</tr>
<tr>
<td>CSTR</td>
<td>9.50</td>
<td>61</td>
<td>(Voelklein, Rusmanis and Murphy, 2019)</td>
</tr>
<tr>
<td>TBR</td>
<td>0.18</td>
<td>67</td>
<td>(Asimakopoulos et al., 2020)</td>
</tr>
<tr>
<td>TBR</td>
<td>0.18</td>
<td>86</td>
<td>(Asimakopoulos et al., 2020)</td>
</tr>
<tr>
<td>TBR</td>
<td>0.80</td>
<td>96</td>
<td>(Ghofrani-Isfahani et al., 2022)</td>
</tr>
<tr>
<td>TBR</td>
<td>1.20</td>
<td>66</td>
<td>(Kougias et al., 2020)</td>
</tr>
</tbody>
</table>
3.4.2 Advantages and Challenges of Syngas Biomethanation

The most attractive advantage of syngas biomethanation is it can utilize a wide range of biomass to produce methane. These include non-degradable or hardly degradable biomass, that cannot be utilized in general anaerobic digesters. For example, lignocellulosic biomass, industrial waste, municipal solid waste, and agricultural waste etc. Another advantage is accessibility of different upgradation techniques. By this we mean that, syngas biomethanation can incorporate any upgradation system, such as – TBR, CSTR, Fluidized beds, fixed beds etc (Grimalt-Alemany, Skiadas and Gavala, 2018; Asimakopoulos et al., 2020).

Syngas biomethanation creates some significant challenges to produce biomethane. Syngas produces impurities that cause inhibitions. A very recent study shows that, four tar compounds produced from syngas (benzene, toluene, styrene and phenol) affect microbial metabolism thus inhibit biomethanation process. Due to its strong inhibitory action and high solubility in water, phenol was found to be the most harmful contaminant. At doses greater than 0.5 g/L, phenol inhibited both hydrogenotrophic methanogens and carboxydotrophs. In comparison to phenol, benzene, toluene, and styrene had less inhibitory activity and less solubility. Primarily, they prevented hydrogenotrophic methanogens from growing (Figuera et al, 2023). Tar, produced during gasification process, can clog the bioreactor. Hydrogen sulfide or carbon monoxide can corrode the reactor surface and inhibit metabolic process of the methanogens. Halogenated compounds (such as HCl, HF) also make corosions in bioreactor. Ammonia and Hydrogen cyanide
also can cause toxicity in the reactor. (Grimalt-Alemany, Skiadas and Gavala, 2018; Asimakopoulos et al., 2020; Menin et al., 2021)

Another challenge in syngas biomethanation is lower rate of gas-liquid mass transfer which causes low biomethane yields. Increasing the gas pressure, adding surfactants, reducing the volume of the liquid, speeding up the agitation or employing membrane bioreactors can overcome this problem with low transfer rate (Chandolias, Pekgen and Taherzadeh, 2019; Li, Zhu and Angelidaki, 2021). It is difficult to optimize and control the process of syngas biomethanation during scaling up the process. To address this challenge, simulation and sensor based modelling and predictive research are required (Chandolias, Pekgen and Taherzadeh, 2019; Paniagua, Lebrero and Muñoz, 2022). For successful biomethanation, a stable microbial metabolism in the reactor is crucial that can be altered by the composition of syngas, controlling abiotic environmental factors such as pH, temperature, nutrients and contaminants. These factors need to be considered during syngas biomethanation (Grimalt-Alemany, Skiadas and Gavala, 2018; Paniagua, Lebrero and Muñoz, 2022)

4 Factors Affecting the Biomethanation

Various biological or non-biological factors impact the biomethanation process. The main key factor is microbial community which is governed by the type of microbial culture; their diversity, growth, metabolic pathways, synergistic and inhibitory effects, capacity of mass transfer, and methanogenesis etc. The biotic community and dynamics are also dependent on the reactor configuration and operational conditions, such as pH, temperature, H₂ partial pressure, and biomass-gas ratio. The metabolic pathways and gas-liquid mass transfer can be impacted by different carrier materials and catalysts. These factors will be described in this section.

4.1 Microbial Community

In the course of biomethanation, different categories of microorganisms execute each stage. Fermenting bacteria, bacteria that oxidize organic acids, and methanogenic archaea are the
three primary physiological types of microorganisms that are participating in methane generation (Angelidaki et al., 2011). To prevent the build-up of any intermediary metabolite in the system, the various biological transformation processes must remain sufficiently interconnected in a balanced digestion system. Initially, hydrolytic bacteria catalyze the breakdown of solid organic substances with the aid of exoenzymes, resulting in the conversion of proteins, carbohydrates and fats into amino acids, monosaccharides, organic acids such as fatty acids and alcohols. Monosaccharides are further converted into short-chain fatty acids, alcohols, CO₂ and hydrogen by fermentative and hydrolytic bacteria. Glycerol is transformed into pyruvate, while fatty acid chains undergo β-oxidation reaction process to degrade into acetic acid. Certain groups of bacteria employ coupled oxidation-reduction reactions (Stickland reaction) to break down amino acids. The conversion of hydrolyzed compounds into volatile fatty acids by clostridia and other hetero-fermentative bacteria is called acidogenesis. Accumulation of propionate is highly toxic to the process (at low temperature) compared to other volatile fatty acids (Dhaked, Singh and Singh, 2010).

But in our review, we will focus how the H₂ addition in in-situ will change the factors affecting the AD process and then for ex-situ how the reactions between microbes, CO₂ and H₂ are affected by different factors.

Figure 7: Biological Process of Biomethanation
4.1.1 Microorganisms in Different Biomethanation

We can see from the table 2 that *Methanobacterium* is the most common type of microorganism used in different bioreactors. In some studies the bacteria also show some unique features such as removal of phosphorus (Phosphorus is typically provided by the organic substrate that is digested, such cow manure, which contains phosphorus in a variety of forms, including organic phosphorus, inorganic phosphorus, and polyphosphate. Phosphorus can be limiting for microbes if the ratio is increased) and upgrading of biogas via DIET process (Zafiri, Kornaros and Lyberatos, 1999; Liu et al., 2019; Xu et al., 2020). Different species of this microorganism shows various features such as growing in both mesophilic and thermophilic conditions (*Methanobrevibacter arboriphilus, Methanobacterium formicicum*), surviving in high sulfur environment (*Methanothermobacter marburgensis*), accumulating propionate in nutrient limiting environment (*Methanobacterium sp, Methanothermobacter sp*) showed in table 2.

*Methanosarcinales* also have unique functions. Depending on the quantity and quality of substrates, *Methanosarcinales* can shift between various metabolic routes. To produce hydrogenotrophic methanation, acetoclastic methanation, or methylotrophic methanation, they might employ the reductive acetyl-CoA pathway, the aceticlastic pathway, or the methylotrophic pathway (Schütz, Seiler and Conrad, 1989; Conrad, 2020; Ernst et al., 2022).

Microbes also plays important role in syngas biomethanation, for example converting carbon monoxide into methane and carbon dioxide, synergistic association and co-culture formation with other methanogens can make syngas biomethanation more prospective (Novak et al., 2021; Song et al., 2021; Liu et al., 2020; Kato et al., 2021; Diender et al., 2018; Zipperle et al., 2021)

<table>
<thead>
<tr>
<th>Types of Biomethanation</th>
<th>Microorganisms</th>
<th>Features</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>In situ Biomethanation</td>
<td><em>Methanobacterium</em> (45.21% to 50.88%)</td>
<td><em>Acinetobacter</em> genus is significant in phosphorus</td>
<td>(Derakhshesh et al., 2022)</td>
</tr>
<tr>
<td><strong>Acinetobacter</strong> (19.8%)</td>
<td>removal (Zafiri, Kornaros and Lyberatos, 1999) <em>Methanosaeta</em> (also called <em>Methanothrix</em>) could improve biogas upgrading via DIET (Liu et al., 2019; Xu et al., 2020)</td>
<td></td>
<td></td>
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<td>--------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Methanomicrobiales, Methanobacteriales and Methanosarcinales</strong></td>
<td>Can shift between metabolic pathways depending on the substrates (Schütz, Seiler and Conrad, 1989; Conrad, 2020; Ernst et al., 2022)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Methanosarcina</strong> (43.2 %)</td>
<td>Dominant methanogens under acid condition (pH 5.5) (Yang et al., 2023)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ex-situ Biomethanation</strong></td>
<td><strong>Methanobrevibacter arboriphilus</strong> hydrogenotrophic methanogenesis both under mesophilic and thermophilic conditions (Karakashev, Batstone and Angelidaki, 2005) capable of thriving in environments with elevated H₂S concentrations (Kaster et al., 2011) (Dupnock and Deshusses, 2017)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Methanobacterium formicicum</strong></td>
<td>(Porté et al., 2019a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Methanothermobacter marburgensis</strong></td>
<td>(Seifert, Rittmann and Herwig, 2014b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Methanothermobacter wolféii</strong></td>
<td>(Guneratnam et al., 2017)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Methanoculleus sp</strong></td>
<td>Found frequently in high salt and ammonia AD processes (maize and manure as a substrate) (Maus et al., 2012; Burkhardt et al., 2019)</td>
<td>(Wang et al., 2013; Ashraf et al., 2020; Logroño et al., 2020)</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
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<td></td>
</tr>
<tr>
<td><strong>Methanothermobacter thermautotrophicus</strong></td>
<td>Frequent in thermophilic ex-situ BM (Martin et al., 2013) (Kougias et al., 2020)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Methanospirillum</strong></td>
<td></td>
<td>(Kim, Choi and Chung, 2013)</td>
<td></td>
</tr>
<tr>
<td><strong>Methanobrevibacter arboriphilus</strong></td>
<td>capacity to endure oxygen (O2) exposures for up to three days (Dupnock and Deshusses, 2017)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Methanobacterium, Methanothermobacter</strong></td>
<td>Accumulation of propionate accumulated occurs when nutrient is limited for Methanogens</td>
<td>(Laguillaumie et al., 2022)</td>
<td></td>
</tr>
<tr>
<td><strong>SHA-98</strong></td>
<td>Combined with <em>Methanothermobacter</em>, assist syntropic acetate oxidation process which stabilize the upgradation.</td>
<td>(Wu et al., 2021)</td>
<td></td>
</tr>
<tr>
<td><strong>Coprothermobacter and Caldanaerobacter</strong></td>
<td>Functional under alkaline condition (pH 8.5–9.0) Maximum CH$_4$ Production</td>
<td>(Chen, Du and Xie, 2021)</td>
<td></td>
</tr>
<tr>
<td><strong>Methanosarcina and Methanoculleus</strong></td>
<td>Fluctuates over different phases which also alters the acetate accumulation.</td>
<td>(Jiang et al., 2022)</td>
<td></td>
</tr>
<tr>
<td><strong>Methanothermobacter thermautotrophicus</strong></td>
<td></td>
<td>(Alfaro et al., 2018)</td>
<td></td>
</tr>
<tr>
<td>Syngas Biomethanation</td>
<td>Methanobacterium sp. MBA03</td>
<td>synergy of MBA03 with Methanobacterium promote more stable performance.</td>
<td>(Laguillaumie et al., 2022)</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------</td>
<td>-----------------------------------------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td></td>
<td>Clostridiaceae DTU-pt_113</td>
<td>Associated in biofilm formation as a result of the rich-nutrient media that was dripping from the top of the reactor at the topmost layer of packing material.</td>
<td>(Tsapekos et al., 2021)</td>
</tr>
<tr>
<td>Methanoculleus bourgensis</td>
<td></td>
<td>(Thapa, Park and Jun, 2022)</td>
<td></td>
</tr>
<tr>
<td>Clostridium, Acetobacterium and Sporomusa</td>
<td></td>
<td>CO metabolization, generate acetate and alcohols</td>
<td>(Novak et al., 2021; Song et al., 2021)</td>
</tr>
<tr>
<td>Rhodospirillum, Thermincola, Desulfotomaculu, Carboxydothermu, C aboxydocella and Moorella</td>
<td>Carboxydothermus hydrogenoformans, Methanothermobacter thermoautotrophicus.</td>
<td>carboxydrotrophic hydrogenogenesis, convert CO to H2/ CO2</td>
<td>(Liu et al., 2020; Kato et al., 2021)</td>
</tr>
<tr>
<td></td>
<td>Thermococcus onnuriqqqneus and Methanocaldococcus jannaschii, Methanocaldococcus vulcanius, or Methanocaldococcus villosus</td>
<td>The synergistic association or co-culture of both microbes convert syngas into biomethane.</td>
<td>(Diender et al., 2018)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Co-culture of these microbes convert syngas into biomethane effectively</td>
<td>(Zipperle et al., 2021)</td>
</tr>
</tbody>
</table>
4.1.2 Effect of Starvation on Microbes

Some research shows that methane content in biomethanation process perhaps decreased due to starvation. It also causes accumulation of volatile fatty acids and changes in microbial community (Braga Nan et al., 2020; Logroño et al., 2021). But other research found that methane production can be boosted up after starvation of a period up to 14 days. This might be resulted from the redundant of methanogens that can adapt to intermittent hydrogen supply and avoid acetate accumulation (Logroño et al., 2021). The variables stated above may also affect how much methane content is produced because of starvation. Generally speaking, this situation decreases the generation of methane via influencing the activity and abundance of hydrogenotrophic methanogens. Nevertheless, some research has demonstrated that methane content production can be maintained or even increased after starvation periods, particularly when using inocula from wastewater treatment plants or biogas plants with high hydrogenotrophic methanogen diversity (Braga Nan et al., 2020; Logroño et al., 2021).

4.2 Abiotic Factors

Abiotic factors for biomethanation means the physical, chemical and environmental factors such as temperature, pH, pressure, nutrient requirements, reactor configurations, different types of materials that affect biomethanation in different stages.

4.2.1 Temperature and pH

Temperature significantly affects the metabolic activity of the methanogenic microbes, gas-liquid mass conversion of H₂ and CO₂, as well as the structure and diversity of microbial population. The microorganisms performing biomethanation are affected by temperature in terms of growth rate, metabolic activity, and substrate usage. There are three categories of temperature ranges: psychrophilic (20 °C), mesophilic (20–40 °C), and thermophilic (40–70 °C). In general, higher temperatures increase the yields and rates of methane generation, but they also need more energy and have lesser stability.
The major substrates for hydrogenotrophic methanogens are carbon dioxide and bicarbonate, and pH has an impact on their balance. pH has an impact on biomethanation-related microbial metabolism and enzyme function as well. Between 6.5 to 8.5 is the ideal pH range for biomethanation, depending on the temperature and microbial community. A deviation from this range could thwart the biomethanation process and result in a failure of the process. Scientists are currently interested in the ex-situ upgrading of biogas under alkaline conditions. Studies show that, hydrogenotrophic methanogen enrichment is preferable at pH range 8.0 to 9.0, which may yield more than 97% of methane at mesophilic temperature (35 °C) (Logroño et al., 2020). Temperature and pH have significant role in Biomethanation that are presented in table 7.

Table 8: Effect of temperature and pH on effluent methane content.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Working volume</th>
<th>pH</th>
<th>Gas ratio (H₂: CO₂)</th>
<th>Product gas CH₄ content (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>55 ± 1</td>
<td>58.1 L</td>
<td>6.0–8.0</td>
<td>3.75:1/4:1</td>
<td>71.4 ± 12.1–99.1 ± 1.3</td>
<td>(Strübing et al., 2017)</td>
</tr>
<tr>
<td>55 ± 1</td>
<td>58.1 L</td>
<td>7.0 ± 0.25</td>
<td>3.78:1</td>
<td>72.8 ± 26.1–97.5 ± 0.9</td>
<td>(Strübing et al., 2018)</td>
</tr>
<tr>
<td>55 ± 1</td>
<td>58.1 L</td>
<td>7.7 ± 0.1</td>
<td>3.78:1</td>
<td>98.1 ± 2.1</td>
<td>(Strübing et al., 2019)</td>
</tr>
<tr>
<td>54 ± 1</td>
<td>1 L</td>
<td>8.29 ± 0.03 – 8.60 ± 0.09 / 8.12 ± 0.14 – 8.63 ± 0.11</td>
<td>4.13:1:1.53 (CH₄)</td>
<td>95.1 ± 0.5–98.7 ± 0.3 / 94.9 ± 0.6–99.1 ± 0.1</td>
<td>(Porté et al., 2019a)</td>
</tr>
<tr>
<td>52</td>
<td>0.291 L</td>
<td>7.19 ± 0.02 – 9.49 ± 0.34</td>
<td>3.155–4.065:1:0.5–1 (N₂)</td>
<td>80—98 (with N₂)</td>
<td>(Ashraf et al., 2020)</td>
</tr>
<tr>
<td></td>
<td>Volume mL</td>
<td>Temperature °C</td>
<td>Ratio N₂</td>
<td>Percentage</td>
<td>Reference</td>
</tr>
<tr>
<td>------</td>
<td>-----------</td>
<td>----------------</td>
<td>----------</td>
<td>------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>52</td>
<td>0.291 L</td>
<td>7.5–8.0</td>
<td>3.71:1:1 (N₂)</td>
<td>67.1 (without N₂)</td>
<td>(Sieborg et al., 2020b)</td>
</tr>
<tr>
<td>52</td>
<td>0.291 L</td>
<td>7.73–8.51</td>
<td>4:1:1 (N₂) / 4:1:2 (N₂)</td>
<td>95.3±4.4 (with N₂)</td>
<td>(Dahl Jønson et al., 2020)</td>
</tr>
<tr>
<td>38 ± 1</td>
<td>61 L</td>
<td>7 ± 1</td>
<td>4:1</td>
<td>94</td>
<td>(Burkhardt et al., 2019)</td>
</tr>
<tr>
<td>37 ± 2</td>
<td>5.78 L</td>
<td>6.8–7.0</td>
<td>6.7–3.7:1</td>
<td>95.4–97</td>
<td>(Rachbauer et al., 2016)</td>
</tr>
<tr>
<td>37 ± 0.5</td>
<td>61 L</td>
<td>7.2–7.4</td>
<td>4:1</td>
<td>98</td>
<td>(Burkhardt, Koschack and Busch, 2015)</td>
</tr>
<tr>
<td>37</td>
<td>26.8 L</td>
<td>n.a</td>
<td>4:1</td>
<td>92.8–97.9</td>
<td>(Burkhardt and Busch, 2013)</td>
</tr>
<tr>
<td>35</td>
<td>0.2258 L</td>
<td>4.0–9.0</td>
<td>3.6:1 (N₂)</td>
<td>0–44</td>
<td>(Dupnock and Deshusses, 2017)</td>
</tr>
<tr>
<td>55</td>
<td>0.05</td>
<td>7</td>
<td>4:1</td>
<td>99.9</td>
<td>(Wu et al., 2021)</td>
</tr>
<tr>
<td>37</td>
<td>0.05</td>
<td>7.5–8.5</td>
<td>4:1</td>
<td>97.6</td>
<td>(Rafrafi, Laguillaumie and Dumas, 2021a)</td>
</tr>
<tr>
<td>55</td>
<td>0.08</td>
<td>7.05</td>
<td>4:1</td>
<td>98.9</td>
<td>(Wu et al., 2021)</td>
</tr>
<tr>
<td>55</td>
<td>0.04</td>
<td>7.05</td>
<td>6:1</td>
<td>98</td>
<td>(Wu et al., 2021)</td>
</tr>
</tbody>
</table>
| 65   | 10        | 6.85            | 4:1      | 85         | (Seifert, Rittmann and}
4.2.2 Graphene and Pyrochar

Pyrochar and graphene both have different effects on methane generation. The direct interspecies electron transfer (DIET) between hydrogen-producing bacteria and hydrogenotrophic methanogens can be improved by graphene, a carbon-based material with excellent electrical conductivity and a significant surface area (Wu et al., 2020). In addition to increasing the biomethanation process' stability and resilience in the presence of intermittent hydrogen supply (Wang et al., 2020), this can increase the rate and yield of methane generation. In addition, graphene helps stabilize ex-situ biomethanation after getting inconsistent gas supply possibly because of graphene's strong electrical conductivity.
and substantial specific surface area. The integration of graphene increased production rate by 267% and the efficiency of gas conversion by 18.2% in response to the shock of intermittent gas input. The addition of pyrochar, however, had no positive benefits on the upgrading performance (Wu et al., 2021). Pyrochar is a carbon-rich product of biomass pyrolysis that can increase the surface area and electrical conductivity of the reactor (Wang et al., 2020). Pyrocar, on the other hand, might not have the same favorable impact on methane generation as graphene since it might have impurities or a poorer conductivity. (Wang et al., 2020; EUScienceInnov, 2021)

4.2.3 Using Carrier Materials

Carrier materials assist to speed up and increase the methanogenesis rate. It also helps in DIET process (described in 4.2.2). Some examples of carrier materials are pumice, polyurethane foam, expanded clay and lava rock. Some carrier materials are shown in figure 8. It is showing wood-straw ash filter material after vulcanization, and clay pellets. These materials help biomethanation process by increasing the Hydrogen gas-liquid mass transfer coefficient. In a study, pumice was found to be the most effective carrier material for gas liquid mass transfer and highest 99.8% methane was obtained by using this material (Jensen, Poulsen, et al., 2021). Some other carrier materials, including activated carbon, clay-based carriers, wood-ash, zeolite, stainless steel mesh, filter material, etc., have been studied for biomethanation and found to be useful in reducing H₂ consumption rate, increasing biofilm formation and contributing reactor performance and stability (Jensen, Poulsen, et al., 2021; Sieborg et al., 2021; Kusnere, Spalvins and Bataitis, 2023).
Carrier materials help in biofilm formation which results in making more surface area for contact between methanogenic archaea and reactant inflow gases. Moreover, shape and positioning of the carrier materials are important as well to prevent unexpected wetting or recirculation of liquid (Porté et al., 2019b). Sometimes clogging derived from long term use of polyurethane foam may cause problems accumulating solids in the liquid phase (Ashraf et al., 2020).

4.2.4 Reactor Configuration

Reactor configuration is a vital consideration for determining implementation of biomethanation from lab or bench scale to pilot and full-scale. Because, during scaling-up, the equipment costs increase and also the maintenance becomes crucial. Different types of reactors are described with comparative analysis in 3.2 section. According to the most current studies TBR has the highest potential among all other types of reactors. For reactor selection, it is important to consider such as the variety and structure of the feedstock, the hydraulic retention duration, the speeds at which organic materials are loaded, the technique for injecting hydrogen, and the expense of operations (Braga Nan et al., 2020).
5 Discussion and Conclusion

This review gives an overview of different types of biomethanation, their methane yield, advantages and challenges in scaling up the systems. Although Europe has introduced demonstration and pilot plants, the area of biological methanation remains at a low Technology Readiness level (TRL) and requires substantial research, as evidenced by the abundance of articles on the topic. However, there has been a recent surge in interest in this field, with a most of the articles on the topic being published in the last few years. Ex-situ biomethanation has achieved more advancement in successful up-scaling compared to in-situ biomethanation, which tends to be more effective with Trickle bed reactors capable of providing around 95-99% methane (Aryal et al., 2021; Sposob, Wahid and Fischer, 2021). In-situ biomethanation faces challenges due to low gas-liquid transfer rate, process instability and microbial inhibition by high hydrogen partial pressure (Wang and Lee, 2021; He et al., 2022). These can be solved by utilizing carrier materials, applying DIET, optimizing operational conditions by controlling biotic and abiotic factors (He et al., 2022; Kutlar, Tunca and Yilmazel, 2022). These solutions significantly decrease the hydrogen consumption adding extra economic benefit.

Syngas biomethanation has been also gaining popularity in recent years. This approach can utilize a wide range of biomass as carbon source and can be incorporated with any of the existing biomethanation system. However, the impurities from syngas and lower rate of gas-liquid mass transfer seem to limit the process with low biomethane yield (Grimalt-Alemany, Skiadas and Gavala, 2018; Asimakopoulos et al., 2020; Menin et al., 2021). Further research is required to minimize these problems for successful implementation of this highly potential method.

Finally, this review provides information about the dominant microorganisms that are used in different biomethanation plants including their unique features (Novak et al., 2021; Song et al., 2021; Liu et al., 2020; Kato et al., 2021; Diender et al., 2018; Zipperle et al., 2021). These potential microbial functions also open a way to research on how nutrients and minerals can be recovered from the biomethanation plants and how different co-digestion and synergies can be utilized for boosting biomethanation process.
The limitations of this thesis are the unavailability of energy efficiency or electricity requirements data. This review only provides information about the CO\textsubscript{2} hydrogen ratio for ex-situ plants, not for syngas and in-situ cases. It is also based on only 12 in-situ, 16 ex-situ and, 12 syngas biomethanation cases that have been developed recently.

This thesis provides many answers to existing problems of up-scaling and the potential solutions from small scale research as well. DIET, Carrier materials, syngas incorporation or co-digestive microbial models together can make breakthrough in future by replacing the energy intensive systems with highly efficient, economic and sustainable biological biomethanation. Researchers and industries should focus to implement more optimized full-scale In-situ biomethanation so that many existing biogas reactors can be used for producing biomethane.
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