In situ Biogas Upgrading by CO_2-to-CH_4 Bioconversion

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Abstract:	Biogas produced by anaerobic digestion is an important renewable energy carrier. Nevertheless, the high CO2 content in biogas limits its utilization to mainly heat and electricity generation. Upgrading biogas into biomethane broadens its potential as vehicle fuel or substitute for natural gas. CO2-to-CH4 bioconversion represents one cutting-edge solution for biogas upgrading. In-situ bioconversion can capture endogenous CO2 directly from the biogas reactor, is easy to operate, and provides an infrastructure for renewable electricity storage. Despite these advantages, several intrinsic challenges need to be addressed to move in-situ upgrading technologies closer to applications at scale. This opinion article reviews the state-of-art of this technology and identifies some obstacles and opportunities of biological in-situ upgrading technologies for future development.

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Dear Editor,

We appreciate yours and the reviewers' efforts for commenting and editing our paper entitled "In-situ Biogas Upgrading by CO_2-to-CH_4 Bioconversion".

We are happy to hear that both reviewers consider the manuscript interesting, which greatly encouraged us for the revision of our work. The editor and the reviewers have done an excellent job which helps us to make the manuscript much better.

All the comments are considered, and the manuscript was carefully modified to meet the suggestions. We hope that the revised manuscript could fully comply with the suggestions of the editor and reviewers. The point-by-point response to the comments is enclosed and the changes are highlighted in the revised manuscript.

We thank again our editor and reviewers for giving us such a precious chance to improve our work.

Yours faithfully,

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Opinion

In-situ Biogas Upgrading by CO_2-to-CH_4 Bioconversion

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Abstract

Biogas produced by anaerobic digestion is an important renewable energy carrier. Nevertheless, the high CO_2 content in biogas limits its utilization to mainly heat and electricity generation. Upgrading biogas into biomethane broadens its potential as vehicle fuel or substitute for natural gas. CO_2-to-CH_4 bioconversion represents one cutting-edge solution for biogas upgrading. In-situ bioconversion can capture endogenous CO_2 directly from the biogas reactor, is easy to operate, and provides an infrastructure for renewable electricity storage. Despite these advantages, several intrinsic challenges need to be addressed to move in-situ upgrading technologies closer to applications at scale. This opinion article reviews the state-of-art of this technology and identifies some obstacles and opportunities of biological in-situ upgrading technologies for future development.

Keywords: In-situ biogas upgrading; Bioconversion; Renewable electricity; Mechanisms; Challenges

Anaerobic digestion and the needs of biogas upgrading

Anaerobic digestion (AD) (see Glossary) is a complex biochemical process, during which organic matter is digested to form **biogas** through four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Box 1) [1]. The produced biogas has a **lower calorific value (LCV)** of 20–25 MJ/m^3 based on the CH_4 content, and it is considered to be one of the most important renewable energy carriers [2, 3]. AD technology has been used worldwide for organic waste treatment and **bioenergy** production [4]. Facing the problems of energy shortage and environmental pollution caused by the over-consumption of **fossil fuels**, the global biogas industry has developed significantly in recent years [5, 6]. The annual biogas production in Europe and China is expected to reach 300 billion m^3 by 2020 [7], and the global power production capacities from commercial biogas facilities are predicted to reach 29.5 GW in 2022 [8].

The produced biogas is composed of approximately 60% CH_4 and 40% CO_2, with minor concentrations of other gases including H_2, NH_3, H_2S, and others [9]. Traditionally, most of the raw biogas is used to produce electricity and heat [10]. To reach the specification of natural gas, the CH_4 content in biogas should be upgraded at least to 95%, at which point it is called biomethane or bio-natural gas [11, 12]. The biomethane can be used directly as vehicle fuel or injected into the natural gas grid, thereby broadening the range of applications and increasing the value of biogas [13]. For this purpose, **biogas upgrading**-increasing its CH_4 content by removing CO_2 or converting CO_2 into CH_4 is necessary [14]. The rapid development of the biogas industry and the growth of using upgraded biogas as vehicle fuel has created a golden period of development for biogas upgrading technologies and plants [15]. The number of biogas upgrading plants in Europe has increased by 51% in 2 years, from 483 in 2018 to 729 in 2020, among which Germany has the most biogas upgrading plants (232), followed by France (131) and the UK (80) [Gas Infrastructure Europe (GIE); European Biogas Association (EBA). EBA-GiE biomethane map 2020. Available online: https://www.europeanbiogas.eu/wp-content/uploads/2020/06/GIE_EBA_BIO_2020_A0_FULL_FINAL.pdf.]. In Sweden, the use of biogas as a vehicle fuel has exceeded the use of natural gas [16].

Along with public awareness for green transition solutions biogas upgrading has become in focus as a key technology. Therefore, this opinion mainly focuses on one current developments and the

cutting-edge solution for biological biogas upgrading by in-situ CO_2-to-CH_4 conversion. The recent understanding of the pathways and mechanisms that are involved in the in-situ biogas upgrading processes are reviewed. The obstacles and opportunities of this research field are identified for future development. Furthermore, perspectives and suggestions are provided to move the in-situ biogas upgrading technologies closer to applications at scale.

Technologies for biogas upgrading

In general, biogas can be upgraded using physical, chemical, or biological means, or a combination of these approaches [3]. Physical biogas upgrading (Box 2) is based on removing CO_2 from biogas using organic solvent absorption, water scrubbing, pressure swing adsorption, cryogenic separation, or membrane separation [2]. Chemical biogas upgrading (Box 2) occurs by reducing the CO_2 to CH_4 via the **Sabatier reaction**, using H_2 as a reductant [17]. Biological biogas upgrading (Box 2) covers **photosynthetic** or **chemoautotrophic reactions** [3]. Physical and chemical technologies have been commercially used in the biogas industry due to their advantages of high selectivity, efficiency, and high CH_4 content in the upgraded biogas. However, these technologies also have disadvantages, such as high investment and energy demand, and in some cases need for toxic solvents [18, 19]. In addition, the CO_2 removed from the physical upgrading processes is released to the air, which wastes the carbon source and contributes to global warming [20]. Comparatively, biological technologies, which use microorganisms as catalysts to drive the conversion of CO_2 into CH_4 or other valuable bio-commodities at moderate conditions (e.g., room temperatures and atmospheric pressure), have gained increasing attention due to the unique advantages of much smaller carbon and energy footprints [21, 22]. The most important advantage of the biological upgrading methods is that CO_2 is captured and recycled to new products. Biological upgrading methods can involve methanogens binding CO_2 into CH_4, dicarboxylic acid-producing bacteria binding CO_2 into dicarboxylic acids such as bio-succinic acid, or algae binding CO_2 into algal biomass [3].

Microalgae, bacteria or archaea have widely been used to upgrade biogas in recent years [23-26]. Based on the upgrading process configurations, these technologies can be classified into **in-situ, ex-situ**, and hybrid biogas upgrading. In-situ H_2 injected directly in the biogas reactor captures the endogenously produced CO_2, while ex-situ H_2, together with biogas, are injected in a separate reactor [27]. Finally, hybrid upgrading is a combination of the in-situ and ex-situ approaches, where an initial

in-situ upgrading results in an acceptable pH for biological processes, while the last polishing upgrading happens in an ex-situ process [28]. In-situ biogas upgrading can integrate biomethanation and biogas upgrading in the same reactor, which is easier to operate and more economically attractive, and therefore is more attractive [22].

In-situ biogas upgrading by CO_2-to-CH_4 Bioconversion: the pathways and mechanisms

There are three pathways for in-situ CO_2-to-CH_4 bioconversion: the direct hydrogenotrophic methanogenesis pathway, the indirect pathway through homoacetogenic acetate formation followed by acetoclastic methanogenesis, and the direct electron transfer (DET) pathway (Figure 1, Key Figure). The direct hydrogenotrophic methanogenesis pathway is driven by hydrogenotrophic methanogenic archaea using H_2 as an electron donor to directly reduce CO_2 into CH_4 through the so-called Wolf cycle (Equation 1, Figure 1A), which is also the most widely studied pathway for in-situ biogas upgrading [23]. In the indirect pathway, the CO_2 is first converted into acetate, mediated by homoacetogenic bacteria via the Wood-Ljungdahl reaction (Equation 2, Figure 1B) [29], and then the formed acetate is degraded by acetoclastic methanogenic archaea to generate CH_4 (Equation 3). The DET pathway occurs at the **biocathode** of a microbial electrochemical system through a so-called **electromethanogenic** process, in which methanogens obtain electrons directly from the biocathode via physical contact with the electrode (Equation 4, Figure 1D) [30]. Hydrogenotrophic methanogenesis and homoacetogenesis are affected and regulated by many factors including H_2 partial pressure, temperature, and other operating parameters of the reactor. The H_2 partial pressure may affect the metabolism directly. Hydrogenotrophic methanogenesis and homoacetogenesis could only take place at high H_2 partial pressure (≥500 Pa), while **syntrophic acetate oxidation** to form H_2 and CO_2 would occur at low H_2 partial pressure [31]. Temperature is another crucial factor affecting the CO_2-to-CH_4 bioconversion pathway. Hydrogenotrophic methanogens are more active at thermophilic conditions, while homoacetogens are better adapted to lower temperatures [3, 31]. As Zhu and colleagues reported, most of the added H_2 was consumed to reduce CO_2 to CH_4 according to the indirect pathway under mesophilic conditions, while the direct hydrogenotrophic methanogenesis pathway predominated under thermophilic condition [12]. Some other operating parameters such as pH and ammonia concentration may also affect the bioconversion pathway. The alkaline pH favors the conversion of H_2 and CO_2 to acetate by homoacetogens rather than CH_4 by hydrogetrophic

methanogens [32]. Wang and colleagues reported that the ammonia level can affect the CO_2-to-CH_4 bioconversion pathway. A shift from aceticlastic pathway to hydrogenotrophic pathway occurred when the ammonia level increased (1-7g NH^{4+}-N/L) [24].

Exogenous H_2 addition is the most widely used approach for in-situ CO_2-to-CH_4 bioconversion (Figure 1C) [34-37]. Usually, the H_2 can be obtained from coal gasification, petroleum refinery, petrochemical plants, soda manufacture or directly electrolysis of water [15]. Recently, using renewable excess electricity, such as excess wind or solar energy for H_2 production has drawn attentions due to economically and environmentally sustainability [38]. In this process, H_2 supply and mass transfer play key roles. Insufficient H_2 supply leads to incomplete conversion of CO_2 into CH_4 and inadequate CO_2 removal from biogas, while excess H_2 supply could lead to high residual H_2 content in biogas [39]. Based on Equation 1, an H_2:CO_2 ratio of 4:1, which corresponds to the stoichiometric ratio, is usually recommended [40]. Recently, the bioelectrochemical power-to-gas (Box 3) process has been studied as a promising alternative for in-situ biogas upgrading (Figure 1D) [41-43]. Similarly to the other in-situ methods, renewable electricity can be used to hydrolyze water to form O_2 and H^+ at the anode, and then the H^+ and e^- are transferred to the biocathode to reduce CO_2 into CH_4 [44]. The main difference is that the reducing equivalents are provided as H^+ and e^- rather than H_2 [45-47].

$CO_2+4H_2 \rightarrow CH_4+2H_2O$ ΔH^0= -130.7 kJ/mol [1]

$4H_2+2CO_2 \rightarrow CH_3COOH+2H_2O$ ΔH^0= -104.5 kJ/mol [2]

$CH_3COOH \rightarrow CH_4+CO_2$ ΔH^0= -31.0 kJ/mol [3]

$CO_2+8H^++8e^- \rightarrow CH_4+2H_2O$ [4]

Challenges and opportunities

Luo and colleagues first mentioned the concept of in-situ biogas upgrading in 2012 [48], and since then, the research interest in in-situ biogas upgrading has rapidly increased due to its advantages such as easy integration with existing AD reactors, simple operation, high efficiency, and environmental compatibility. Though promising, there are still several technical and economic barriers that need to be addressed before widespread applications.

The low gas to liquid phase transfer rate caused by the low solubility of H_2

All gas-liquid fermentations suffer from the poor transfer of the gases to the liquid phases, where

the biological processes take place. This is a common problem for all biological upgrading technologies. The dissolved H_2 is a key factor, as microorganisms can only use the dissolved H_2 for the reaction [49]. The gas-to-liquid mass transfer of H_2 can be described by Equation 5 [50].

$$R_t = 22.4 k_L a (H_{2gTh} - H_{2l}) \quad (5)$$

Here, R_t, 22.4, k_La, H_{2gTh} and H_{2l} represent the gas-to-liquid mass transfer rate [L/(L·day)], the volume of 1 mol gas at standard temperature and pressure (L/mol), gas transfer coefficient (day^{-1}), H_2 concentration in the gas phase (mol /L) and dissolved H_2 in the liquid phase (mol /L), respectively.

Equation 5 shows that the gas-to-liquid mass transfer rate of H_2 has a positive correlation with the gas transfer coefficient. Therefore, increasing the gas transfer coefficient can be a simple and convenient method to improve the H_2 gas-to-liquid mass transfer rate. Intense stirring [48], minimizing the gas bubble size by adding packing materials [50], biogas recirculation [51], and increasing the pressure in reactors [52] have been reported to increase the gas transfer coefficient. In addition, some novel methods including membrane modules and bioelectrochemical systems have also been recently used to overcome this challenge. For instance, using a hollow-fiber membrane increased the H_2 gas transfer coefficient from 30 to 430 h^{-1}, and the final CH_4 concentration in biogas was upgraded to 95% [53]. Even so, membrane fouling and short service life are still problems, more efforts should be paid on new material selection, and materials and surface modification. Direct electron uptake or in-situ H_2 generation in the cathode of **microbial electrolysis cell (MEC)** could also be an efficient solution [18]. However, the electron transfer mechanisms of technology are not fully clarified, low **cathode potential** and energy efficiency limit its application. In addition, reactor design, using bio-surfactants to reduce the surface tension without effect on the biological process are all potential solutions for the low gas to liquid phase transfer rate, which need to be further studied.

Inhibition on the metabolism of intermediate products with increased hydrogen partial pressure

A challenge of in-situ upgrading is that the process in the main biogas reactor might be disturbed by the direct addition of exogenous H_2 into the biogas reactor. During the biogas fermentation process, the degradation of volatile fatty acids (VFAs) and alcohols is prevented at standard conditions (1 atm partial pressures, molar concentrations of reactants and products, and 0 ℃ temperature) because these processes are endergonic at standard conditions ($\Delta G>0$, Equation 6, Equation 7). Therefore, syntrophy between fermentative microorganisms and methanogens is needed to constantly reduce the product

concentrations, create a negative ΔG at reactor conditions, and make the reactions exergonic [54]. Degradation of VFAs and alcohols requires a rather low H_2 concentration, which can only be achieved by syntrophic growth [54]. Exogenous H_2 addition into the AD reactor could lead to increased H_2 concentration (unless it is consumed quickly), which could inhibit the syntrophic degradation of VFAs and alcohols, lead to their accumulation, and eventually inhibit the AD process. Moreover, exogenous H_2 addition could stimulate the production of acetate via the Wood-Ljungdahl pathway, potentially inhibiting AD if the produced acetate could not be converted into CH_4 quickly [39]. Luo and colleagues observed the inhibition of propionate and butyrate degradation by H_2 addition with a pressure of 1 atm [48]. Nevertheless, the inhibition of syntrophic degradation of VFAs and alcohols by exogenous H_2 addition could also be reversed after longer-term H_2 exposure due to the improved relative abundance of hydrogenotrophic methanogens in the microbial communities [3]. Therefore, the enrichment of hydrogenotrophic methanogens via long term acclimatization or bioaugmentation with hydrogenotrophic methanogens directly could be promising countermeasures for this challenge by rapidly consuming the added exogenous H_2.

Furthermore, recent studies reported **direct interspecies electron transfer (DIET)** between fermentative microorganisms and methanogens mediated by pili or cytochrome C on the cell membrane or aid of conductive materials [55]. DIET could thus establish syntrophic degradation of VFAs and alcohols ($\Delta G<0$, Equation 8, Equation 9) according to direct electron transfer between fermentative microorganisms and methanogens without the diffusion of H_2, thereby avoiding H_2 accumulation [56]. However, further studies are needed to prove whether DIET counteracts H_2 accumulation in practice.

$$CH_3CH_2OH+H_2O \rightarrow CH_3COO^-+H^++2H_2 \quad \Delta G^0 = +9.7\ kJ/mol \quad (6)$$

$$CH_3CH_2COO^-+3H_2O \rightarrow CH_3COO^-+HCO_3^-+H^++3H_2 \quad \Delta G^0 = +76.5\ kJ/mol \quad (7)$$

$$CH_3CH_2OH+H_2O \rightarrow CH_3COO^-+5H^++4e^- \quad \Delta G^0 = -149.6\ kJ/mol \quad (8)$$

$$CH_3CH_2COO^-+3H_2O \rightarrow CH_3COO^-+HCO_3^-+7H^++6e^- \quad \Delta G^0 = -162.5\ kJ/mol \quad (9)$$

Increased pH caused by CO_2 consumption during in-situ biogas upgrading

The most serious challenge of in-situ biogas upgrading is the increase of pH due to the binding of the endogenously produced CO_2. The CO_2 dissolved in the liquid phase of the AD reactor can dissociate into H^+ and HCO_3^- (Equation 10), which could affect the pH during the AD process [57].

The reduction of CO_2 into CH_4 by adding exogenous H_2 directly into a biogas reactor (in-situ biogas upgrading), would lower the level of endogenously produced CO_2 in the liquid and thereby increasing pH above 8.5, which in return would inhibit the biological processes [31]. In general, the ideal pH for AD ranges from 6.6 to 7.8 [58], and therefore the increased pH during in-situ biogas upgrading might inhibit the AD process. pH control with chemicals or co-digestion with a substrate of low pH could be a promising solution to this challenge. Luo and colleagues reported that co-digestion of manure with acidic whey could maintain the pH of the AD system around 7.8 with the addition of exogenous H_2 for in-situ biogas upgrading, which was in the optimal pH range for AD [59]. Moreover, ex-situ biogas upgrading by decoupling the main biogas process from the upgrading process is also a potential solution, which is under development [28].

$$H_2O + CO_2 \leftrightarrow H^+ + HCO_3^- \quad (10)$$

Economic aspects

In-situ biogas upgrading by CO_2-to-CH_4 bioconversion relies on an accessible, available, and cost-effective reducing agent. H_2 assisted biogas upgrading is a well-studied method for bioconversion of CO_2 into CH_4, and the newly emerging bioelectrochemical bioconversion of CO_2 has also attracted attention. At present, the H_2 production processes are highly costly. Thus, in-situ biogas upgrading by bioconversion of CO_2 into CH_4 currently seems to be more expensive than physicochemical biogas upgrading. However, the environmental benefit of CO_2-to-CH_4 bioconversion could be regarded as an offset of CO_2 emissions [60], and CO_2-to-CH_4 bioconversion usually leads to improved CH_4 yield, which could compensate part of the cost for H_2 production. In addition, in-situ biogas upgrading also allows integrating biogas production and upgrading in a single reactor without further investment in additional reactors [22].

Using intermittent renewable energy to power in-situ biogas upgrading by CO_2-to-CH_4 bioconversion can valorize renewable energy by converting intermittent or excessive electricity along with CO_2 into CH_4 for later use. Wind energy and solar energy are the most plentiful renewable energy sources [61]. However, the electrical energy produced from wind and solar is intermittent, so it cannot meet the variable consumption demands [31]. **Power-to-gas** (P2G) technology, which utilizes excessive electricity to split the water and generate H_2 for later use (Equation 11), represents an attractive method for electricity storage [62]. Several inherent disadvantages e.g. low volumetric

energy, safety and storage issues limit the utilization of H_2 [39]. Meanwhile, the utilization technology of H_2 as vehicle fuel is still immature [62]. Using H_2 to reduce the CO_2 into CH_4 (Equation 1, Equation 2, and Equation 3) could simultaneously be considered as both H_2 storage and biogas upgrading. In addition to H_2 production, renewable surplus electricity could also be used as a power source to convert CO_2 into CH_4 in the bioelectrochemical systems, thereby upgrading the biogas. In the long term, the production cost for renewable electricity is expected to decrease. The decreased cost of renewable energy, the need for storage of intermittent or excessive renewable energy, the shortage of fossil fuels, and the increasing requirements for a sustainable circular bioeconomy, combined with the rapid development of CO_2 bioconversion technology, provides a golden opportunity for in-situ biogas upgrading technology as an economically favorable avenue compared with the traditional physicochemical biogas upgrading processes in the near future.

$2H_2O \rightarrow O_2 + 2H_2$ $\quad \Delta H^0 = +237.2$ kJ/mol [11]

Concluding Remarks and Future Perspectives

Biogas upgrading to convert raw biogas into biomethane broadens the scope of biogas applications and increases the value of biogas. In-situ CO_2-to-CH_4 bioconversion represents a promising avenue for biogas upgrading due to its advantages e.g. simple and easy operating, carbon capture and utilization, renewable electricity storage, economic and environmental-friendly, etc. Despite recent advances, several challenges need to be addressed and certain questions need to be answered (see Outstanding Questions) before its practical application.

Though the pathways and main microorganisms of the CO_2-to-CH_4 bioconversion have been identified, the molecular mechanism, metabolic process, and microbial responses under different operating parameters are not fully clarified, especially for the electromethanogenesis process. Further researches using advanced techniques such as genome-centric metatranscriptomics, isotope tracer technique, and high-throughput sequencing are needed to uncover the phylogenetic and metabolic properties of the CO_2-to-CH_4 bioconversion processes. Metabolic regulation and genetic engineering approaches are also expected to improve the bioconversion efficiency of CO_2.

Besides, recent studies of CO_2-to-CH_4 bioconversion are limited to bench-scale. For scaling-up and industrial implementation, the performance and stability of in-situ biogas upgrading processes at long-term continuous operation are still unknown. Future research in this direction will help to further

identify the potential challenges and assess the relevant countermeasures of this technology, which could also provide essential parameters for further evaluation of the process sustainability through **life-cycle assessment (LCA)**.

Moreover, the practical application of the technology is quite dependent on the availability of renewable electricity which is the main source of H_2 or electrons. Thus, the improvement of the efficiency in solar and wind energy technologies or other renewable energy sources could also promote the in-situ biogas upgrading. So far, commercial H_2 gas or commercial power supply is used in most of the studies. In these studies, it is always claimed that H_2 or electrons could be obtained from renewable energy, but the real integration of these two processes is rarely reported. In the future, integration of solar panel or even development of photoelectrode should be pursued, since we need this information to further evaluate the applicability of the whole concept. Also, the recent developed nanoparticles such as cadmium sulfide nanoparticles [63] could be used for the in-situ harvesting solar energy and power in-situ CO_2-to-CH_4 conversion without external power supply, which may provide an alternative avenue for the in-situ biogas upgrading.

Furthermore, conversion of the CO_2 into higher-value chemicals (e.g., higher alcohols) [15] or downstream technology that could further convert methane to higher-value products such as microbial protein using methanotrophic bacteria [64] could help to make the whole process more profitable.

In summary, it is a long journey for a novel technology to reach real-life implementation. Having identified the challenges that exist, and as solutions are developed, the in-situ biogas upgrading technology by CO_2-to-CH_4 bioconversion is experiencing rapid development and should reach practical application in the near future.

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Glossary

Anaerobic digestion (AD): a biological process that can convert the organic substrates into CH_4, CO_2, and other gases in small amounts, under oxygen-free conditions. Typically, the AD process could be divided into four steps including hydrolysis, acidogenesis, acetogenesis, and methanogenesis, which are accomplished by a complex microbial community involving hydrolytic, fermenting, homoacetogenic, syntrophic, and methanogenic microorganisms.

Biogas: the end-product of the AD process, which is a mixture of CH_4 (approximately 60%), CO_2 (approximately 40%), and small amounts of H_2, NH_3, H_2S, etc. Applications of biogas are including heating, electricity generation, cooking fuel, or after upgrading as vehicle fuel, which is considered as one of the most important renewable energy carriers.

Bioenergy: renewable energy produced from biomass.

Biogas upgrading: a process to remove CO_2 (or convert it into CH_4), H_2S, steam, and other impurities from biogas, thus to improve the calorific value of biogas. Upgraded biogas with CH_4 content higher than 95% could be called as bio-methane or bio-natural gas.

Biocathode: cathode that using the microorganisms attached on its surface as catalysts for biosynthesis.

Cathode potential: the applied potential needed at the cathode to overcome the overpotentials, thus provide electrons to the microorganisms, which is a key factor that affects the performance of bioelectrochemical system.

Chemoautotrophic reaction: a process of synthesizing cell biomass from carbon dioxide using energy derived from inorganic reactions.

Direct interspecies electron transfer (DIET): a syntrophic metabolism between fermentative microorganisms and methanogens, among which the electron transfer is realized without mediating diffusive electron carriers such as molecular hydrogen or formate. The DIET could be realized via conductive pili, membrane-bound electron transport proteins or abiotic conductive materials.

Electromethanogenesis: CH_4 produced by the direct transfer of electrons to methanogens.

Ex-situ biogas upgrading: biogas upgrading conducted in a separate reactor from the biogas reactor.

Fossil fuels: hydrocarbons, primarily coal, fuel oil, or natural gas.

In-situ biogas upgrading: biogas upgrading conducted directly in the biogas reactor.

Life-cycle assessment (LCA): a methodology for assessing potential environmental impacts and

resources consumed in each step of the life-cycle of a commercial product, process, or service.

Lower calorific value (LCV): the amount of heat released by combusting a specified quantity (initially at 25°C) and returning the temperature of the combustion products to 150°C, which assumes the latent heat of vaporization of water in the reaction products is not recovered, which is also known as net calorific value.

Microbial electrolysis cell (MEC): a type of bioelectrochemical system which can produce hydrogen in the cathode chamber with external power supply.

Photosynthetic reaction: the process by plants and photosynthetic microorganisms that can reduce CO_2 with H_2O into chemical energy in the form of carbohydrates by extracting reducing equivalents from light. The process is catalyzed by chlorophyll.

Power-to-gas (P2G): a process that can convert electrical power to gas fuels, such as H_2 or CH_4, which in turn can be further converted to other fuels or bio-products.

Sabatier reaction: a chemical process for converting H_2 and CO_2 to CH_4. The process requires elevated temperatures and pressures and the presence of specific catalysts ($CO_2+4H_2 \rightarrow CH_4+2H_2O$).

Syntrophic acetate oxidation: A two-step reaction in which the acetate is first oxidized by syntrophic acetate oxidizing bacteria to form H_2 and CO_2, which are subsequently converted into CH_4 by hydrogenotrophic methanogens.

Box 1 Methanogenesis

Methanogenesis is the process that methanogens use simple substrates e.g. H_2, CO_2, formate, acetate, and a variety of methyl-containing compounds to produce CH_4 under oxygen-free condition [65]. Although the substrates for methanogenesis are simple, the CH_4 formation is a complex biochemistry process, among which at least six unusual coenzymes and about 200 genes are involved [66]. Based on the substrate, the methanogens could be typically classified into three groups: hydrogenotrophic methanogens, aceticlastic methanogens, and methylotrophic methanogens (Table I). Methanogenesis is largely affected by many parameters, among which pH and temperature are two of the most important factors. The optimal pH for methanogenesis is in a range of 6.6-7.8 [59]. Based on the optimal temperature the methanogens could be divided into psychrotolerant methanogens (around 18°C), mesophilic methanogens (around 37 °C), thermophilic methanogens (around 55 °C) and hyperthermophilic methanogens (around 65 °C).

Table I Methanogenesis

Methanogens	Substrate	Reactions	Typical Methanogens	Typical habitat
Hydrogenotrophic methanogens	H_2 and CO_2 Formate Methanol	$4H_2+CO_2 \rightarrow CH_4+2H_2O$ $4HCOOH \rightarrow CH_4+3CO_2+2H_2O$ $4CH_3OH \rightarrow 3CH_4+CO_2+2H_2O$	*Methanobacterium bryantii* *Methanobacterium formicicum* *Methanobacterium thermoalcaliphium* *Methanothermobacter thermoautotrophicum* *Methanothermobacter wolfeii* *Methanobrevibacter smithii* *Methanobrevibacter ruminantium* Methanococcoides methylutens	Deep marine sediments Termite hindguts Human gastrointestinal tracts Animal gastrointestinal tracts
Aceticlastic methanogens	Acetate	$CH_3COOH \rightarrow CH_4+CO_2$	*Methanosaeta concilii (soehngenii)* *Methanosaeta thermophila*	Anaerobic digesters Rice fields Wetlands
Methylotrophic methanogens	Trimethylamine Dimethyl sulfate Methylated ethanolamines	$4(CH_3)_3N+6H_2O \rightarrow 9CH_4+3CO_2+4NH_3$ $2(CH_3)_2NH+2H_2O \rightarrow 3CH_4+CO_2+2NH_3$ $4(CH_3)NH_2+2H_2O \rightarrow 3CH_4+CO_2+4NH_3$ $2(CH_3)_2S+2H_2O \rightarrow 3CH_4+CO_2+H_2S$	*Methanosarcina barkeri* *Methanosarcina mazei* *Methanosarcina thermophile*	Marine Hypersaline habitat Sulfate-rich sediments

Box 2 Biogas upgrading technologies

Biogas upgrading technology could widen the scope of biogas application e.g. transport vehicle fuel or substitute for natural gas, which attracts increasing attention. An increasing number of biogas plants with biogas upgrading units have been built in recent years. Germany is leading the biomethanation plants with about 232 of biomethanation plants existing in 2020, while utilization of biogas exceeds natural gas as a vehicle fuel in Sweden. Many biogas upgrading technologies including physical, chemical, and biological technologies have been developed, among which physical biogas upgrading technologies are most widely used and biological biogas upgrading technologies are developing rapidly in recent years. The mechanisms, advantages, and disadvantages of different biogas upgrading technologies are shown in table Ⅰ.

Table Ⅰ Mechanisms, advantages and disadvantages of different biogas upgrading technologies

Strategies	Mechanism		Advantage	Disadvantage
Physical technologies	Water scrubbing	Based on the difference in solubility in water of different gases. Compared with CH_4, CO_2 and H_2S have a much higher solubility in water.	✓ High CH_4 purity up to 99% ✓ Simultaneous removal of CO_2 and H_2S ✓ Simple process, easy operation, low CH_4 loss (less than 2%) ✓ No need of harmful chemicals, the water could be regenerated	✓ High investment ✓ High pressure and energy needed ✓ Huge amount of water needed ✓ High risk of biological contamination
	Pressure swing adsorption	Based on molecular characteristics of different and the affinity of the adsorbent material. The gases could be attracted to solid surfaces under high pressure and released with the decrease of pressure.	✓ Low energy cost and capital investment ✓ High CH_4 purity ranged 96–98% ✓ Safety and easy operation ✓ water-free process and clean gas produced ✓ simplicity of operation	✓ Higher CH_4 loss (up to 4%) ✓ Pre- purification equipment need to remove H_2S from biogas ✓ Pre-drying need to remove water from biogas ✓ High risk of adsorbent material contamination by impurities in the biogas
	Absorption using amine solutions	CO_2 could be bound into the solvent by an exothermic chemical reaction. The formed chemical bonds could be disrupted under a high temperature of 120-160 °C.	✓ High efficiency ✓ High CH_4 purity up to 99% ✓ Easy operation, low CH_4 loss (less than 0.1%) ✓ Simultaneous removal of CO_2 and H_2S	✓ High investment for amine solvents ✓ Toxic solvents needed and loss due to evaporation ✓ High energy needed for chemical solutions regeneration ✓ Waste chemical solutions need to reasonable disposal
	Absorption using organic solvents	Based on the difference in solubility in the organic solvent of different gases.	✓ Less liquid inputs, smaller dimensions of the upgrading unit ✓ High CH_4 purity up to 98% ✓ Simultaneous removal of CO_2 and H_2S ✓ Simple process, easy operation, low CH_4 loss	✓ Toxic organic solvents needed ✓ Difficult regenerated of organic solvents due to the high solubility of CO_2 ✓ High temperatures are required for H_2S separation
	Cryogenic separation	Different gases condense at different temperature and pressure domains	✓ High CH_4 purity > 97% ✓ Recovery of CO_2 with high purity ✓ Environmental friendly	✓ High investment and operation costs ✓ High energy needed ✓ This technology is still under developing
	Membrane separation	Based on the selective permeability properties of membranes allowing the separation of the biogas components	✓ Small space requirements ✓ Fast installation and start-up, available at low capacities ✓ Less operational and energy cost ✓ High reliable and cheap process ✓ Simple and environmentally friendly	✓ High cost and easy fragility of the membranes ✓ For high purity product, multiple steps of the membrane are required ✓ Low membrane selectivity ✓ Pre-treatment necessary
Chemical technologies	chemical hydrogenation/Sabatier reaction	Reduction of CO_2 with H_2 based on Sabatier reaction.	✓ High selectivity and process efficiency ✓ Conversion of CO_2 into CH_4, reduction of CO_2 emission ✓ High CH_4 purity	✓ Easily affected by the trace gasses in the biogas ✓ High amount of pure H_2 gasses needed ✓ Expensive catalysts needed ✓ High energy cost to maintain the operational conditions
Biological technologies	Photosynthetic reaction	Photosynthesis by photoautotrophic biology to uptake CO_2 utilizing solar irradiation, water and nutrients to produce biomass, oxygen, and heat	✓ High methane recovery up to approximately 97% ✓ Transformation of CO_2 into other products ✓ Production of active biomass ✓ low requirements in terms of land and water	✓ High investment cost and energy demands ✓ Low photosynthetic CO_2 uptake and high natural sources requirements ✓ High risk of biological contamination
	Chemoautotrophic reaction	Methanogenesis of CO_2 based on the action of methanogens	✓ High selectivity, process efficiency, and CH_4 purity ✓ Conversion of CO_2 into CH_4, reduction of CO_2 emission ✓ Moderate temperatures and atmospheric pressure ✓ Easily integrate with the AD process	✓ High amount of reductant needed ✓ This technology is still under developing

			✓ Environmentally friendly	

Box 3 Bioelectrochemical power-to-gas

Bioelectrochemical power-to-gas is a cutting-edge technology that relays on microbial reduction of CO_2 into CH_4 with an additional supply of electrical energy. The bioelectrochemical power-to-gas system mainly consists of two compartments: anodic compartment where water splits into O_2, H^+, and e^-; cathodic compartment where CO_2 is reduced into CH_4 by microorganisms [42]. Though a cation exchange membrane is usually used to separate anodic and cathodic compartments in most of the cases, it is not essential for the smoothly running of the system.

Figure Ⅰ summarizes the methanogenesis pathways in the bioelectrochemical power-to-gas system. The methanogenesis pathways include acetoclastic methanogenesis and hydrogenotrophic methanogenesis, during which H_2 is used as an electron donor to transport the electrons from the cathode to the methanogens; and direct electron transfer (DET) from cathode to methanogens via physical contact without the intermediate production of H_2 is also reported [65]. Many factors include cathode potential, electron donor, operational parameters, and inoculum used are highly associated with the methanogenesis performance of bioelectrochemical power-to-gas system. Until now, the bioelectrochemical power-to-gas is limited to lab-scale study, its molecular mechanism is still not fully identified, and some shortages such as low cathode potential and energy efficiency limit its industrial application. However, this technology has drawn increasing attention and is expected to be a promising option for in-site biogas upgrading.

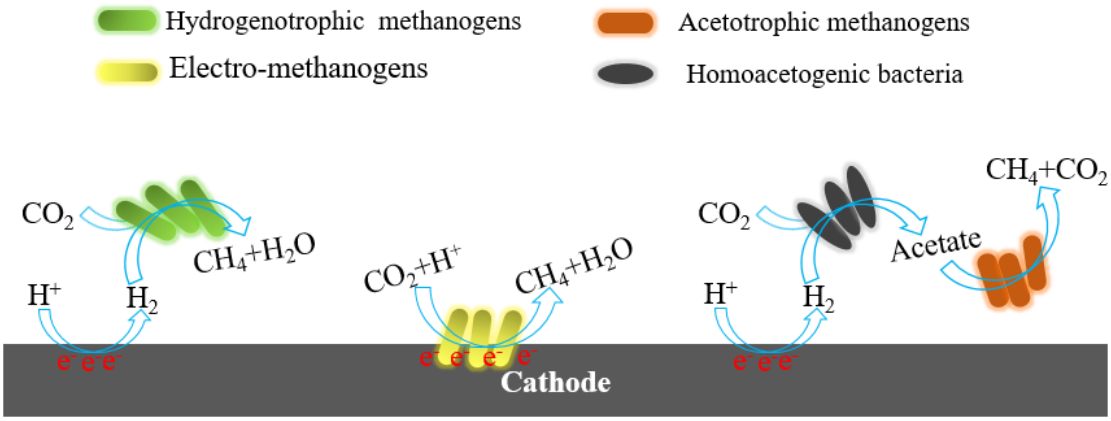

Figure Ⅰ Methanogenesis pathways in the bioelectrochemical power-to-gas system

Figure Captions

Figure 1. (Key Figure) The pathways and mechanisms for in-situ biogas upgrading through bioconversion of CO_2 into methane. (A) Wolf cycle for reduction of CO_2 into CH_4 (H4MPT: tetrahydromethanopterin; MFR: methanofuran; F_{420}: coenzyme F_{420}; Fd: specific ferredoxin). Adapted from Thauer (2012) [33]. (B) Wood-Ljungdahl reaction for homoacetogenic acetate production and acetate split for CH_4 and CO_2 production (FH_4: tetrahydrofolate). Adapted from Can (2014) [29]. (C) In-situ biogas upgrading by injection of H_2 directly into the AD reactor. (D) bioelectrochemical biogas upgrading systems.

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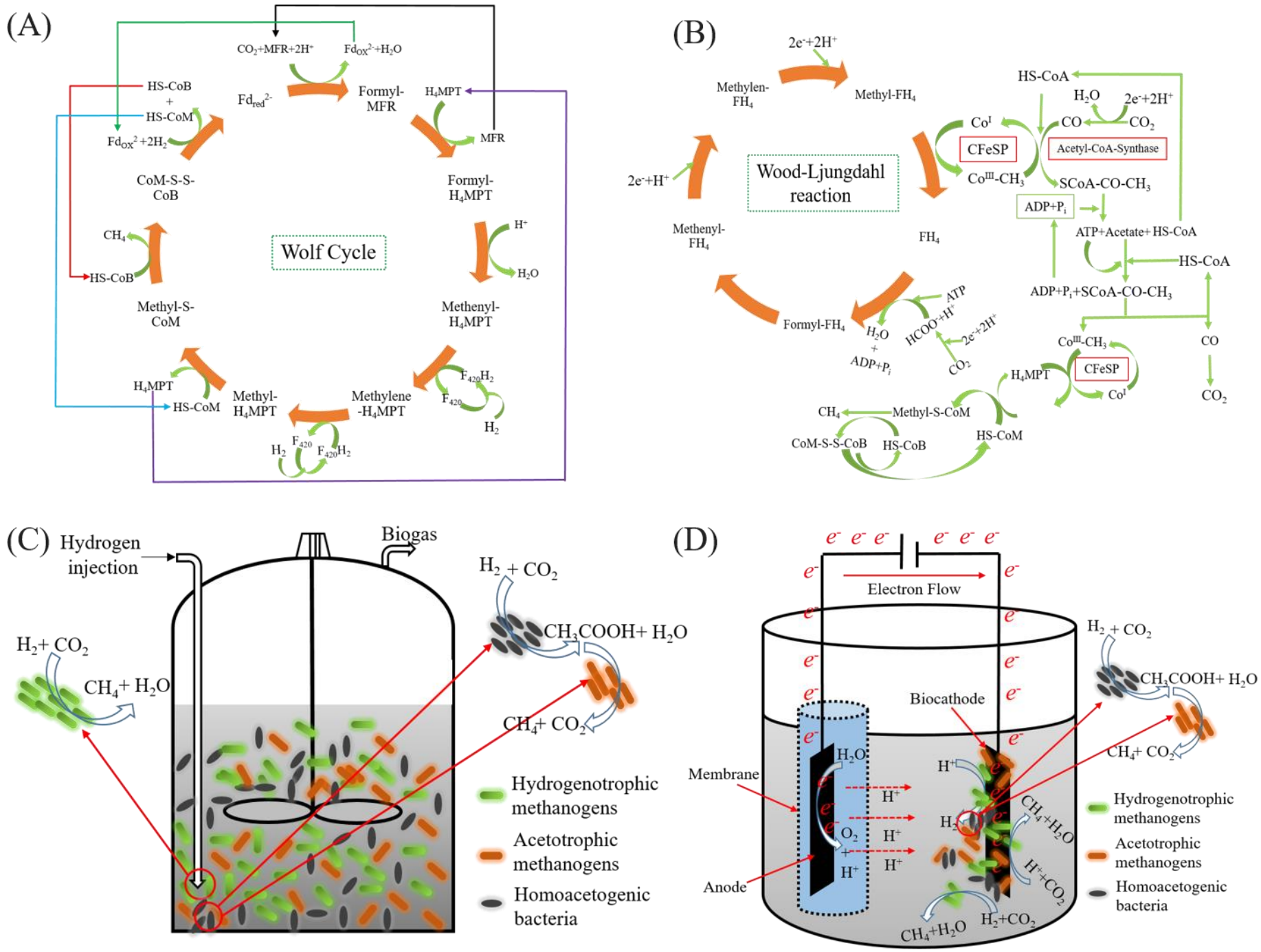
Figure 1, key figure



Outstanding Questions

1. What are the main factors that limit the industrial application of in-situ biogas upgrading by CO_2-to-CH_4 bioconversion? What preparations should be made before its industrial application?
2. Could the membrane technology provide the solution for the barrier of gas to the liquid transfer? What is the main technological difficulty for this technology?
3. Could DIET be a solution for the metabolism barriers caused by the increased hydrogen partial pressure? Would the existing strategies for boosting DIET in the typical AD processes be applicable for the in-situ CO_2-to-CH_4 bioconversion?
4. Are the microbiome and interspecies interactions associated with the CO_2-to-CH_4 bioconversion fully understood?
5. What are the electron transfer mechanisms between the cathode electrode and methanogens in the bioelectrochemical systems? How to boost the electron uptaking by methanogens? What is the maximum CO_2-to-CH_4 conversion rate in the bioelectrochemical systems and what could be done to improve the efficiency of biogas upgrading to meet the large-scale application?
6. How to advance the development of the in-situ biogas upgrading process using the tons of information from genomics, metagenomics, transcriptomics, proteomics, and metabolomic analysis?

The editor and reviewers' comments and the corresponding responses are shown as following. Each change is highlighted in the revised manuscript.

Response to the editor

Title Page

1. *We encourage our authors to list contact information such as ORCIDs, Twitter or social media handles, or links to lab websites, which will be linked to in the online version of the manuscript on the title page; feel free to add this information if you are interested.*

   Response: Thanks for your kindly suggestion. We have added the all the authors' ORCIDs in the revised manuscript.

Content

1. *Please note that I have added a few comments on the document asking for clarifications. Please re-write these sections as requested, keeping non-expert readers in mind.*

   Response: We are so grateful for your meaningful comments. We have revived the whole manuscript according your suggestion.

2. *I recommend adding a few sentences of "thesis statement" at the end of the introductory section to introduce the subject of the review: what will the article be about? Why is this an interesting topic to write about now—for instance, have there been significant recent developments in how this topic is understood or practiced, does this article provide a new interpretation of an existing field, or is it simply the first comprehensive review of a timely subject? Finally, what will a reader learn from this article that he or she couldn't learn simply by reading the references?*

   Response: Thanks for your suggestion. The thesis statement has been added in our revised manuscript as follows (L86-92).

   "Along with public awareness for green transition solutions biogas upgrading has become in focus as a key technology. Therefore, this opinion mainly focuses on one current developments and the cutting-edge solution for biological biogas upgrading by in-situ CO_2-to-CH_4 conversion. The recent understanding of the pathways and mechanisms that are involved in the in-situ biogas upgrading processes are reviewed. The obstacles and opportunities of this research field are identified for future development. Furthermore, perspectives and suggestions are provided to move the in-situ biogas upgrading technologies closer to applications at scale".

3. *I think the manuscript would be improved with an expanded concluding section to provide a stronger perspective on the field rather than simply recapitulating the rest of the manuscript. For instance, you might describe your hypothesis for how the field might develop in the future, what the "next round" of experiments should look like, what the prospects are for large-scale implementation, and so on.*

   Response: The Concluding Remarks and Future Perspectives section of our manuscript has been largely improved according to the comments.

4. *Otherwise, I have no significant comments beyond the points that the reviewers raised; please read them carefully and revise the manuscript accordingly.*

   Response: We have read the reviewers' comments carefully and revised the manuscript accordingly (please find it in the following sections).

Figures/Tables/Text Boxes

1. *Please upload individual image files for each of your figures (including figures contained within Text Boxes) along with the revised manuscript. Please see our Author Instructions under the Figure Guidelines tab for more information about acceptable file types and resolution. If possible, please include both a finalized image (.jpg, .tif, or .pdf preferred) and an original file that the image was created from (for instance, .ppt or .ai) for each figure.*
   Response: Both the finalized image (pdf) and the original file (ppt) have been uploaded as individual files.
2. *In the revised version, please attach the caption for Figure 1 to the end of the main text, near the tables and text boxes, rather than including it with the figure file.*
   Response: Revised, as suggested.
3. *I'm happy to inform you that we offer complimentary professional illustration support in collaboration with the Elsevier Webshop; please email me* ***prior to resubmission*** *if you are interested in this service.*
   Response: Thanks for the information.
4. *Figure360 video (optional): Create a narrated, animated version of your figure that helps the reader zoom in on the most important take-home message in a matter of minutes. For guidelines and examples, please click here.*
   Response: Thanks for the information.

Outstanding Questions

1. *Please call out the Outstanding Questions as (see Outstanding Questions) somewhere in the concluding section.*
   Response: Revised, as suggested.

References and Length

1. *During revision, please keep the main text under 3500 words. This limit does not include the Abstract, References, or any additional elements (Figures, Tables, Text Boxes).*
   Response: The total words of the main text in our revised manuscript is 3370.
2. *Please ensure that the revised manuscript cites no more than 75 references.*
   Response: The total references of the main text in our revised manuscript is 66.
3. *Please include any references to websites, trade publications, or other non-peer* reviewed documents (such as the current Reference 16) as parenthetical URL citations rather than including them in the main References list.
   Response: Revised, as suggested (Line 81-84).

Clarity and Accessibility

1. *Finally, please review the changes I have suggested in the main text and make any modifications necessary if the intended scientific meaning has been altered.*
   Response: Thanks for your kindly modification, we have revised our manuscript thoroughly according the editor's and reviewers' comments.

<u>Responses to reviewers</u>

Comments from reviewer #1

This is a timely and informative paper that focuses on an important subject in bioenergy production from the wastes. There is a broad interest in converting CO2 to an energy

compound and biogas upgrading holds much promise. I think the paper can be accepted after some minor changes, mostly about writing.

Response: Thanks for your kindly comments, we have revised the manuscript thoroughly including the language.

1. *Line 101: is "bind" after "methanogens" a correct word here?*
   Response: Apologize for this mistake. Revised, as suggested.
2. *Line 120: "a most studied" may be changed to "the most widely studied"*
   Response: Revised, as suggested.
3. *Line 121: "During" can be changed to "In" and "the" before "CO2" can be removed*
   Response: We have changed this sentence as follows (L128-129).
   "In the indirect pathway, the CO_2 is first converted into acetate, mediated by homoacetogenic bacteria via the Wood-Ljungdahl reaction."
4. *Line 124: "an electrochemical" may be changed to "a microbial electrochemical"*
   Response: Revised, as suggested.
5. *Line 128: "technology" may be changed to "method" or "approach"*
   Response: Revised, as suggested.
6. *Line 129: choose to use "methane" or "CH4" throughout the manuscript*
   Response: Revised, as suggested. "CH_4" was used throughout the manuscript.
7. *Line 131: should "could not" be changed to "could"?*
   Response: We have changed this sentence as follows (L154-156).
   "Insufficient H_2 supply leads to incomplete conversion of CO_2 into CH_4 and inadequate CO_2 removal from biogas, while excess H_2 supply could lead to high residual H_2 content in biogas."
8. *Line 132: "do not succeed adequate" can be changed to "inadequate"*
   Response: Revised, as suggested.
9. *Line 315: "described" can be changed to "studied"*
   Response: Revised, as suggested.
10. *Line 135-139: this paragraph may be combined with the sentences in 124-126, because they are very relevant to each other.*
    Response: Thanks for your suggestion. The sentences in Line 124-126 described the DET pathway for *in-situ* bioconversion of CO_2 into CH_4, while the sentences in line 135-139 described the approach used for bioconversion of CO_2 into CH_4. These are two different concepts. In the revised manuscript, we have clarified the points and we have combined the sentences in line 135-139 with the sentences in line 128-134.
11. *Line 148: add "and" before "since"*
    Response: Revised, as suggested.
12. *Line 151: "application" can be changed to "applications"*
    Response: Revised, as suggested.
13. *Line 165: change "is" to "can be"*
    Response: Revised, as suggested.
14. *Line 166: "Strategies e.g.," can be changed to "Strategies such as"*
    Response: We have changed this sentence as follows (L184-186).

"Intense stirring, minimizing the gas bubble size by adding packing materials, biogas recirculation, and increasing the pressure in reactors have been reported to increase the gas transfer coefficient."

15. *Line 17-178: This sentence "On the contrary ex-situ is decoupling the main biogas process from the upgrading process" is confusing. Is the following content that talks about H2 inhibition happening in the 'ex situ" configuration?*
    Response: We apologize for this mistake. We have removed this sentence in our revised manuscript.
16. *Line 188: add "and" before "if"*
    Response: We have changed this sentence as follows.
    "Moreover, exogenous H_2 addition could stimulate the production of acetate via the Wood-Ljungdahl pathway, potentially inhibiting AD if the produced acetate could not be converted into CH_4 quickly."
17. *Line 218: "is put" should be modified*
    Response: We have changed this sentence as follows (L233-235).
    "The reduction of CO_2 into CH_4 by adding exogenous H_2 directly into a biogas reactor (in-situ biogas upgrading), would lower the level of endogenously produced CO_2 in the liquid and thereby increasing pH above 8.5, which in return would inhibit the biological processes."
18. *Line 219: add "and" before "therefore"*
    Response: Revised, as suggested.

Comments from reviewer #2

It is a very interesting opinion paper regarding in-situ biogas upgrading by CO2-to-CH4 bioconversion. The manuscript was very well crafted, right on cutting edge of the field. Based on the state of the art and excellent discussions, I believe that the contents of the manuscript interest the reader of the journal. Motivation and future perspectives are also clearly described. This paper has no weaknesses and is well-prepared. In my opinion, this manuscript should be published in a current form, after considering minor revisions stated below.

Response: Thanks for your kind comments. We have revised the manuscript according to the specific comments.

Specific comments

1. *Line 92-94. This sentence is incorrect, for physical biogas upgrading e.g. water scrubbing, membrane separation, toxicity solvents are not needed.*
   Response: We have modified this sentence as follows (L101-102).
   "However, these technologies also have disadvantages, such as high investment and energy demand, and in some cases need for toxic solvents".
2. *Line 96-98. The catalysts in biological biogas upgrading could also be microalgae etc.*
   Response: We agree that the biogas upgrading could also be realized by microalgae through the fixation of CO_2 into biomass. However, the microalgae could not realize

the conversion of CO_2 into CH_4. Since the focus of the current paper was in CO_2 capture to CH_4, algae was not been included.

3. *Line 108, remove "biogas".*
   Response: Revised, as suggested.
4. *Section "In-situ biogas upgrading by CO_2-to-CH_4 Bioconversion: the pathways and mechanisms". As the authors described, there are three pathways for biogas upgrading. Please clarify which is the main pathway under a certain condition.*
   Response: The relationship between the CO_2-to-CH_4 bioconversion pathways and H_2 partial pressure, temperature, and other operating parameters of the reactor have been added in the revised manuscript as follows (L133-148).
   "Hydrogenotrophic methanogenesis and homoacetogenesis are affected and regulated by many factors including H_2 partial pressure, temperature, and other operating parameters of the reactor. The H_2 partial pressure may affect the metabolism directly. Hydrogenotrophic methanogenesis and homoacetogenesis could only take place at high H_2 partial pressure (≥500 Pa), while **syntrophic acetate oxidation** to form H_2 and CO_2 would occur at low H_2 partial pressure [31]. Temperature is another crucial factor affecting the CO_2-to-CH_4 bioconversion pathway. Hydrogenotrophic methanogens are more active at thermophilic conditions, while homoacetogens are better adapted to lower temperatures [3, 31]. As Zhu and colleagues reported, most of the added H_2 was consumed to reduce CO_2 to CH_4 according to the indirect pathway under mesophilic conditions, while the direct hydrogenotrophic methanogenesis pathway predominated under thermophilic condition [12]. Some other operating parameters such as pH and ammonia concentration may also affect the bioconversion pathway. The alkaline pH favors the conversion of H_2 and CO_2 to acetate by homoacetogens rather than CH_4 by hydrogetrophic methanogens [32]. Wang and colleagues reported that the ammonia level can affect the CO_2-to-CH_4 bioconversion pathway. A shift from aceticlastic pathway to hydrogenotrophic pathway occurred when the ammonia level increased (1-7g NH^{4+}-N/L) [24]".
5. *Line 130, remove "produced"*
   Response: Revised, as suggested.
6. *Line 131-133, this sentence is inconsistent.*
   Response: This sentence has been revised as follows (L154-156).
   "Insufficient H_2 supply leads to incomplete conversion of CO_2 into CH_4 and inadequate CO_2 removal from biogas, while excess H_2 supply could lead to high residual H_2 content in biogas".
7. *The direct interspecies electron transfer (DIET) could be described in detail in Glossary or the Box.*
   Response: Thanks for your suggestion. We have added the detail of DIET in Glossary (Line 340-343).
8. *Line 172-174, does the MEC also have some shortages?*
   Response: The challenges of MEC have been added in Box 3 (Line 416-419).
9. *Line 257, please using consistent words either bio-natural gas or biomethane.*
   Response: We have used a consistent word of biomethane.
10. *Line 267-268, this sentence here seemed to be contradictory to the topic of this manuscript.*
    Response: This sentence has been removed in the revised manuscript.

11. *Line 273-276, remove "still has".*

    Response: Revised, as suggested.

12. *There are also some works about ex-situ upgrading. It is better to give some suggestions for at which conditions the ex-situ should be pursued. Or ex-situ upgrading could be an option to overcome some present challenges such as pH issues.*

    Response: Ex-situ biogas upgrading means the biogas upgrading is conducted in a separate reactor, where biogas and a suitable amount of H_2 together are injected. Ex-situ biogas upgrading could be an option for the pH issues, which has been added in our revised manuscript as follows (L240-242).

    "Moreover, ex-situ biogas upgrading by decoupling the main biogas process from the upgrading process is also a potential solution, which is under development".

Highlights

1. Upgrading biogas into biomethane broadens its applications and increases the value of biogas. Among others, *in-situ* CO_2-to-CH_4 bioconversion could capture the endogenous CO_2 directly from the biogas reactor, which is easy to operate, and provides infrastructure for renewable electricity storage.
2. Though promising, several intrinsic challenges need to be addressed to move in-situ upgrading technologies closest to scale applications.
3. *In-situ* CO_2-to-CH_4 bioconversion powered by renewable electricity could integrate of multidisciplinary approaches including wind or solar energy technology, P2G technology, AD technology, and biogas upgrading technology.
4. Bioelectrochemical systems represent a potential technology for biogas upgrading as well as the storage of discontinuous wind/solar energy. However, its molecular mechanism and scale-up feasibilities need to be further explored.

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