On-site CO₂ bio-sequestration in anaerobic digestion: current status and prospects

Suyun Xuᵃ, Zihao Qiaoᵃ, Liwen Luoᵇ,ᶜ, Yongqi Sunᵃ, Jonathan Woon-Chung Wongᵇ,ᶜ⁺, Xueyu Gengᵈ, Jing Niᵃ

ᵃ School of Environment and Architecture, University of Shanghai for Science and Technology, Shanghai, 200093, China
ᵇ Institute of Bioresource and Agriculture, Hong Kong Baptist University, Hong Kong SAR
ᶜ Department of Biology, Hong Kong Baptist University, Hong Kong SAR
ᵈ School of Engineering, University of Warwick, Coventry CV4 7AL, United Kingdom

⁺Corresponding author: J.W.C. Wong (Tel: 852 34117056, jwcwong@hkbu.edu.hk)
Abstract

The advantages of anaerobic digestion (AD) technology in organic solid waste treatment for bioenergy recovery are evidenced in worldwide. Recently, more attention has been paid to on-site biogas research, as well as biogenic CO₂ sequestration from AD plant, to promote “carbon neutral”. Single-phase and two-phase AD system can be incorporated with various CO₂ bioconversion technologies through H₂ mediated CO₂ bioconversion (in-situ and ex-situ biogas upgrading), or other emerging strategies for CO₂ fixation without exogenous H₂ injection; these include in-situ direct interspecies electron transfer reinforcement, electromethanogenesis, and off-gas reutilization. The existing and potential scenarios for on-site CO₂ bio-sequestration within the AD framework are reviewed from the perspectives of metabolic pathways, functional microorganisms, the limitations on reaction kinetics. This review concluded that on-site CO₂ bio-sequestration is a promising solution to reduce greenhouse gas emissions and increase renewable energy recovery.

Keywords

Anaerobic digestion; Biogas upgrading; CO₂ sequestration; Hydrogen; Methanogenesis
1. Importance of CO₂ sequestration and conversion in biogas plant

Various organic wastes, such as agricultural wastes, food waste, and organic fractions of household wastes, have been utilized as feedstocks for anaerobic digestion (AD) to harvest biogas. AD treatment of organic waste can effectively solve the global problem of biomass disposal, generating biogas as an alternative to fossil fuels; thus, it has attracted more attention in recent decades (Xu et al., 2017; Zhou et al., 2019). Biogas enriched with methane (CH₄) is an important renewable bioenergy with clear advantages, including high calorific value and flexible application such as heating, power production, and transport fuels (Agnèessens et al., 2018). In general, CH₄ and carbon dioxide (CO₂) accounted for 50–70% and 30–50% of biogas, respectively (Kadam and Panwar 2017). The removal of CO₂ and other trace contaminants from biogas known as “biogas upgrading” processes, is necessary before biogas utilisation (Sun et al., 2015).

Previous researchers have focused mainly on fossil CO₂ sources as greenhouse gases (GHG), whereas biogenic CO₂ is considered as “carbon neutral” (Bajón Fernández et al., 2017). The recent studies also revealed that biogenic CO₂ can be produced in significant quantities. In Europe, an estimated 69.7 Mt/a of CO₂ are produced by biogas upgrading, biogas combustion, bioethanol, and other fermentation processes, in contrast to carbon emissions (437 Mt/a CO₂) from solid biomass combustion (Rodin et al., 2020). There are two main sources of CO₂ emitted from AD plant, i.e., the CO₂ contained in biogas and the CO₂ generated from the combustion of CH₄ in combined heat and power (CHP) engines or flares, which concentrations are ~35% and 8–15%, respectively (Bajón Fernández et al., 2017). For an AD plant with daily treatment
capacity of 100 tonnes biowaste, the average biogas yield is 300 m$^3$/tonne, and the annual biogenic CO$_2$ emission is estimated to be ~0.02 Mt/a CO$_2$. The number of biomethane production plants and commercial biogas upgrading plants worldwide has increased in the recent years. In Europe, there are approximately 577 biogas upgrading plants distributed among the member countries (IEA, 2019). While in China, supporting policies encourage investment in the construction of biogas projects; 172 new biomethane projects are anticipated to be deployed by 2020 (Schimdt et al., 2019).

The CO$_2$ in biogas emission of AD plants was identified as an easily recoverable direct emission due to its higher concentration (Alibardi et al., 2017), and its point source nature reduces capture costs. Currently, physio-chemical methods for biogas upgrading, such as water scrubbers, chemical scrubbers, pressure swing adsorption, and membrane separation are prevailed due to their high level of technology readiness (Allegue et al., 2012; Lemmer et al. 2015; Sun et al., 2015). However, these technologies may cause methane losses of 0.1–8% and increase biogas production costs by 20–72% due to the high operating pressure, electricity demand, or chemical/water requirements (Linville et al., 2016). Thus, the renewable energy-driven carbon capture and utilization of CO$_2$ from green sources is a strategic approach for CO$_2$ mitigation.

The development of biological biogas upgrading technology and CO$_2$ bioconversion process have increased rapidly in recent years, indicating significant potential in reducing the carbon footprint and supporting energy supply decarburisation (Bajón Fernández et al., 2017; Sarker et al., 2018). The present work provides a comprehensive overview of various on-site
CO₂ sequestration methods, in which the innovative reactor configurations, major factors for CO₂ conversion, and energy utilisation efficiency are discussed. In addition, this review summarises the potential challenges for developing biological biogas upgrading technology.

2. CO₂ bio-sequestration: mechanism and assumption

2.1. CO₂ generation pathways during anaerobic process

Theoretically, there are four stages throughout the AD process targeting methane recovery: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Liu et al., 2012; Shin et al., 2010). Table 1 lists the amount of CO₂ produced by the anaerobic digestion system and related operating parameters. In the acidogenesis stage, facultative anaerobic bacteria such as Clostridium, Ruminococcus, Paenibacillus, and Streptococci transform the metabolites produced during the hydrolysis stage into volatile fatty acids (VFAs) such as alcohols, acetate, propionate and butyrate, and concomitantly generate gases, such as H₂, CO₂, and H₂S (Deublein and Steinhauser, 2011; Ziganshin et al., 2013).

**Insert Table 1**

Based on the distribution of major liquid products, acidogenic metabolic pathways are generally classified as, (1) acetate-ethanol type, (2) propionate-type, (3) butyrate-type, (4) mixed-acid, and (5) lactate-type metabolic pathways (Zhou et al., 2019). As shown in Table 1, the cleavage of complex organic matter is always coupled with H₂ and CO₂ as by-products. H₂ production is generally performed by microorganisms belonging to the genera Clostridium, e.g.
C. buytricum, C. thermolacticum, C. pasteurianum, C. paraputrificum and C. bifermantans (Akutsu et al., 2009). Usually, H₂ account for 18.3–50.6% of the total volume produced, while CO₂ ranges from 30.7 to >52% (Zhou et al., 2019). Later, in the acetogenesis phase, microorganisms such as Aminobacterium, Acidaminococcus, and Desulfovibrio, convert intermediate products, such as propionic acid and butyric acid, into acetic acid, CO₂, and H₂.

Finally, in the methanogenic stage, methane is generated in two ways: first, H₂ and CO₂ generate methane through the action of hydrogenotrophic methanogens such as Methanobacteriales, Methanococcages, and Methanomicrobials, and the second is accomplished by aceticlastic methanogens producing methane from acetic acid (Seon et al., 2014). Typically, approximately 30% of the methane compounds of biogas is produced via hydrogenotrophic methanogenesis (HM), this proportion can increase to 40~90% when the digester is directly injected with hydrogen (Luo and Angelidaki, 2012).

2.2. CO₂ bio-sequestration microorganisms and metabolic pathways in AD system

CO₂ sequestration technologies can generally be classified as chemoautotrophic or photosynthetic. Non-photosynthetic microorganisms with CO₂ assimilation ability are placed in archaea (phyla crenarchaeota and euryarchaeota) and bacterial domain, (phyla actinobacter, chloroflexi, chlorobi, firmicutes, proteobacteria, and Thermodesulfobacteria) (Salehizadeh et al., 2020). During the acetogenic process, CO₂ and H₂ are produced along with other metabolites. Meanwhile, there are also some microorganisms with CO₂-assimilating capacity coexisted in AD system. The most studied metabolic pathway is Wolf cycle performed by
hydrogenotrophic methanogens, reducing CO$_2$ and H$_2$ to CH$_4$ (Fig. 1). Another Wood-Ljungdahl pathway is mediated by homoacetogens, in which CO$_2$ is first converted to acetate and then the acetate is utilized by acetylactic methanogenesis to generate CH$_4$ (Thauer, 2012; Fu et al. 2021).

Various hydrogenotrophic methanogens are involved in Wolf cycle, including *Methanosarcinaceae, Methanomicrobials, Methanobacteria*, and *Methanococcales* (Aryal et al., 2018). Previous studies have shown that the injection of exogenous H$_2$ can enhance the activity of hydrogenotrophic methanogens such as *Methanobacterium, Methanoculleus* and *Methanomicrobium* (Agnessens et al., 2018). Exogenous H$_2$ can also modify the structure of archaea dominated by *Methanothermobacter thermotrophicus* (Luo and Angelidaki, 2012a).

Acetogenic mixotrophs, such as *Acetobacterium woodi, C. scatologenes, C. aceticum, C. carboxydivorans, C. ljungdahlii, and Thermoanaerobacter kivi* can also be used to fix CO$_2$ through an anaerobic, non-photosynthetic route (Fast et al., 2015). As shown in Table 1, homoacetogenesis refers to the process in which CO$_2$ is reduced to acetate with H$_2$ via the acetyl-CoA pathway from autotrophic and/or heterotrophic substrate, including sugars, alcohols, organic acids, CO and H$_2$/CO$_2$ (Pan et al., 2021). The Gibb’s free energy of heterotrophic homoacetogenesis ($\Delta G_0^\prime$ = -310.9 kJ/mol) is more negative than that of autotrophic homoacetogenesis ($G_0^\prime$ = -104.6 kJ/mol) (Saady et al., 2013).

In the methanogenic reactor fed with CO$_2$/H$_2$, there are two possible pathways for methane generation: 1) the direct utilization of CO$_2$ and H$_2$ by HM, and 2) the indirect metabolic pathway, where autotrophic homoacetogenic bacteria converts CO$_2$ into acetic acid, and the acetoclastic methanogens converts acetic acid into CH$_4$ (Angelidaki et al. 2018; Aryal et al. 2018). Although
\[ \Delta G_0 \] of hydrogenotrophic methanogenesis (-135 kJ/mol) is more negative than autotrophic homoacetogenesis, autotrophic homoacetogenesis is more favorable under high \( p_{H_2} \) (>500 Pa), due to its higher H\(_2\) threshold concentration (350–700 nM) than hydrogenotrophic methanogens (20–75 nM) (Schink 1997; Montiel-Corona et al., 2020). Thus, homoacetogens can outcompete with hydrogenotrophic methanogens, but interact syntropically with acetoclastic methanogens under high \( p_{H_2} \) for facilitating CO\(_2\) conversion (Aryal et al. 2018).

Furthermore, the ability of some heterotrophic homoacetogens such as *C. ljungdahlii*, *C. autoethanogenum*, *Eubacterium limosum* and *Moorella thermoacetica* etc. for concurrent utilizing organic sugars and inorganic CO\(_2\) is verified (Jones, et al., 2016). By controlled cofeeding of glucose as adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) generators, the CO\(_2\) reduction capacity of *Moorella thermoacetica* is stimulated via mixtrophic pathway (Park et al., 2019). In fructose fermentation, CO\(_2\) emission is completely exhausted when provided with enough H\(_2\) as reductant, indicating the general trait of mixotrophy among acetogens (Jones, et al., 2016). Thus, the mixtrophic capacity of acetogens can be further explored to reduce CO\(_2\) emissions from fermenters.

**Insert Table 2**

**Insert Figure 1**

2.3. CO\(_2\) capture scenarios for single-phase and two-phase anaerobic digestion

Generally, AD configurations are divided into single-phase and two-phase systems. In two-phase system, hydrolytic and acidogenic processes are separated from methanogenesis,
whereas, in a single-phase system, all four stages occur simultaneously in one reactor (Grimberg et al., 2015; Luo et al., 2019). Both single-phase and two-phase digestion system can be incorporated into CO\textsubscript{2} capture technologies (as shown in Fig. 2). Besides biogas upgrading, CO\textsubscript{2} emission reduction from biogas plants and the bioconversion of CO\textsubscript{2} to CH\textsubscript{4} or other metabolites can also be coordinated to promote the overall energy recovery from biogas plant.

**Insert Figure 2**

In single-phase AD, biogas upgrading can be performed via in-situ or ex-situ strategies. In the ex-situ biogas upgrading process, biogas and external H\textsubscript{2} are injected into a separate bioreactor. The H\textsubscript{2} required for CO\textsubscript{2} reduction can be obtained from electrolysis utilising surplus electricity from wind and solar energy, or other alternative sources enriched in H\textsubscript{2} (i.e, coke oven gas: 92% H\textsubscript{2} and 8% CO). Three strategies can be utilised to promote the bioconversion of CO\textsubscript{2} to CH\textsubscript{4} in in-situ biogas upgrading: external H\textsubscript{2} injection, promoted HM via electromethanogenesis, or conductor-mediated direct interspecies electron transfer (DIET) (Angelidaki et al., 2018; Fu et al., 2021). Either Wolf cycle or acetyl-CoA pathway can concurrent in the single-phase CO\textsubscript{2} sequestration process. Generally, the highest biogas upgrading efficiency (~99%) can be expected in the route of external H\textsubscript{2} injection, as the manipulation of gas composition and flow rate is more viable and the regulation of metabolic kinetics is easier (Luo and Angelidaki et al., 2012a). Electromethanogenesis is also effective for CO\textsubscript{2} fixation and biogas upgrading, and it is a sustainable electrons provider if renewable energy power is adopted (Jiang et al., 2013; Zhang et al., 2019). Comparatively, the DIET also
has great potential in CO$_2$ reduction as it’s an efficient way for electrons transportation that
more energy is possibly conserved for metabolite productions (Rodríguez et al., 2008). The
pros and cons of each scenario will be discussed in the following sections.

In the two-phase AD of food waste, off-gas (H$_2$ and CO$_2$) produced in an acidogenic
reactor is usually neglected; however, a recent study reported that this proportion could
constitute up to 30% of the total decomposed organic matter (in terms of COD) (Jones et al.,
2016). The overall methane yield from two-phase AD can be increased utilising the off-gas of
an acidogenic reactor (Clark et al., 2012). When handling the biomass of high solids, VFA
accumulation and gas production stagnation occur when digesters are subjected to a high
organic loading rate or short hydraulic retention time (Oliveira and Doelle 2015). The two-
phase system is more flexible and stable when dealing with an acid crisis (Xu et al. 2017).
Therefore, more research and applications for two-phase AD system should be conducted in
the near future to improve energy efficiency from two-phase AD.

As shown in Fig. 2d and 2e, to harnessing the energy of H$_2$ and CO$_2$ from the acidogenic
reactor could be an efficient way to boost up the feasibility of two-phase technology. Off-gas
(H$_2$ and CO$_2$) harvested from the first acidogenic reactor can be converted to CH$_4$ in the second
methanogenic reactor via HM process (Salomoni et al., 2011). Nevertheless, related practice
showed that the ratio between autogenic H$_2$ and CO$_2$was not enough for efficient reuse in
methanogenic reactor, as the theoretical H$_2$/CO$_2$ ratio of 4 is required for HM reaction (Yan et
al., 2017). Alternatively, the acidogenic fermentation parameters (e.g. partial pressure of H$_2$ and
CO$_2$, pH) can be manipulated to control the product distribution favorable for methanogenesis
(Yan et al., 2016, 2020). Approximately 30% increment of \( \text{CH}_4 \) yield was obtained in the two-phase system with the headspace pressure manipulation (3~6 psi) in the acidogenic phase by autogenic \( \text{H}_2 \) and \( \text{CO}_2 \) (Yan et al., 2017). Until now, only a few papers have demonstrated the effectiveness of this approach, and more studies are needed to focus on parameter optimization and metabolic pathways for AD system with synergistic substrate cofeeding (i.e. organic substrate and inorganic \( \text{CO}_2 \)).

3. \( \text{CO}_2 \) bio-sequestration technology

As indicated above, \( \text{H}_2 \) represents a key intermediate in the \( \text{CO}_2 \) reduction to \( \text{CH}_4 \) process, working as an electron donor in the Wolf cycle and Wood-Ljungdahl pathway. Thus, several research groups have assessed the possibility of enhancing \( \text{CH}_4 \) production from AD by \( \text{CO}_2 \) reduction with \( \text{H}_2 \) injection (Luo and Angelidaki 2012a; Bassani et al. 2015; Corbellini et al., 2018). This kind of process can be classified as “exogenous \( \text{H}_2 \) mediated \( \text{CO}_2 \) bioconversion to \( \text{CH}_4 \)”.

However, the high cost and the low water solubility of \( \text{H}_2 \) hinder the full exploitation of \( \text{CO}_2 \) bioconversion into \( \text{CH}_4 \) at AD sites. To overcome these limitations, an alternative approach could be the strengthened bioconversion of \( \text{CO}_2 \) into \( \text{CH}_4 \) in an AD reactor without the addition of exogenous \( \text{H}_2 \). Several biological conversion technologies of \( \text{CO}_2 \) into \( \text{CH}_4 \) in the presence or absence of exogenous \( \text{H}_2 \) are reviewed and discussed here: (i) exogenous \( \text{H}_2 \) mediated \( \text{CO}_2 \) bioconversion to \( \text{CH}_4 \), (ii) the utilisation of acidogenic off-gas for \( \text{CO}_2 \) fixation, (iii) electromethanogenesis with a microbial electrolysis cell (MEC), (iv) a DIET-strengthened methanogenic reactor, and (v) AD reactor with \( \text{CO}_2 \) sparging.
3.1 Exogenous H$_2$ mediated CO$_2$ bioconversion to CH$_4$

The H$_2$-mediated bioconversion process of CO$_2$ to CH$_4$ can be generally classified into ex-situ and in-situ (Adnan et al., 2019). Ex-situ biogas process means that the collected biogas is transferred to an external closed reactor, and CO$_2$ is biologically converted to CH$_4$ by injecting H$_2$ or other reagents (Baena-Moreno et al. 2019). In-situ process increases CH$_4$ content by regulating the operation conditions (e.g. H$_2$ injection) in the original anaerobic digester (Kougias et al. 2017). The in-situ and ex-situ here refer to the two forms of biogas upgrading, and the on-site CO$_2$ bio-sequestration mentioned later means that the CO$_2$ produced is directly used on the basis of the original process equipment, which refers to how CO$_2$ is harvested and used.

The advantages of ex-situ biogas upgrading depend on the reactor’s stability, where the exhausted CO$_2$ from other sources such as flue gas and syngas can also be utilised (Angelidaki et al. 2018). More than 95% of the CH$_4$ content can be obtained through ex-situ biogas upgrading (Adnan et al., 2019). The primary shortcoming of ex-situ biogas upgrading is the additional reactor maintenance cost. As biogas upgrading requires additional purchase of related supporting equipment and chemicals, it is estimated that this part of the cost can only be offset when the biogas production capacity exceeds 100 m$^3$/h (Lindeboom et al., 2011). A major advantage of the in-situ technique is that it can be employed in existing biogas plants and the current natural gas infrastructure for H$_2$/CO$_2$ injection and biomethane utilisation, thus, the cost of in-situ biogas upgrading is much lower, attracting much attentions in recent years (Mulat et al., 2017; Fu et al., 2021). Nevertheless, the application of this in-situ conversion
technology thus far is limited to lab-scale studies due to its low conversion rate (Table 2 and Table 3).

**Insert Table 2, Table 3**

The exogenous H\textsubscript{2} injection into the AD digester, can affect the parameters of digester and the composition and activity of syntrophic microbial community, such as pH increase due to the CO\textsubscript{2} removal and process inhibition due to higher p\textsubscript{H2}. Moreover, due to the low solubility of H\textsubscript{2}, a technical challenge is the limitation of H\textsubscript{2} transfer-rate to the liquid fraction (Wahid et al., 2019). Thus, technical challenges associated with the biogas upgrading include the optimization of biogas upgrading reactor’s configuration and various environmental factors, such as temperature, gas residence time, and pH value, play a vital role in the (Corbellini et al., 2018; Wahid et al., 2019).

3.1.1 Reactor configurations

Due to the low solubility of H\textsubscript{2}, one of the most important issues in biological biogas upgrading is the limited gas-liquid mass transfer rate of H\textsubscript{2} (Park et al., 2021). Apart from continuous stirred tank reactors (CSTRs), there are several types of biogas upgrading reactors have been developed to solve the gas-liquid mass transfer rate limitations of H\textsubscript{2}, including biotrickling filters (BTFs), bubble column reactors (BCRs) and membrane reactors (MRs), their schematic diagrams and performances are shown in Fig. 3 and Tables 2-3.

**Insert Figure 3**

In the BTF and BCR reactors, various carrier materials (e.g. polypropylene and glass rings) with large specific surface areas have been packed to provide sufficient attachment sites for
microorganisms to grow and multiply (Burkhardt et al., 2015). Moreover, the reactive groups on the microorganism’s surface interact with the carrier material to be firmly fixed to the material’s surface, thereby avoiding biomass loss (Burkhardt et al., 2019). As shown in Table 2, by improving the reactor configuration, high H₂ and CO₂ bioconversion rates have been achieved in ex-situ biogas upgrading, i.e. ~98% in BCR reactors (Kougias et al., 2017) and 95% in ceramic membrane biofilm reactors (Alfaro et al., 2018). The CH₄ production rate also varied among different reactors, with the highest rate found in ceramic membrane biofilm reactor as 0.22 m³CH₄/m³H₂ (Alfaro et al., 2018).

For in-situ biogas upgrading, the injection of exogenous H₂ can potentially improve methane content to 75–86% in a continuously operated CSTR (Corbellini et al., 2018) and 89%-94% in a batch reactor (Mulat et al., 2017). Despite the significant enhancement, some limiting factors remain in the in-situ biocoversion of CO₂ to CH₄. For example, excessive H₂ injection might yield reduced VFAs degradation efficiencies, leading to the stagnation of methane generation (Agnéessens et al., 2017). Although research on biogas upgrading technology is being conducted based on different reactor structures, as long as the reaction process can be adjusted in the most suitable environmental range, considerable upgrading effects can be obtained specifically in practical applications. The type of reactor used in the process needs to be determined according to the specific economic and environmental conditions.

3.1.2 Temperature

Among various environmental factors, operating temperature is considered as the critical factor controlling the conversion rate of CO₂ and H₂ in AD digester (Xu et al. 2020a). For
example, Bassani et al. (2015) found that 89% of CH$_4$ content could be achieved under mesophilic conditions and further increased to 95% under thermophilic conditions (Luo and Angelidaki, 2012b). Guneratnam et al. (2017) also investigated ex-situ biological methanation operation at two thermophilic temperatures (55 °C and 65 °C) yielding 85% -88% CH$_4$ content. Hydrogenotrophic methanogens have higher competence in thermophilic reactors and are thus, preferable for microbiological ex-situ biogas upgrading process with H$_2$ (Hao et al., 2013; Guneratnam et al., 2017). The high abundance of *Methanothermobacter wolfeii*, comprising 85% of the archaeal community, indicates a likely and resilient candidates for thermophilic ex-situ biogas upgrading (Guneratnam et al., 2017). Although a better upgrading efficiency can be achieved under thermophilic conditions, the mesophilic reactor’s stability was greater in the comparative study (Xu et al. 2020a), indicating the suitability of mesophilic reactor for in-situ biogas upgrading process.

Higher temperature can also accelerate the mass transfer of H$_2$ and CO$_2$, however, the solubility of H$_2$ and CO$_2$ are adversely affected. Higher utilization efficiencies for H$_2$ have been found in mesophilic reactors, whereas their CO$_2$ utilization rate are relatively low (Luo and Angelidaki, 2012b). Although the higher diversity of microbial communities is found in mesophilic reactors, it is difficult to determine which microorganisms are the most significant. As such, this might be a focus for future research.

### 3.1.3 H$_2$ injection rate and dispersion method

The bioconversion rate of CO$_2$ is limited by the poor solubility of H$_2$ in the aqueous phase, as only the dissolved H$_2$ in liquid can be utilised by the microorganisms. Therefore, the extent of
bioconversion strongly depends on the transformation efficiency of gaseous H\textsubscript{2} into liquids (Guiot et al. 2011). The H\textsubscript{2} liquid mass transfer rate is typically expressed as follows (Fu et al., 2020),

\[ r_t = 22.4 k_{La}(H_{2gTh} - H_{2l}) \]

where, \( r_t \): H\textsubscript{2} liquid mass transfer rate (L/(L/d))

22.4: gas-volume-to-mole ratio (1 mol of gas corresponds to 22.4 L of gas at standard temperature and pressure)

\( k_{La} \): gas transfer co-efficient (per day)

\( H_{2gTh} \): H\textsubscript{2} concentration in the gas phase (mol/L)

\( H_{2l} \): H\textsubscript{2} dissolved in the liquid phase (mol/L)

As this equation suggests, \( r_t \) can be enhanced by increasing \( k_{La} \). Several attempts have been made to improve \( k_{La} \) including the modulation of the mixing speeds and gas recirculation (Alitalo et al. 2015), changing the diffusion device (Bassani et al. 2016; Alfaro et al., 2018), adding packing materials (Burkhardt et al., 2015).

Although prolonging the gas residence time promotes the utilisation rate of H\textsubscript{2}, the residence time dependent on the reactor type. For the ex-situ biogas upgrading with BTF reactors, Burkhardt et al. (2015) found that limiting the injection gas (H\textsubscript{2}/CO\textsubscript{2}=3.76) retention time to 2.25 h yield a 94-99% H\textsubscript{2} conversion efficiency. Similarly, Rachbauer et al. (2016) reported that when the gas (H\textsubscript{2}/CO\textsubscript{2}=3.67–4.17) retention time was controlled at 2.3 h, the CH\textsubscript{4} content of the biogas reached up to 96%. In the newly developed biofilm plug flow reactor, the retention time could be reduced to 0.16–0.24 h (100–150 v/v/d), and a 90-99% conversion
efficiency is obtained for the total gas throughputs (Savvas et al., 2017). The volumetric
capacity can be significantly improved with a shortened residence time. When the gas recycling
rate reaches 12 $L/h$, high-quality biogas with a methane content of >98% was obtained in the
BCR reactor (Voelklein et al. 2019). In a BTF reactor with gas recirculation, the $H_2$ injection
rate could be adjusted to 25.2 $L/L/d$ (Alitalo et al. 2015).

When exogenous $H_2$ is injected into the AD of food waste for in-situ biogas upgrading, it
can promote the enrichment of hydrogenotrophic methanogens. Zabranska and Pokorna (2018)
reported a $CH_4$ content increased from 27.6% to 71.7% after injecting exogenous $H_2$. In the in-
situ biogas upgrading process with sewage sludge as feedstock, $H_2$ utilization efficiency can be
increased to >94% and the $CH_4$ content of off-gas can be increased to 73% when the gas
recycling rate reaches 202 $L/L/d$ (Alfaro et al. 2019). However, the negative impact of the rate
and amount of $H_2$ injected on the reactor’s stability should be investigated.

3.1.4 Impact of $H_2$ injection on the system’s stability

Apart from the limited conversion rate, the adverse impact on the stability of the $CO_2$-$CH_4$
bioconversion system should also be considered, that is the acidic pH and inhibition of
syntrophic metabolism due to the accumulated $H_2$.

In biogas upgrading processes, the depletion of $CO_2$ by hydrogenotrophic methanogens
tends to increase the reactor’s pH, consequently leading to the inhibition of the microbial
community (Kadam and Panwar, 2017). To balance the pH buffer system, the stoichiometric
ratio between $H_2$ and $CO_2$ should be carefully optimised. Agnesens et al. (2017) reported that
$CO_2$ content in the headspace lower than 12% inhibits the activity of hydrogenotrophic
methanogens, reducing methane production. This is consistent with the conclusion of Voelklein et al. (2019) that the bioconversion rate of CO$_2$ to CH$_4$ decreased when the concentration of CO$_2$ was lower than 9%. Generally, H$_2$/CO$_2$ ratio of around 4:1 was estimated as the optimal ratio for H$_2$ injection in biogas upgrading processes (Burkhardt et al., 2015; Rachbauer et al., 2016). Alternatively, H$_2$ addition has been suggested as a co-digestion remedy for acidic substrates to elevate the pH. Luo and Angelidaki (2012a) investigated the co-digestion of manure and whey by adding H$_2$, their results showed that the reactor pH could be maintained below 8. Therefore, reactor pH can also be regulated by the co-digestion with other readily acidified substrates (e.g. food waste).

Furthermore, H$_2$ has a direct influence on products and reactants at different AD stages. Theoretically, the syntrophic oxidation of VFAs (e.g. propionate and butyrate) is thermodynamically favourable only when the $p_{H2}$ is reduced to 2.6 and 74 Pa, respectively (Zabranska and Pokorna 2018). Thus, direct H$_2$ injection into the anaerobic reactor via in-situ biogas upgrading may pose a significant risk to the digestion system by increase of $p_{H2}$, inhibiting the accumulation of syntrophic bacteria, propionate, and butyrate, and in the worst-case, causing process failure (Cazier et al. 2015; Agneessens et al. 2017).

Nevertheless, H$_2$ injection can also stimulate the production of acetate by the homoacetogenic bacteria via the Wood–Ljungdahl reaction. Recent studies have found that the cooperation between homoacetogens and acetoclastic methanogens increases the overall CH$_4$ yield (Fu et al., 2021). HM and homoacetogenesis are affected and regulated by many factors, including $p_{H2}$, temperature, and other reactor operating parameters. In an efficient AD system
with an in-situ biogas upgrading setup, there is a collaborative symbiosis among the syntrophic bacteria, homoacetogens, and hydrogenotrophic/acetoclastic methanogenesis. However, the impact of $H_2$ injection on the carbon flow among various $H_2$-utilizing metabolic pathway not sufficiently understood, and more research is required in this area.

3.2. Utilisation of acidogenic off-gas

When two-phase AD is used to treat organic waste, the biogas generated by the acidogenic phase during the preliminary hydrolysis and fermentation of food waste is mainly composed of $H_2$ and $CO_2$. Previous studies have pointed out that over one-third of sugar carbon is lost as $CO_2$ due to the decarboxylation of pyruvate to acetyl-CoA (Jones et al., 2016), and the energy carried by $H_2$ in the acidogenesis accounts for up to 30% of the total recovered energy (Alfaro et al. 2018). In previous experiments, this part of $H_2$ was wasted because of factors such as actual operation and gas-liquid mass transfer. Furthermore, as the hydrolysis and fermentation processes in the acidogenic reactor continue, $CO_2$ and $H_2$ continue to build up in the reactor headspace. Previous research has shown that excessively high $p_{H2}$ can have a more severe inhibitory effect on the hydrolysis fermentation and acidogenesis processes (Huang et al., 2016; Yan et al., 2017), inhibiting the production of $H_2$. Thus, the in-situ consumption or diversion of $H_2$ is necessary to improve the hydrolysis rate and convey electron donors for methanogenic processes.

Emerging technologies inject acidogenic off-gas ($H_2$ and $CO_2$) into the methanogenic reactor to improve the final biogas quality. Research on diverting off-gas into methanogenic
reactors has resulted in a 38.6% methane yield increase (Yan et al., 2016). Additionally, the potential of in-situ CO$_2$ fixation in the acidogenic phase has also been explored. Salomoni et al., (2011) investigated the injection of CO$_2$ into the fermentation phase of a pilot-scale two-phase AD plant, where off-gas from the fermentation phase was recirculated into the methanogenic phase to sustain CO$_2$ reduction, an overall 25% increase in CH$_4$ yield was observed. Maintaining the headspace pressure of the acidogenic phase (3~6 psi) by autogenic H$_2$ and CO$_2$ could optimise the acidogenic product, leading to an indirect improvement in the CH$_4$ yield of 10–30% (Yan et al., 2017). In addition, different types of substrates have been digested in a two-phase AD system to test the H$_2$ production and reutilization potential (Yan et al., 2020). In this study, under a headspace pressure of ~ 3.3 psi in LBR, favourable hydrolysis and acidification with reused acidogenic off-gas ensured the highest specific CH$_4$ production of 0.42 L/g·VS$_{\text{added}}$.

Compared to external H$_2$, the source of H$_2$ is self-sufficient, and only the gas transfer system needs to be set in the reactor, which is relatively simple and cost-effective. However, a disadvantage is that the H$_2$ yield from the acidogenic phase is limited, which is hard to meet the optimal H$_2$/CO$_2$ ratio of 1:4 (Bassani et al. 2017). Thus, CO$_2$ in off-gas cannot be utilized completely and the increase in methane concentration is less than that of other technologies (Alfaro et al. 2018). Additionally, if sporadic H$_2$ injections are performed, a significant fraction of the consumed H$_2$ is converted to acetate by homoacetogenesis; at a higher injection rate the abundance of hydrogenotrophic methanogens increases, reducing the possibility of acetate accumulation (Agneessens et al., 2018). Therefore, follow-up research should focus on
maintaining the optimal H₂/CO₂ ratio using internal H₂. Internal H₂ technology can be combined with other technologies, such as the pressure reactor technology as described below, to achieve the simultaneous increase in methane production and content.

3.3 Electromethanogenesis with MEC

Emerging technology for converting CO₂ into methane through microbial electrolytic cells (MECs) has shown significant potential in the last decade (Jiang et al., 2013; Zhang et al., 2019). In MECs, external energy is supplied to promote a thermodynamically nonspontaneous reaction, such as bioelectrochemical CO₂ conversion into CH₄ (known as electromethanogenesis). The most critical components in the electromethanogenesis process are hydrogenotrophic methanogens developed in the MEC cathode compartment (i.e. biocathode) (Cerrillo et al. 2017). There are two different mechanisms of extracellular electron transfer involved in the CO₂ to CH₄ bioconversion in MEC:

(i) Indirect conversion, where H₂ is formed from free protons through intermediate abiotic electrochemical and/or microbial catalysis in the cathodic compartment, and then utilised by hydrogenotrophs to generate CH₄.

(ii) Direct conversion, CH₄ is formed via electromethanogenesis by taking the electrons from the cathode:

When utilised as a biogas upgrading technology, MECs are essential as a sustainable H₂ provider for HM. Apart from the ex-situ biogas upgrading (using CO₂ as feedstock), MEC can also be introduced into an AD with a complex substrate. By inserting electrodes used in a MEC
into an AD system and applying voltage to generate electrical simulations and hydrogen gas, the MEC-assisted AD system (MEC-AD) has been proved to improve the overall AD performance. MEC-AD system use exoelectrogenic bacteria on the anode are to oxidize organic substrate and reduce VFA concentrations, while the generated electrons are utilised by the biocathode in the electromethanogenesis process (Holmes and Smith 2016; Lu and Ren 2016; Angelidaki et al. 2018). The cathode potential required to enhance the electromethanogenic process is usually ranges from -0.4 to -1.4 V (vs the standard hydrogen electrode) (Cerrillo et al., 2017). At more negative potentials, acetate may also be produced simultaneously with CH₄ and H₂ in a microbial biocathode based on mixed cultures (Jiang et al., 2013). In the electro-fermentation system, VFA production from complex substrates can be increased by the in-situ reuse of anodic off-gases (mainly CO₂ and H₂) in the cathode (Zhou et al., 2019).

With the biogas upgrading process in MEC, the CH₄ purity of the biogas can be elevated to 83% at a flow rate of 1 m³/m³/d (Cerrillo et al., 2018). The biogas conversion rate and energy efficiency can be further increased by improving the configuration and operation of the MEC reactor. For example, 99% of the energy was recovered by incorporating a polarisation strategy in the MEC (Zeppilli et al., 2019). A 99–100% methane purity can be obtained in a microbial electrochemical separation cell, however, a high biogas rate injection adversely affects the upgrading efficiency (Kokkoli et al., 2018). The CH₄ yield could be increased by ~30% for the MEC-AD reactor when transferring AD to MEC-AD under an applied voltage of 0.5 V (Xu et al., 2019). Bioelectrodes can alleviate ammonia inhibition for VFA degradation in anaerobic digester in the MEC-AD system, thus increasing the CH₄ yield (Luo et al., 2016).
In general, MEC technology can achieve very significant effects for biogas upgrading. Among various in-situ biogas upgrading technologies, the configuration of MEC-AD is relatively simple, and its reaction process can consistently provide sustainable \( \text{H}_2 \) with relatively high energy recovery; thus, it has very significant development prospects. However, the electron transfer mechanisms of MEC technology have not been fully clarified, and low cathode potential and energy efficiency limit its application. Furthermore, the current research on MEC is still limited to a laboratory size, and much work is need before its actual production. Therefore, amended MECs technology in AD process can be used to achieve the goal of biogas upgrading. Among various in-situ biogas upgrade technologies, the combination of MEC and AD presents more advantages than others: the configuration of MEC-AD is simple, and bio-hydrogen reaction is sustainable, and the energy recovery efficiency is high. However, the electron transfer mechanisms of MEC technology are not fully clarified. The finitude of cathode potential and energy efficiency limit its application. Furthermore, present studies regarding MEC are still in lab-scale, to apply in practical industry, it needs more efforts.

### 3.4 DIET-strengthened methanogenic reactor

In recent years, DIET has emerged as an important electron pathway for incorporated reactions in AD and anaerobic papers, and studies on its mechanisms have greatly increased (Lee and Lee, 2019; Tsui and Wang, 2019). Carbon materials and nano-iron materials serve as intermediate to accelerate the electron transfer between electron donors and acceptors by their cyclic transformation of oxidation and reduction states (Charalambous and Vyrides, 2020; Pan
et al., 2021; Wang et al., 2021). In an AD digester supplemented with biochar, the methane production lag time was shortened and the methane yield was increased under a high organic load (Yang et al., 2020).

The major mechanisms involved in a DIET-strengthened AD system are enhanced direct electron transfer in syntrophic acetate oxidation-HM (SAO-HM) dominated systems (Fig. 6). SAO-HM has reportedly become the dominant pathway to overcome acetate accumulation from initially acetoclastic methanogenesis dominant anaerobic systems under stressed environments, i.e. high organic loading rates or ammonia stress (Sun et al., 2014). In the DIET-strengthened SAO-HM systems, $p_{H2}$ is reduced, and the overall reaction rate is increased (Pan et al., 2021). Thus, DIET can simultaneously promote the conversion of CO$_2$ into methane by accepting electrons from conductor or capacitive conducting material, and acetate cleavage by acetoclastic methanogens due to reduced $p_{H2}$ (Charalambous and Vyrides, 2020). Moreover, anaerobic fermentative reactions proceed close to thermodynamic equilibrium, and the anaerobic microbial ecosystems are generally under energy-limited conditions (Rodríguez et al., 2008). If multiple electron transfer routes occur at the same time, the energy-based ecological niche of microorganism could be widened, and more energy would be conserved for metabolite productions. Thus, it is essential an opportunity to enhance CO$_2$ fixation/conversion efficiency in AD systems with DIET strengthening strategies.

Nevertheless, in most studies, the effects of DIET on the methanation of VFAs and other complex substrates are focused, whereas the conversion potential of CO$_2$ reduction in DIET-strengthened reactor has got less attention (Yang et al., 2020). A recent study reported that the
methane production rate from CO\textsubscript{2} and H\textsubscript{2} was increased by 20-70% in methanogenic reactor supplemented with biochar (Ren et al., 2020). It is worthy to further discuss the effect of biochar (or other mediators) supplementation on the digester cofeeding CO\textsubscript{2} and organic substrate. Furthermore, the microorganism involved in DIET and the effective bioaugmentation strategies on in-situ CO\textsubscript{2} fixation require more exploration. In most studies, \textit{Geobacter} species (e.g. \textit{G. metallireducens} or \textit{G. hydrogenophilus}) were identified as electron-donating bacteria in the DIET-mediating methanogenic reactors; \textit{Thauera}, \textit{Corynebacterium}, \textit{Spirochaeta}, \textit{Clostridium}, \textit{Coprothermobacter}, and \textit{Syntrophomonas} species have also been identified (Pan et al., 2021). \textit{Methanosaeta}, \textit{Methanosarcina} and other methanogens (such as \textit{Methanospirillum}, \textit{Methanobacterium}, \textit{Methanolinea}, \textit{Methanothrix} and \textit{Methanoregula} species) have been identified in the DIET-stimulating methanogenic reactors (Rotaru et al., 2014; Ma et al., 2019; Baek et al., 2021). The simulating effect of biochar on DIET in AD process is associated with the abundance of surface oxygen-containing functional groups on biochar, which is helpful for promoting the syntrophic anaerobic metabolism, substrate hydrolysis and VFAs consumption rate (Xu et al., 2020b). The capacitive properties of biochar might be associated with the selectively attached microorganism (Xu et al., 2020c). Nevertheless, the interaction between conducting materials and microbial metabolism and the interfacial electron transport mechanism in AD system are still unclear, which requires further study.

\textbf{3.5. AD reactor with CO\textsubscript{2} sparging}
Recently, the strategy of gas sparing during AD fermentation has attracted the attentions of some researchers. As gas mixing efficiently improves the uniform distribution of the substances within digesters, it significantly enhances the biological reactions and intensifies the production of CH$_4$ (Boontawee et al., 2016). However, the strategy of gas sparging should be carefully maintained, as the overall effect is complicated by multiple intermediate reaction steps and the bacteria’s relative populations, facilitating each step (as shown in Fig. 1).

### 3.5.1. Reduced $p_{H_2}$ in CO$_2$ sparging reactor

Thermodynamically, syntrophic propionate and butyrate oxidation ($\Delta G^0$ = +76.1 kJ and 48.3 kJ/mol, respectively) are extremely unfavourable, thus, low $p_{H_2}$ is essential for VFA degradation (Xu et al., 2020b). Values of $p_{H_2} < 6 \times 10^{-4}$ bar positively affect the reaction feasibility of syntrophic VFAs oxidation, whereas higher values will reduce the feasibility “niche” (Ceron-Chafla et al., 2020). Usually, the low $p_{H_2}$ is maintained by timely hydrogenotrophic consumption, nevertheless the activity of hydrogenotrophic methanogens might be suppressed under unfavorable conditions such as acid crisis. Therefore, it may achieve the goal of reducing $p_{H_2}$ through alternative regulation methods. For example, the positive effect of recirculating H$_2$-removed biogas into AD digester has been proved (Hao et al., 2013). In recent years, promising results have also been reported in increased CH$_4$ production with CO$_2$ injection/sparging in lab-scale and pilot-scale AD systems (Al-mashhadani et al., 2016; Alibardi et al., 2017). Al-mashhadani et al. (2016) found that less CH$_4$ was produced in the N$_2$ sparging reactor than CO$_2$ sparging, because CO$_2$ and H$_2$ are stripped during N$_2$ sparging, which are necessary substrate for other bacteria involved in methane production. CO$_2$ enrichment
increased CH₄ production by ~12% and a production rate of 371 L/kgVS/d in a pilot-scale continuous anaerobic digester treating sewage sludge (Alibardi et al., 2017). Under the changing $p_{CO_2}$ and $p_{H_2}$ conditions, a new thermodynamic equilibrium is established, further modifying the biochemical energy distribution among partners such as acetogens and methanogens (Ceron-Chafla et al., 2020).

3.5.2. Potential electron donors for CO₂ reduction in CO₂ sparging reactor

Although the positive results are reported for the AD reactors with CO₂ injection, in most studies, the accompanying electron donors were not highlighted (Li et al., 2017). Some scholars hold that the activities of homoacetogens and hydrogenotrophic methanogens can be stimulated by CO₂ injection; homoacetogens can reduce CO₂ into acetic acid, which is further transformed into CH₄ by acetoclastic methanogens (Liu et al., 2012) or through syntrophic acetate oxidation followed by HM (Schnürer and Nordberg, 2008). Alimahmoodi and Mulligan (2008) suggested the possibility of utilising VFAs as electron donors for hydrogenotrophic methanogens to convert CO₂ to CH₄, as evidenced by a 69–86% CO₂ uptake. *Methanosarcinaceae*, a family of acetoclastic methanogens, gradually became the dominant strain after the injection of CO₂ and the CH₄ production increased by 20%, indicating that exogenous CO₂ injection could enhance homoacetogenesis through Wood-Ljungdahl pathway, leading to the formation of acetic acid and thereby enhancing acetoclastic methanogenesis (Bajón Fernández et al., 2019). As discussed in Section 2.1, the activities of heterotrophic/mixtrophic homoacetogens might be stimulated in the CO₂ sparging reactor, which help to increase the acidogenic efficiency and
enhance the overall CH$_4$ production. Nevertheless, the complex mechanism requires further validation.

### 3.5.3. CO$_2$ pressurized methanogenic reactor

Owing to the higher solubility of CO$_2$ and other undesirable gas components (such as NH$_3$) compared to CH$_4$, the CH$_4$ content in the gaseous phase can be increased in pressurized methanogenic reactors. Thus, it is proposed as a beneficial strategy for biogas purification and compression (Merkle et al., 2017). For example, when increasing the pressure from 10 to 50 bar in a two-phase anaerobic reactor, the CH$_4$ content of the biogas increased from 79.08% to 90.45% (Merkle et al., 2017). Chen et al. (2014) also found that biogas upgraded from 66% to 75% of CH$_4$ when pressure was increased from 1.07 to 8.91 bar. The impact of moderate $p_{CO2}$ on methanogenesis has also been observed in oil reservoirs. Under the operational conditions of 50 bar pressure, a temperature of 55 °C and 10% $p_{CO2}$ resulted in a shift from syntrophic acetate oxidation to acetoclastic methanogenesis (Mayumi et al., 2013). However, this method also poses a problem: as more and more CO$_2$ is dissolved in the solution, the pH value in the reactor decreases significantly, causing the methanogens activity and stabilising the reactor’s negative effects. Lemmer et al. (2017) found that, despite a decrease in pH from 7.0 (1 bar) to 6.31 (10 bar) and 6.25 (30 bar), there was no significant influence on the degradation of organics and specific methane yield. These intriguing results suggest the possibility of enhancing the overall CH$_4$ production via CO$_2$ injection under regulated headspace pressure.

Nonetheless, further studies are required to clarify the bacterial tolerance and stress response
to headspace pressure, e.g. the selectively enriched high CO\textsubscript{2} pressure-tolerant strains and pressure-tolerant strains, the changes of enzymatic activity.

4. Perspective and outlooks

On-site CO\textsubscript{2} bio-sequestration is a novel and effective method to reduce GHG emissions from AD plants. Although successful lab-scale and pilot-scale demonstrations have already been made in for biological biogas upgrading process with H\textsubscript{2} injection, the inadequate understanding of in-situ CO\textsubscript{2} bioconversion and reaction mechanisms hampers the efficient CO\textsubscript{2} conversion rate and maintenance of the process’s stability.

The advantages and disadvantages of different on-site biological biogas upgrading technologies are compared in Table 4. Generally, the higher biogas upgrading efficiency can be realized in the scenario with exogenous H\textsubscript{2} injection, as the regulation of metabolic kinetics is more viable via manipulating gas injection rate. Nonetheless, the cost of H\textsubscript{2} is about 9 €c/kWh (considering that ~13.3 kWh can be generated from 1 kg H\textsubscript{2}), which is more expensive than the operation cost of 7€c/kWh in physiochemical removal of CO\textsubscript{2} in biogas (Angelidaki et al., 2018). Currently, as the cost of H\textsubscript{2} is judged to be dominating in the biological hydrogenation process, the total cost of biogas upgrading with external H\textsubscript{2} injection is hardly to be covered from the increased energy amount in upgraded biomethane. Thus, the technology of using H\textsubscript{2} to achieve CO\textsubscript{2} bio-sequestration should focus on the long-term economic benefits. Various countries are gradually increasing their support for renewable energy plants, such as feed-in tariffs and tax exemptions, and energy policy (Nguyen et al., 2021). It is expected that the cost of green H\textsubscript{2} will be consistently decreasing, and the maturity and total amortized investment of
The H₂-injection biogas upgrading technology can be reduced by scaling.

The alternative H₂ sources or endogenous reductant should also be explored to promote the sustainability and economics of this technology. Electromethanogenesis is also an effective way to provide electrons for CO₂ fixation, especially when renewable energy power is adopted (Zhang et al., 2019). Meanwhile, simultaneous CO₂ reduction and enhancement of methane yield can be realized in the DIET strengthened digesters (Xu et al., 2015; Shen et al., 2021). Nonetheless, the electron transfer mechanisms and exchange among various CO₂-fixation systems are still unclear, and more studies are required for the influence of mediators on the interactions between homoacetogenes and methanogens. The flow of carbon and reductant electrons needs to be clarified in-depth and more comprehensively to further reveal the mechanisms of CO₂ conversion to CH₄ and the pathways to use electrons.

Moreover, to promote the overall efficiency and reduce the production costs of bio-based products through CO₂ fixation, the selection and manipulation of feasible metabolic pathways is necessary. Some intriguing results are obtained from the CO₂ fixation in pressurized reactor (Chen et al., 2014; Lemmer et al., 2017) and mixtrophic cofeeding reactor (Jones, et al., 2016; Park et al., 2019). The landmark changes might be occurred by utilizing the specific microorganisms, e.g. extremely thermophilic strains, mixotrophic strains, high tolerance strains to CO₂ pressure and headspace pressure etc. Furthermore, the development of enabling technologies such as metabolic engineering, genetic engineering, and synthetic biology tools is be required.

5. Conclusion
Simultaneous CO\textsubscript{2} reduction and enhancement of CH\textsubscript{4} production in biogas plants is a new way to cope with GHG reduction and sustainable bioenergy demand. Wolf cycle and acetyl-CoA pathways are the main mechanisms utilized for on-site CO\textsubscript{2} bio-sequestration. Apart from exogenous H\textsubscript{2} injection, the alternative H\textsubscript{2} sources or endogenous reductant should be explored to promote the bioconversion rate of CO\textsubscript{2} to CH\textsubscript{4}, e.g. electron supplied with electromethanogenesis or strengthened DIET. Moreover, intensive work is required for understanding the interaction among symbiotic bacteria and their metabolism under changing \(p_{CO2}\) and \(p_{H2}\) conditions, to ensure the stability of reactors.

Acknowledgement

The authors of this study would like to thank the financial support from National Natural Science Foundation of China (51978595).

Declaration on conflict of interest

We declare that there is no conflict of interest.

References


32


Grimberg, S. J., Hilderbrandt, D., Kinnunen, M., Rogers, S., 2015. Anaerobic digestion of food waste through the operation of a mesophilic two-phase pilot scale digester--assessment of


Xu, J., Bu, F., Zhu, W., Luo, G., Xie, L., 2020a. Microbial consortiums of hydrogenotrophic methanogenic mixed cultures in lab-scale ex-situ biogas upgrading systems under different conditions of temperature, pH and CO. Microorganisms 8(5), 772.


### Figures and Tables

Table 1. Metabolic pathways involved CO\(_2\) generation and conversion in AD (Zhou et al., 2019; Pan et al., 2021)

<table>
<thead>
<tr>
<th>Metabolic stage</th>
<th>Acetate-ethanol type</th>
<th>(\Delta G^0)(^+) kJ/mol</th>
<th>Eq.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acidogenesis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetogenesis</td>
<td>(\text{Acetate-ethanol type} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\text{C}_6\text{H}_12\text{O}_6 + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH} + 2\text{CO}_2 + 4\text{H}_2)</td>
<td>-135.6</td>
<td>(1)</td>
</tr>
<tr>
<td></td>
<td>(\text{C}_6\text{H}_12\text{O}_6 + 4\text{H}_2\text{O} + 2\text{NAD}^+ \rightarrow 2\text{CH}_3\text{COO}^- + 2\text{HCO}_3^- + 2\text{NADH} + 2\text{H}^+)</td>
<td>+215.7</td>
<td>(2)</td>
</tr>
<tr>
<td></td>
<td>(\text{C}_6\text{H}_12\text{O}_6 + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COO}^- + 2\text{HCO}_3^- + 2\text{NADH} + 2\text{H}_2)</td>
<td>+234.8</td>
<td>(3)</td>
</tr>
<tr>
<td><strong>Propionate-type</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\text{C}_6\text{H}_12\text{O}_6 \rightarrow \text{CH}_3\text{COO}^- + \text{CH}_3\text{CH}_2\text{COO}^- + \text{CO}_2 + 2\text{H}_2 + 2\text{H}^+)</td>
<td>+287.0</td>
<td>(4)</td>
</tr>
<tr>
<td><strong>Butyrate-type</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\text{C}_6\text{H}_12\text{O}_6 \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{CO}_2 + 2\text{H}_2)</td>
<td>-257.1</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
<td>(\text{C}_6\text{H}_12\text{O}_6 + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + 2\text{HCO}_3^- + 2\text{H}_2 + 3\text{H}^+)</td>
<td>-261.5</td>
<td>(6)</td>
</tr>
<tr>
<td><strong>Lactate-type</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\text{C}_6\text{H}_12\text{O}_6 \rightarrow 2\text{CH}_3\text{CH}(_2\text{H})\text{COOH})</td>
<td>-217.4</td>
<td>(7)</td>
</tr>
<tr>
<td></td>
<td>(\text{C}_6\text{H}_12\text{O}_6 \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + \text{CH}_3\text{CH}_2\text{OH} + \text{CO}_2)</td>
<td>+287.0</td>
<td></td>
</tr>
<tr>
<td><strong>Acetogenesis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetogenesis</td>
<td><strong>Propionate:</strong> (\text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + 3\text{H}_2 + \text{CO}_2)</td>
<td>+76.2</td>
<td>(8)</td>
</tr>
<tr>
<td></td>
<td><strong>Butyrate:</strong> (\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH} + 2\text{H}_2)</td>
<td>+48.4</td>
<td>(9)</td>
</tr>
<tr>
<td></td>
<td><strong>Lactate:</strong> (\text{CH}_3\text{CHOHCOOH} + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + \text{HCO}_3^- + 2\text{H}_2)</td>
<td>-4.2</td>
<td>(10)</td>
</tr>
<tr>
<td></td>
<td><strong>Ethanol:</strong> (\text{CH}_3\text{CH}_2\text{OH} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2)</td>
<td>+9.6</td>
<td>(11)</td>
</tr>
<tr>
<td><strong>Homo-acetogenesis</strong></td>
<td><strong>Autotrophic:</strong> (4\text{H}_2 + 2\text{CO}_2 \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2\text{O})</td>
<td>-104.6</td>
<td>(12)</td>
</tr>
<tr>
<td></td>
<td><strong>Heterotrophic:</strong> (\text{C}_6\text{H}_12\text{O}_6 \rightarrow 3\text{CH}_3\text{COO}^- + 3\text{H}^+)</td>
<td>-310.9</td>
<td>(14)</td>
</tr>
<tr>
<td><strong>Methanogenesis</strong></td>
<td><strong>Acetoclastic methanogenesis:</strong> (\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2)</td>
<td>-31.0</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td><strong>Hydrogenotrophic methanogenesis:</strong> (4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O})</td>
<td>-135.0</td>
<td>(16)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>Reactors</td>
<td>CH$_4$ (%)</td>
<td>CO$_2$ removal (%)</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------</td>
<td>------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>55</td>
<td>MR</td>
<td>96%</td>
<td>71</td>
</tr>
<tr>
<td>55</td>
<td>Up-flow reactor</td>
<td>98</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>CSTR</td>
<td>79</td>
<td>33</td>
</tr>
<tr>
<td>55</td>
<td>FBR</td>
<td>&gt;90</td>
<td>--</td>
</tr>
<tr>
<td>37</td>
<td>BTF</td>
<td>&gt;96</td>
<td>&gt;96</td>
</tr>
<tr>
<td>37</td>
<td>BTF</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td>35</td>
<td>FBR</td>
<td>--</td>
<td>68-100</td>
</tr>
<tr>
<td>37</td>
<td>BPFR</td>
<td>98</td>
<td>--</td>
</tr>
</tbody>
</table>

BTF: Biotrickling filter; BCR: Bubble column reactor; BPFR: Biofilm plug flow reactor; CSTR: Continuous stirred tank reactor; FBR: Fixed bed reactor; MR: Membrane reactor
<table>
<thead>
<tr>
<th>Upgrading technology</th>
<th>Application properties</th>
<th>Substrate</th>
<th>Temperature</th>
<th>H₂ injection rate</th>
<th>pH</th>
<th>CH₄ (%)</th>
<th>CO₂ (%)</th>
<th>Limitation</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSTR with H₂ injection</td>
<td>Cattle manure and whey</td>
<td>55 °C</td>
<td>1.5 &amp; 1.7 L-H₂/L/d</td>
<td>7.9</td>
<td>75%</td>
<td>6.6-13% (decrease with H₂ injection)</td>
<td>Overall H₂ utilization efficiency is low</td>
<td>(Luo and Angelidaki, 2012a)</td>
<td></td>
</tr>
<tr>
<td>CSTR with H₂ injection</td>
<td>Cattle manure</td>
<td>55°C (T) 35°C (M)</td>
<td>0.192, 0.51 L-H₂/L/d</td>
<td>7.78→7.95 85% (T) 89% (M)</td>
<td>9% (T) 7% (M)</td>
<td>Incomplete mass transfer between liquid and gas</td>
<td>(Bassani et al., 2015)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSTR</td>
<td>Cattle manure and potato-starch</td>
<td>55°C</td>
<td>0.41 L-H₂/L/d</td>
<td>86.4% 10.7%</td>
<td>-</td>
<td>-</td>
<td>(Corbellini et al., 2018)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSTR with H₂ injection</td>
<td>Pig manure</td>
<td>55°C</td>
<td>0.085 L-H₂/L/d</td>
<td>7.63→7.85 80% 269 L/k g VS</td>
<td>-</td>
<td>H₂ addition inhibited acetate but not propionate production</td>
<td>(Zhu et al., 2019)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch serum bottle</td>
<td>Maize leaf</td>
<td>52°C</td>
<td>Headspace pressure 56 to 138 kPa</td>
<td>8.0</td>
<td>89%</td>
<td>10-12%</td>
<td>Excessive H₂ causes VFAs accumulation</td>
<td>(Mulat et al., 2017)</td>
<td></td>
</tr>
<tr>
<td>Batch serum bottle</td>
<td>Glucose</td>
<td>35°C</td>
<td>Pulsed H₂ feeding 0.05-0.18 g COD/L/d H₂:CO₂ = 4:1</td>
<td>pH&gt;8</td>
<td>94%</td>
<td>3%</td>
<td>-</td>
<td>(Wahid et al., 2019)</td>
<td></td>
</tr>
<tr>
<td>UASB</td>
<td>Glucose</td>
<td>35°C</td>
<td>1.8 H₂ +2.0 glucose (g-COD/L/d) recirculation rate (L/min): Liquid: 10 Gas: 1.5</td>
<td>-</td>
<td>96.1% 3.9% H₂</td>
<td>Improve maximum D₂H₂ concentration and Kₐ value by 1.71 and 1.72 times</td>
<td>(Park et al., 2021)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4 Advantages and disadvantages of biological biogas upgrading strategies

<table>
<thead>
<tr>
<th>Strategies</th>
<th>Mechanism</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exogenous H₂ mediated CO₂ bioconversion to CH₄</td>
<td>(i) H₂ can interact with CO₂ to generate CH₄ under the action of hydrogenotrophic methanogens (ii) H₂ can interact with CO₂ to produce acetate under the action of homoacetogens, thereby enhancing the activity of acetoclastic methanogens</td>
<td>(i) High CH₄ content up to ~95% (ii) Will not produce other harmful substances (iii) No need to add additional chemicals</td>
<td>(i) The supply and transportation cost of exogenous H₂ is too high (ii) Excessive H₂ partial pressure will inhibit hydrolysis fermentation, acidogenesis processes and the activity of methanogens</td>
</tr>
<tr>
<td>Utilisation of acidogenic off-gas</td>
<td>The H₂ in the acidogenic off-gas stimulates the activity of hydrogenotrophic methanogens and acetoclastic methanogens</td>
<td>(i) The source of H₂ is self-sufficient (ii) No need for additional equipment, simple operation and high economic efficiency</td>
<td>(i) The H₂ content in the acidogenic off-gas is limited, and it cannot meet the best H₂/CO₂ ratio of 1:4, resulting in a lower biogas upgrading effect than other strategies.</td>
</tr>
<tr>
<td>Electromethanogenesis with a microbial electrolysis cell (MEC)</td>
<td>(i) Indirect conversion: H₂ is formed from free protons through intermediate abiotic electrochemical and/or microbial catalysis, and then utilised by hydrogenotrophs to generate CH₄ (ii) Direct conversion: CH₄ is formed via electromethanogenesis by taking the electrons from the cathode</td>
<td>(i) The configuration of MEC-AD is simple, and the biogas upgrade effect is excellent (ii) The biological H₂ reaction is sustainable and the energy recovery efficiency is high</td>
<td>(i) The electron transfer mechanism in this strategy has not yet been elucidated (ii) Low cathode potential and low energy efficiency limit its application</td>
</tr>
<tr>
<td>Methanogenic reactor</td>
<td>The injection of exogenous CO₂ will enhance homoacetogenesis through</td>
<td>(i) Provides new ideas for on-site utilisation of CO₂</td>
<td>(i) Excessive carbon dioxide injection will cause the accumulation of VFA</td>
</tr>
<tr>
<td>DIET-strengthened methanogenic reactor</td>
<td>(i) Enhanced direct electron transfer in syntrophic acetate oxidation-HM (SAO-HM) dominated systems</td>
<td>(i) AD system can adapt to higher organic load</td>
<td>(i) The interaction between conductive substances and microbial metabolism and the interface electron transport mechanism are still unclear</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>--------------------------------------------------------</td>
<td>---------------------------------------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>injected with CO₂</td>
<td>the Wood-Ljungdahl pathway, causing higher acetic acid concentrations, thereby enhancing acetoclastic methanogenesis</td>
<td>(ii) CO₂ promotes the acid production and metabolic process of microorganisms, and can effectively increase the conversion rate of organic matter</td>
<td>and decrease the pH value, thereby inhibiting the activity of methanogens and reducing the stability of the reactor</td>
</tr>
<tr>
<td>(ii) CO₂ promotes the acid production and metabolic process of microorganisms, and can effectively increase the conversion rate of organic matter</td>
<td>(iii) Exogenous CO₂ will combine with ammonia nitrogen to form weakly basic ammonium bicarbonate, which can effectively improve the buffering capacity of the methane-producing intermediate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

957 958
Fig. 1 Metabolic pathway for on-site CO\textsubscript{2} sequestration in methanogenic reactor. (a) Wolf cycle for hydrogenotrophic methanogenesis (CO\textsubscript{2}→CH\textsubscript{4}), (b) Wood-Ljungdahl pathway for homoacetogenesis and methanogenesis (CO\textsubscript{2}→acetate→CH\textsubscript{4}). Adapted from Zhou (2019) and Fu et al. (2021).
Fig. 2 Potential on-site CO₂ sequestration scenarios in AD system: (a) ex-situ biogas upgrading, (b) in-situ biogas upgrading, (c) CO₂ injection with biogas recirculation or external harvested CO₂, (d) acidogenic reactor off-gas utilized by methanogenic reactor, (d) pressurized acidogenic reactor with H₂/CO₂
Fig. 3. Structure diagram of several biogas upgrading technology reactors: (a) Biotrickling filter (BTF), (b) Continuous stirred tank reactor (CSTR), (c) Bubble column reactor (BCR), (d) Membrane reactor (MR) for ex-situ biogas upgrading (Thema et al. 2019)
Fig. 4. (a) Electromethanogenesis with microbial electrolysis cell, (b) utilization of acidogenic off-gas towards CO₂ fixation, (c) pressurized reactor with CO₂ injection
**Highlights**

1. Various on-site biogenic CO\(_2\) sequestration scenarios are discussed for AD.

2. Wolf cycle and acetyl-CoA pathways are the main CO\(_2\) fixation mechanisms.

3. CO\(_2\) capture scenarios for single-/two- phase anaerobic digestion are considered.

4. Influencing factors are discussed for exogenous H\(_2\) mediated CO\(_2\) bioconversion to CH\(_4\).

5. Flow of carbon and reductant electrons needs to be clarified in-depth.