



In situ Biogas Upgrading by CO₂-to-CH₄ Bioconversion

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Trends in Biotechnology

In-situ Biogas Upgrading by CO₂-to-CH₄ Bioconversion

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Abstract:	<p>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.</p>

TECHNICAL UNIVERSITY OF DENMARK
DTU-ENVIRONMENT



Dear Editor,

We appreciate yours and the reviewers' efforts for commenting and editing our paper entitled "In-situ Biogas Upgrading by CO₂-to-CH₄ 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,

Yifeng Zhang

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1 **Opinion**2 **In-situ Biogas Upgrading by CO₂-to-CH₄ Bioconversion**3
4
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30 **Abstract**

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

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

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59 **Anaerobic digestion and the needs of biogas upgrading**

60 **Anaerobic digestion (AD)** (see **Glossary**) is a complex biochemical process, during which organic
61 matter is digested to form **biogas** through four steps: hydrolysis, acidogenesis, acetogenesis, and
62 methanogenesis (**Box 1**) [1]. The produced biogas has a **lower calorific value (LCV)** of 20–25 MJ/m³
63 based on the **CH₄** content, and it is considered to be one of the most important renewable energy
64 carriers [2, 3]. AD technology has been used worldwide for organic waste treatment and **bioenergy**
65 production [4]. Facing the problems of energy shortage and environmental pollution caused by the
66 over-consumption of **fossil fuels**, the global biogas industry has developed significantly in recent years
67 [5, 6]. The annual biogas production in Europe and China is expected to reach 300 billion m³ by 2020
68 [7], and the global power production capacities from commercial biogas facilities are predicted to
69 reach 29.5 GW in 2022 [8].

70 The produced biogas is composed of approximately 60% CH₄ and 40% CO₂, with minor
71 concentrations of other gases including H₂, NH₃, H₂S, and others [9]. Traditionally, most of the raw
72 biogas is used to produce electricity and heat [10]. **To reach the specification of natural gas**, the CH₄
73 content in biogas should be upgraded at least to 95%, at which point it is called biomethane or bio-
74 natural gas [11, 12]. The biomethane can be used directly as vehicle fuel or injected into the natural
75 gas grid, thereby broadening the range of applications and increasing the value of biogas [13]. For this
76 purpose, **biogas upgrading**-increasing its CH₄ content by removing CO₂ or converting CO₂ into CH₄
77 is necessary [14]. The rapid development of the biogas industry and the growth of using upgraded
78 biogas as vehicle fuel has created a golden period of development for biogas upgrading technologies
79 and plants [15]. **The number of biogas upgrading plants in Europe has increased by 51% in 2 years,**
80 **from 483 in 2018 to 729 in 2020, among which Germany has the most biogas upgrading plants (232),**
81 **followed by France (131) and the UK (80) [Gas Infrastructure Europe (GIE); European Biogas**
82 **Association (EBA). EBA-GiE biomethane map 2020. Available online:**
83 **[https://www.europeanbiogas.eu/wp-](https://www.europeanbiogas.eu/wp-content/uploads/2020/06/GIE_EBA_BIO_2020_A0_FULL_FINAL.pdf)**
84 **[content/uploads/2020/06/GIE_EBA_BIO_2020_A0_FULL_FINAL.pdf](https://www.europeanbiogas.eu/wp-content/uploads/2020/06/GIE_EBA_BIO_2020_A0_FULL_FINAL.pdf)].** In Sweden, the use of
85 biogas as a vehicle fuel has exceeded the use of natural gas [16].

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

88 cutting-edge solution for biological biogas upgrading by in-situ CO₂-to-CH₄ conversion. The recent
89 understanding of the pathways and mechanisms that are involved in the in-situ biogas upgrading
90 processes are reviewed. The obstacles and opportunities of this research field are identified for future
91 development. Furthermore, perspectives and suggestions are provided to move the in-situ biogas
92 upgrading technologies closer to applications at scale.

93 **Technologies for biogas upgrading**

94 In general, biogas can be upgraded using physical, chemical, or biological means, or a combination
95 of these approaches [3]. Physical biogas upgrading (Box 2) is based on removing CO₂ from biogas
96 using organic solvent absorption, water scrubbing, pressure swing adsorption, cryogenic separation,
97 or membrane separation [2]. Chemical biogas upgrading (Box 2) occurs by reducing the CO₂ to CH₄
98 via the **Sabatier reaction**, using H₂ as a reductant [17]. Biological biogas upgrading (Box 2) covers
99 **photosynthetic** or **chemoautotrophic reactions** [3]. Physical and chemical technologies have been
100 commercially used in the biogas industry due to their advantages of high selectivity, efficiency, and
101 high CH₄ content in the upgraded biogas. However, these technologies also have disadvantages, such
102 as high investment and energy demand, and in some cases need for toxic solvents [18, 19]. In addition,
103 the CO₂ removed from the physical upgrading processes is released to the air, which wastes the carbon
104 source and contributes to global warming [20]. Comparatively, biological technologies, which use
105 microorganisms as catalysts to drive the conversion of CO₂ into CH₄ or other valuable bio-
106 commodities at moderate conditions (e.g., room temperatures and atmospheric pressure), have gained
107 increasing attention due to the unique advantages of much smaller carbon and energy footprints [21,
108 22]. The most important advantage of the biological upgrading methods is that CO₂ is captured and
109 recycled to new products. Biological upgrading methods can involve methanogens **binding** CO₂ into
110 CH₄, dicarboxylic acid-producing bacteria binding CO₂ into dicarboxylic acids such as bio-succinic
111 acid, or algae binding CO₂ into algal biomass [3].

112 Microalgae, bacteria or archaea have widely been used to upgrade biogas in recent years [23-26].
113 Based on the upgrading process configurations, these technologies can be classified into **in-situ**, **ex-**
114 **situ**, and hybrid biogas upgrading. In-situ H₂ injected directly in the biogas reactor captures the
115 endogenously produced CO₂, while ex-situ H₂, together with biogas, are injected in a separate reactor
116 [27]. Finally, hybrid upgrading is a combination of the in-situ and ex-situ approaches, where an initial

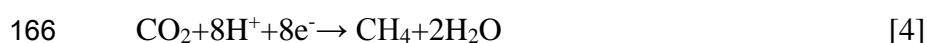
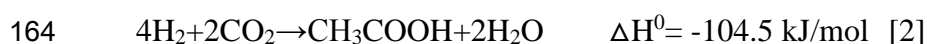
117 in-situ upgrading results in an acceptable pH for biological processes, while the last polishing
118 upgrading happens in an ex-situ process [28]. In-situ biogas upgrading can integrate biomethanation
119 and biogas upgrading in the same reactor, which is easier to operate and more economically attractive,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

121 **In-situ biogas upgrading by CO₂-to-CH₄ Bioconversion: the pathways and mechanisms**

122 There are three pathways for in-situ CO₂-to-CH₄ bioconversion: the direct hydrogenotrophic
123 methanogenesis pathway, the indirect pathway through homoacetogenic acetate formation followed
124 by acetoclastic methanogenesis, and the direct electron transfer (DET) pathway (Figure 1, Key Figure).
125 The direct hydrogenotrophic methanogenesis pathway is driven by hydrogenotrophic methanogenic
126 archaea using H₂ as an electron donor to directly reduce CO₂ into CH₄ through the so-called Wolf cycle
127 (Equation 1, Figure 1A), which is also the most widely studied pathway for in-situ biogas upgrading
128 [23]. In the indirect pathway, the CO₂ is first converted into acetate, mediated by homoacetogenic
129 bacteria via the Wood-Ljungdahl reaction (Equation 2, Figure 1B) [29], and then the formed acetate is
130 degraded by acetoclastic methanogenic archaea to generate CH₄ (Equation 3). The DET pathway
131 occurs at the **biocathode** of a microbial electrochemical system through a so-called
132 **electromethanogenic** process, in which methanogens obtain electrons directly from the biocathode
133 via physical contact with the electrode (Equation 4, Figure 1D) [30]. Hydrogenotrophic
134 methanogenesis and homoacetogenesis are affected and regulated by many factors including H₂ partial
135 pressure, temperature, and other operating parameters of the reactor. The H₂ partial pressure may affect
136 the metabolism directly. Hydrogenotrophic methanogenesis and homoacetogenesis could only take
137 place at high H₂ partial pressure (≥ 500 Pa), while **syntrophic acetate oxidation** to form H₂ and CO₂
138 would occur at low H₂ partial pressure [31]. Temperature is another crucial factor affecting the CO₂-
139 to-CH₄ bioconversion pathway. Hydrogenotrophic methanogens are more active at thermophilic
140 conditions, while homoacetogens are better adapted to lower temperatures [3, 31]. As Zhu and
141 colleagues reported, most of the added H₂ was consumed to reduce CO₂ to CH₄ according to the
142 indirect pathway under mesophilic conditions, while the direct hydrogenotrophic methanogenesis
143 pathway predominated under thermophilic condition [12]. Some other operating parameters such as
144 pH and ammonia concentration may also affect the bioconversion pathway. The alkaline pH favors the
145 conversion of H₂ and CO₂ to acetate by homoacetogens rather than CH₄ by hydrogetrophic

146 methanogens [32]. Wang and colleagues reported that the ammonia level can affect the CO₂-to-CH₄
147 bioconversion pathway. A shift from acetoclastic pathway to hydrogenotrophic pathway occurred when
148 the ammonia level increased (1-7g NH₄⁺-N/L) [24].

149 Exogenous H₂ addition is the most widely used approach for in-situ CO₂-to-CH₄ bioconversion
150 (Figure 1C) [34-37]. Usually, the H₂ can be obtained from coal gasification, petroleum refinery,
151 petrochemical plants, soda manufacture or directly electrolysis of water [15]. Recently, using
152 renewable excess electricity, such as excess wind or solar energy for H₂ production has drawn
153 attentions due to economically and environmentally sustainability [38]. In this process, H₂ supply and
154 mass transfer play key roles. Insufficient H₂ supply leads to incomplete conversion of CO₂ into CH₄
155 and inadequate CO₂ removal from biogas, while excess H₂ supply could lead to high residual H₂
156 content in biogas [39]. Based on Equation 1, an H₂:CO₂ ratio of 4:1, which corresponds to the
157 stoichiometric ratio, is usually recommended [40]. Recently, the bioelectrochemical power-to-gas
158 (Box 3) process has been studied as a promising alternative for in-situ biogas upgrading (Figure 1D)
159 [41-43]. Similarly to the other in-situ methods, renewable electricity can be used to hydrolyze water
160 to form O₂ and H⁺ at the anode, and then the H⁺ and e⁻ are transferred to the biocathode to reduce CO₂
161 into CH₄ [44]. The main difference is that the reducing equivalents are provided as H⁺ and e⁻ rather
162 than H₂ [45-47].



167 **Challenges and opportunities**

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

173 *The low gas to liquid phase transfer rate caused by the low solubility of H₂*

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

175 the biological processes take place. This is a common problem for all biological upgrading
176 technologies. The dissolved H₂ is a key factor, as microorganisms can only use the dissolved H₂ for
177 the reaction [49]. The gas-to-liquid mass transfer of H₂ can be described by Equation 5 [50].

$$178 R_t = 22.4k_{LA}(H_{2gTh} - H_{2l}) \quad (5)$$

179 Here, R_t , 22.4, k_{LA} , H_{2gTh} and H_{2l} represent the gas-to-liquid mass transfer rate [L/(L·day)], the
180 volume of 1 mol gas at standard temperature and pressure (L/mol), gas transfer coefficient (day⁻¹), H₂
181 concentration in the gas phase (mol /L) and dissolved H₂ in the liquid phase (mol /L), respectively.

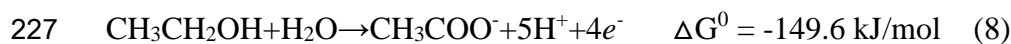
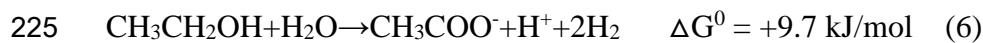
182 Equation 5 shows that the gas-to-liquid mass transfer rate of H₂ has a positive correlation with the
183 gas transfer coefficient. Therefore, increasing the gas transfer coefficient can be a simple and
184 convenient method to improve the H₂ gas-to-liquid mass transfer rate. Intense stirring [48], minimizing
185 the gas bubble size by adding packing materials [50], biogas recirculation [51], and increasing the
186 pressure in reactors [52] have been reported to increase the gas transfer coefficient. In addition, some
187 novel methods including membrane modules and bioelectrochemical systems have also been recently
188 used to overcome this challenge. For instance, using a hollow-fiber membrane increased the H₂ gas
189 transfer coefficient from 30 to 430 h⁻¹, and the final CH₄ concentration in biogas was upgraded to 95%
190 [53]. Even so, membrane fouling and short service life are still problems, more efforts should be paid
191 on new material selection, and materials and surface modification. Direct electron uptake or in-situ H₂
192 generation in the cathode of microbial electrolysis cell (MEC) could also be an efficient solution [18].
193 However, the electron transfer mechanisms of technology are not fully clarified, low cathode potential
194 and energy efficiency limit its application. In addition, reactor design, using bio-surfactants to reduce
195 the surface tension without effect on the biological process are all potential solutions for the low gas
196 to liquid phase transfer rate, which need to be further studied.

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

198 A challenge of in-situ upgrading is that the process in the main biogas reactor might be disturbed by
199 the direct addition of exogenous H₂ into the biogas reactor. During the biogas fermentation process,
200 the degradation of volatile fatty acids (VFAs) and alcohols is prevented at standard conditions (1 atm
201 partial pressures, molar concentrations of reactants and products, and 0 °C temperature) because these
202 processes are endergonic at standard conditions ($\Delta G > 0$, Equation 6, Equation 7). Therefore, syntrophy
203 between fermentative microorganisms and methanogens is needed to constantly reduce the product

204 concentrations, create a negative ΔG at reactor conditions, and make the reactions exergonic [54].
 205 Degradation of VFAs and alcohols requires a rather low H_2 concentration, which can only be achieved
 206 by syntrophic growth [54]. Exogenous H_2 addition into the AD reactor could lead to increased H_2
 207 concentration (unless it is consumed quickly), which could inhibit the syntrophic degradation of VFAs
 208 and alcohols, lead to their accumulation, and eventually inhibit the AD process. Moreover, exogenous
 209 H_2 addition could stimulate the production of acetate via the Wood-Ljungdahl pathway, potentially
 210 inhibiting AD if the produced acetate could not be converted into CH_4 quickly [39]. Luo and colleagues
 211 observed the inhibition of propionate and butyrate degradation by H_2 addition with a pressure of 1 atm
 212 [48]. Nevertheless, the inhibition of syntrophic degradation of VFAs and alcohols by exogenous H_2
 213 addition could also be reversed after longer-term H_2 exposure due to the improved relative abundance
 214 of hydrogenotrophic methanogens in the microbial communities [3]. Therefore, the enrichment of
 215 hydrogenotrophic methanogens via long term acclimatization or bioaugmentation with
 216 hydrogenotrophic methanogens directly could be promising countermeasures for this challenge by
 217 rapidly consuming the added exogenous H_2 .

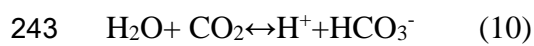
218 Furthermore, recent studies reported **direct interspecies electron transfer (DIET)** between
 219 fermentative microorganisms and methanogens mediated by pili or cytochrome C on the cell
 220 membrane or aid of conductive materials [55]. DIET could thus establish syntrophic degradation of
 221 VFAs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



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

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

233 The reduction of CO₂ into CH₄ by adding exogenous H₂ directly into a biogas reactor (in-situ biogas
234 upgrading), would lower the level of endogenously produced CO₂ in the liquid and thereby increasing
235 pH above 8.5, which in return would inhibit the biological processes [31]. In general, the ideal pH for
236 AD ranges from 6.6 to 7.8 [58], and therefore the increased pH during in-situ biogas upgrading might
237 inhibit the AD process. pH control with chemicals or co-digestion with a substrate of low pH could be
238 a promising solution to this challenge. Luo and colleagues reported that co-digestion of manure with
239 acidic whey could maintain the pH of the AD system around 7.8 with the addition of exogenous H₂ for
240 in-situ biogas upgrading, which was in the optimal pH range for AD [59]. Moreover, ex-situ biogas
241 upgrading by decoupling the main biogas process from the upgrading process is also a potential
242 solution, which is under development [28].



244 *Economic aspects*

245 In-situ biogas upgrading by CO₂-to-CH₄ bioconversion relies on an accessible, available, and cost-
246 effective reducing agent. H₂ assisted biogas upgrading is a well-studied method for bioconversion of
247 CO₂ into CH₄, and the newly emerging bioelectrochemical bioconversion of CO₂ has also attracted
248 attention. At present, the H₂ production processes are highly costly. Thus, in-situ biogas upgrading by
249 bioconversion of CO₂ into CH₄ currently seems to be more expensive than physicochemical biogas
250 upgrading. However, the environmental benefit of CO₂-to-CH₄ bioconversion could be regarded as an
251 offset of CO₂ emissions [60], and CO₂-to-CH₄ bioconversion usually leads to improved CH₄ yield,
252 which could compensate part of the cost for H₂ production. In addition, in-situ biogas upgrading also
253 allows integrating biogas production and upgrading in a single reactor without further investment in
254 additional reactors [22].

255 Using intermittent renewable energy to power in-situ biogas upgrading by CO₂-to-CH₄
256 bioconversion can valorize renewable energy by converting intermittent or excessive electricity along
257 with CO₂ into CH₄ for later use. Wind energy and solar energy are the most plentiful renewable energy
258 sources [61]. However, the electrical energy produced from wind and solar is intermittent, so it cannot
259 meet the variable consumption demands [31]. **Power-to-gas** (P2G) technology, which utilizes
260 excessive electricity to split the water and generate H₂ for later use (Equation 11), represents an
261 attractive method for electricity storage [62]. Several inherent disadvantages e.g. low volumetric

262 energy, safety and storage issues limit the utilization of H₂ [39]. Meanwhile, the utilization technology
263 of H₂ as vehicle fuel is still immature [62]. Using H₂ to reduce the CO₂ into CH₄ (Equation 1, Equation
264 2, and Equation 3) could simultaneously be considered as both H₂ storage and biogas upgrading. In
265 addition to H₂ production, renewable surplus electricity could also be used as a power source to convert
266 CO₂ into CH₄ in the bioelectrochemical systems, thereby upgrading the biogas. In the long term, the
267 production cost for renewable electricity is expected to decrease. The decreased cost of renewable
268 energy, the need for storage of intermittent or excessive renewable energy, the shortage of fossil fuels,
269 and the increasing requirements for a sustainable circular bioeconomy, combined with the rapid
270 development of CO₂ bioconversion technology, provides a golden opportunity for in-situ biogas
271 upgrading technology as an economically favorable avenue compared with the traditional
272 physicochemical biogas upgrading processes in the near future.



274 **Concluding Remarks and Future Perspectives**

275 Biogas upgrading to convert raw biogas into biomethane broadens the scope of biogas applications
276 and increases the value of biogas. In-situ CO₂-to-CH₄ bioconversion represents a promising avenue
277 for biogas upgrading due to its advantages e.g. simple and easy operating, carbon capture and
278 utilization, renewable electricity storage, economic and environmental-friendly, etc. Despite recent
279 advances, several challenges need to be addressed and certain questions need to be answered (see
280 **Outstanding Questions**) before its practical application.

281 Though the pathways and main microorganisms of the CO₂-to-CH₄ bioconversion have been
282 identified, the molecular mechanism, metabolic process, and microbial responses under different
283 operating parameters are not fully clarified, especially for the electromethanogenesis process. Further
284 researches using advanced techniques such as genome-centric metatranscriptomics, isotope tracer
285 technique, and high-throughput sequencing are needed to uncover the phylogenetic and metabolic
286 properties of the CO₂-to-CH₄ bioconversion processes. Metabolic regulation and genetic engineering
287 approaches are also expected to improve the bioconversion efficiency of CO₂.

288 Besides, recent studies of CO₂-to-CH₄ bioconversion are limited to bench-scale. For scaling-up and
289 industrial implementation, the performance and stability of in-situ biogas upgrading processes at long-
290 term continuous operation are still unknown. Future research in this direction will help to further

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

294 Moreover, the practical application of the technology is quite dependent on the availability of
295 renewable electricity which is the main source of H₂ or electrons. Thus, the improvement of the
296 efficiency in solar and wind energy technologies or other renewable energy sources could also promote
297 the in-situ biogas upgrading. So far, commercial H₂ gas or commercial power supply is used in most
298 of the studies. In these studies, it is always claimed that H₂ or electrons could be obtained from
299 renewable energy, but the real integration of these two processes is rarely reported. In the future,
300 integration of solar panel or even development of photoelectrode should be pursued, since we need
301 this information to further evaluate the applicability of the whole concept. Also, the recent developed
302 nanoparticles such as cadmium sulfide nanoparticles [63] could be used for the in-situ harvesting solar
303 energy and power in-situ CO₂-to-CH₄ conversion without external power supply, which may provide
304 an alternative avenue for the in-situ biogas upgrading.

305 Furthermore, conversion of the CO₂ into higher-value chemicals (e.g., higher alcohols) [15] or
306 downstream technology that could further convert methane to higher-value products such as microbial
307 protein using methanotrophic bacteria [64] could help to make the whole process more profitable.

308 In summary, it is a long journey for a novel technology to reach real-life implementation. Having
309 identified the challenges that exist, and as solutions are developed, the in-situ biogas upgrading
310 technology by CO₂-to-CH₄ bioconversion is experiencing rapid development and should reach
311 practical application in the near future.

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320 **Glossary**

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

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

330 **Bioenergy:** renewable energy produced from biomass.

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

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

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

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

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

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

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

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

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

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

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

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

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

356 **Photosynthetic reaction:** the process by plants and photosynthetic microorganisms that can reduce
357 CO₂ with H₂O into chemical energy in the form of carbohydrates by extracting reducing equivalents
358 from light. The process is catalyzed by chlorophyll.

359 **Power-to-gas (P2G):** a process that can convert electrical power to gas fuels, such as H₂ or CH₄, which
360 in turn can be further converted to other fuels or bio-products.

361 **Sabatier reaction:** a chemical process for converting H₂ and CO₂ to CH₄. The process requires
362 elevated temperatures and pressures and the presence of specific catalysts (CO₂+4H₂ → CH₄+2H₂O).

363 **Syntrophic acetate oxidation:** A two-step reaction in which the acetate is first oxidized by syntrophic
364 acetate oxidizing bacteria to form H₂ and CO₂, which are subsequently converted into CH₄ by
365 hydrogenotrophic methanogens.

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378 **Box 1 Methanogenesis**

379 Methanogenesis is the process that methanogens use simple substrates e.g. H₂, CO₂, formate, acetate,
 380 and a variety of methyl-containing compounds to produce CH₄ under oxygen-free condition [65].
 381 Although the substrates for methanogenesis are simple, the CH₄ formation is a complex biochemistry
 382 process, among which at least six unusual coenzymes and about 200 genes are involved [66]. Based
 383 on the substrate, the methanogens could be typically classified into three groups: hydrogenotrophic
 384 methanogens, aceticlastic methanogens, and methylotrophic methanogens (Table I). Methanogenesis
 385 is largely affected by many parameters, among which pH and temperature are two of the most
 386 important factors. The optimal pH for methanogenesis is in a range of 6.6-7.8 [59]. Based on the
 387 optimal temperature the methanogens could be divided into psychrotolerant methanogens (around
 388 18°C), mesophilic methanogens (around 37 °C), thermophilic methanogens (around 55 °C) and
 389 hyperthermophilic methanogens (around 65 °C).

390 **Table I Methanogenesis**

Methanogens	Substrate	Reactions	Typical Methanogens	Typical habitat
Hydrogenotrophic methanogens	H ₂ and CO ₂ Formate Methanol	4H ₂ +CO ₂ →CH ₄ +2H ₂ O 4HCOOH→CH ₄ +3CO ₂ +2H ₂ O 4CH ₃ OH→3CH ₄ +CO ₂ +2H ₂ O	<i>Methanobacterium bryantii</i> <i>Methanobacterium formicicum</i> <i>Methanobacterium thermoalcaliphium</i> <i>Methanothermobacter thermoautotrophicum</i> <i>Methanothermobacter wolfeii</i> <i>Methanobrevibacter smithii</i> <i>Methanobrevibacter ruminantium</i> Methanococcoides methylutens	Deep marine sediments Termite hindguts Human gastrointestinal tracts Animal gastrointestinal tracts
Aceticlastic methanogens	Acetate	CH ₃ COOH→CH ₄ +CO ₂	<i>Methanosaeta concilii</i> (<i>soehngenii</i>) <i>Methanosaeta thermophila</i>	Anaerobic digesters Rice fields Wetlands
Methylotrophic methanogens	Trimethylamine Dimethyl sulfate Methylated ethanolamines	4(CH ₃) ₃ N+6H ₂ O→9CH ₄ +3CO ₂ +4NH ₃ 2(CH ₃) ₂ NH+2H ₂ O→3CH ₄ +CO ₂ +2NH ₃ 4(CH ₃)NH ₂ +2H ₂ O→3CH ₄ +CO ₂ +4NH ₃ 2(CH ₃) ₂ S+2H ₂ O→3CH ₄ +CO ₂ +H ₂ S	<i>Methanosarcina barkeri</i> <i>Methanosarcina mazei</i> <i>Methanosarcina thermophile</i>	Marine Hypersaline habitat Sulfate-rich sediments

391 **Box 2 Biogas upgrading technologies**

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

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

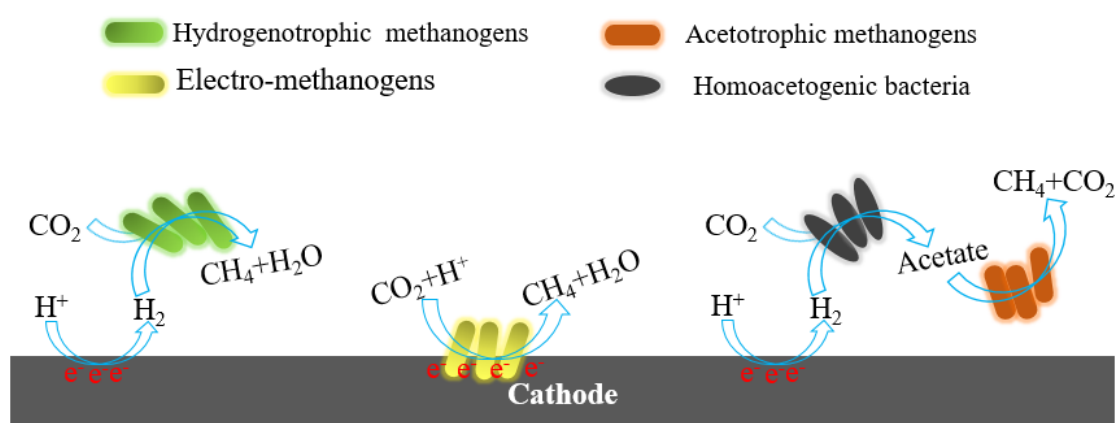
Strategies	Mechanism		Advantage	Disadvantage
Physical technologies	Water scrubbing	Based on the difference in solubility in water of different gases. Compared with CH ₄ , CO ₂ and H ₂ S have a much higher solubility in water.	<ul style="list-style-type: none"> ✓ High CH₄ purity up to 99% ✓ Simultaneous removal of CO₂ and H₂S ✓ Simple process, easy operation, low CH₄ loss (less than 2%) ✓ No need of harmful chemicals, the water could be regenerated 	<ul style="list-style-type: none"> ✓ 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.	<ul style="list-style-type: none"> ✓ Low energy cost and capital investment ✓ High CH₄ purity ranged 96–98% ✓ Safety and easy operation ✓ water-free process and clean gas produced ✓ simplicity of operation 	<ul style="list-style-type: none"> ✓ Higher CH₄ loss (up to 4%) ✓ Pre- purification equipment need to remove H₂S 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.	<ul style="list-style-type: none"> ✓ High efficiency ✓ High CH₄ purity up to 99% ✓ Easy operation, low CH₄ loss (less than 0.1%) ✓ Simultaneous removal of CO₂ and H₂S 	<ul style="list-style-type: none"> ✓ 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.	<ul style="list-style-type: none"> ✓ Less liquid inputs, smaller dimensions of the upgrading unit ✓ High CH₄ purity up to 98% ✓ Simultaneous removal of CO₂ and H₂S ✓ Simple process, easy operation, low CH₄ loss 	<ul style="list-style-type: none"> ✓ Toxic organic solvents needed ✓ Difficult regenerated of organic solvents due to the high solubility of CO₂ ✓ High temperatures are required for H₂S separation
	Cryogenic separation	Different gases condense at different temperature and pressure domains	<ul style="list-style-type: none"> ✓ High CH₄ purity > 97% ✓ Recovery of CO₂ with high purity ✓ Environmental friendly 	<ul style="list-style-type: none"> ✓ 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	<ul style="list-style-type: none"> ✓ 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 	<ul style="list-style-type: none"> ✓ 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.	<ul style="list-style-type: none"> ✓ High selectivity and process efficiency ✓ Conversion of CO₂ into CH₄, reduction of CO₂ emission ✓ High CH₄ purity 	<ul style="list-style-type: none"> ✓ Easily affected by the trace gasses in the biogas ✓ High amount of pure H₂ 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	<ul style="list-style-type: none"> ✓ High methane recovery up to approximately 97% ✓ Transformation of CO₂ into other products ✓ Production of active biomass ✓ low requirements in terms of land and water 	<ul style="list-style-type: none"> ✓ High investment cost and energy demands ✓ Low photosynthetic CO₂ uptake and high natural sources requirements ✓ High risk of biological contamination
	Chemoautotrophic reaction	Methanogenesis of CO ₂ based on the action of methanogens	<ul style="list-style-type: none"> ✓ High selectivity, process efficiency, and CH₄ purity ✓ Conversion of CO₂ into CH₄, reduction of CO₂ emission ✓ Moderate temperatures and atmospheric pressure ✓ Easily integrate with the AD process 	<ul style="list-style-type: none"> ✓ High amount of reductant needed ✓ This technology is still under developing

		✓ Environmentally friendly	
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403 **Box 3 Bioelectrochemical power-to-gas**

404 Bioelectrochemical power-to-gas is a cutting-edge technology that relays on microbial reduction of
405 CO₂ into CH₄ with an additional supply of electrical energy. The bioelectrochemical power-to-gas
406 system mainly consists of two compartments: anodic compartment where water splits into O₂, H⁺ and
407 e⁻; cathodic compartment where CO₂ is reduced into CH₄ by microorganisms [42]. Though a cation
408 exchange membrane is usually used to separate anodic and cathodic compartments in most of the cases,
409 it is not essential for the smoothly running of the system.

410 Figure I summarizes the methanogenesis pathways in the bioelectrochemical power-to-gas system.
411 The methanogenesis pathways include acetoclastic methanogenesis and hydrogenotrophic
412 methanogenesis, during which H₂ is used as an electron donor to transport the electrons from the
413 cathode to the methanogens; and direct electron transfer (DET) from cathode to methanogens via
414 physical contact without the intermediate production of H₂ is also reported [65]. Many factors include
415 cathode potential, electron donor, operational parameters, and inoculum used are highly associated
416 with the methanogenesis performance of bioelectrochemical power-to-gas system. **Until now, the
417 bioelectrochemical power-to-gas is limited to lab-scale study, its molecular mechanism is still not fully
418 identified, and some shortages such as low cathode potential and energy efficiency limit its industrial
419 application.** However, this technology has drawn increasing attention and is expected to be a promising
420 option for in-site biogas upgrading.



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

Figure 1. (Key Figure) The pathways and mechanisms for in-situ biogas upgrading through bioconversion of CO₂ into methane. (A) Wolf cycle for reduction of CO₂ into CH₄ (H₄MPT: tetrahydromethanopterin; MFR: methanofuran; F₄₂₀: coenzyme F₄₂₀; Fd: specific ferredoxin). Adapted from Thauer (2012) [33]. (B) Wood-Ljungdahl reaction for homoacetogenic acetate production and acetate split for CH₄ and CO₂ production (FH₄: tetrahydrofolate). Adapted from Can (2014) [29]. (C) In-situ biogas upgrading by injection of H₂ directly into the AD reactor. (D) bioelectrochemical biogas upgrading systems.

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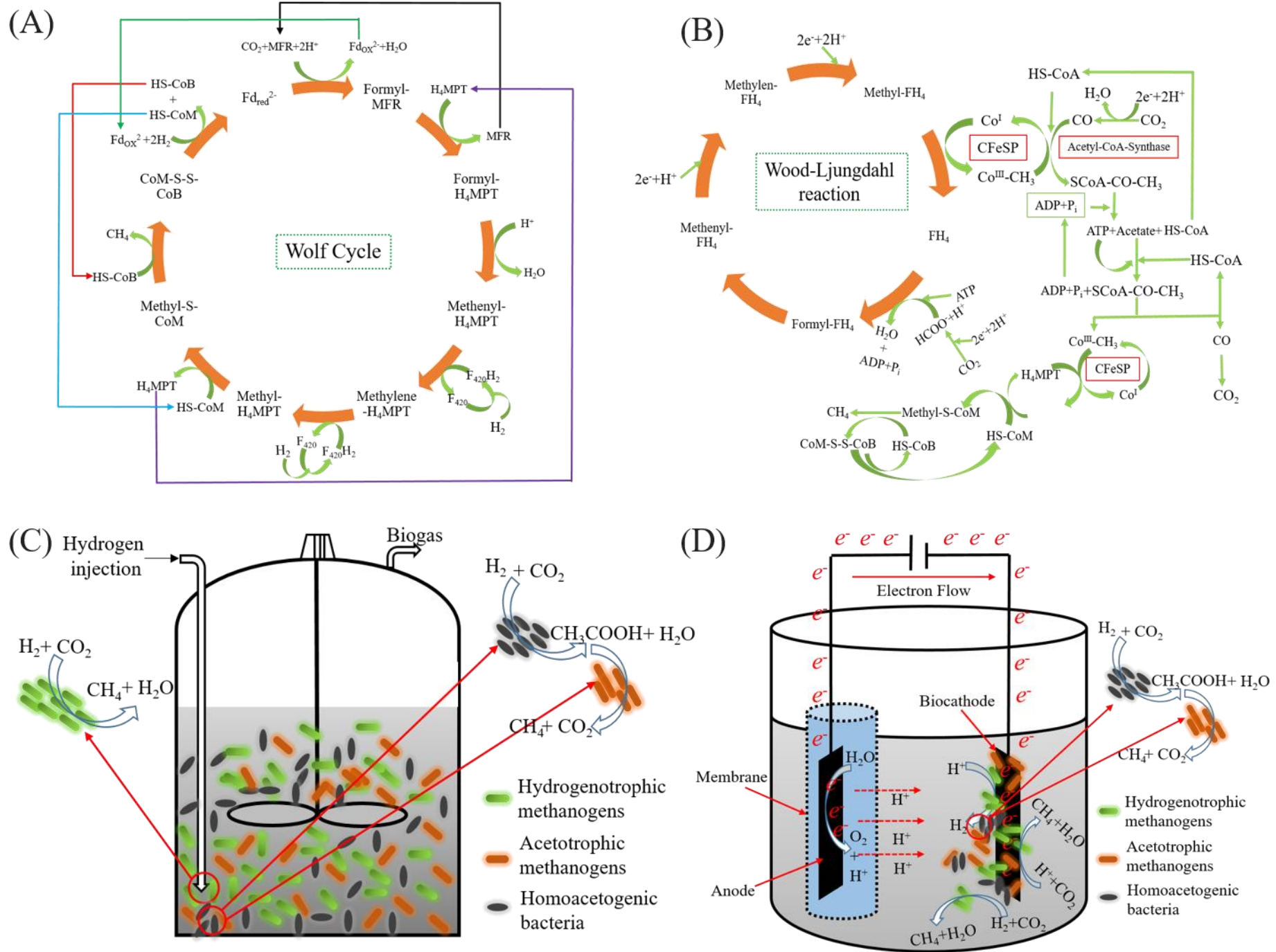
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Figure 1, key figure



Outstanding Questions

1. What are the main factors that limit the industrial application of in-situ biogas upgrading by CO₂-to-CH₄ 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 CO₂-to-CH₄ bioconversion?
4. Are the microbiome and interspecies interactions associated with the CO₂-to-CH₄ 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 CO₂-to-CH₄ 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 revivied 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 CO₂-to-CH₄ 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.

Responses to reviewers

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 CO₂ 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. "CH₄" 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 H₂ supply leads to incomplete conversion of CO₂ into CH₄ and inadequate CO₂ removal from biogas, while excess H₂ supply could lead to high residual H₂ 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 CO₂ into CH₄, while the sentences in line 135-139 described the approach used for bioconversion of CO₂ into CH₄. 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 H₂ addition could stimulate the production of acetate via the Wood-Ljungdahl pathway, potentially inhibiting AD if the produced acetate could not be converted into CH₄ quickly.”

17. Line 218: "is put" should be modified

Response: We have changed this sentence as follows (L233-235).

“The reduction of CO₂ into CH₄ by adding exogenous H₂ directly into a biogas reactor (*in-situ* biogas upgrading), would lower the level of endogenously produced CO₂ 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 CO₂ into biomass. However, the microalgae could not realize

the conversion of CO₂ into CH₄. Since the focus of the current paper was in CO₂ capture to CH₄, algae was not been included.

3. *Line 108, remove "biogas".*

Response: Revised, as suggested.

4. *Section "In-situ biogas upgrading by CO₂-to-CH₄ 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 CO₂-to-CH₄ bioconversion pathways and H₂ 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 H₂ partial pressure, temperature, and other operating parameters of the reactor. The H₂ partial pressure may affect the metabolism directly. Hydrogenotrophic methanogenesis and homoacetogenesis could only take place at high H₂ partial pressure (≥ 500 Pa), while **syntrophic acetate oxidation** to form H₂ and CO₂ would occur at low H₂ partial pressure [31]. Temperature is another crucial factor affecting the CO₂-to-CH₄ 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 H₂ was consumed to reduce CO₂ to CH₄ 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 H₂ and CO₂ to acetate by homoacetogens rather than CH₄ by hydrogetrophic methanogens [32]. Wang and colleagues reported that the ammonia level can affect the CO₂-to-CH₄ bioconversion pathway. A shift from aceticlastic pathway to hydrogenotrophic pathway occurred when the ammonia level increased (1-7g NH₄⁺-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 H₂ supply leads to incomplete conversion of CO₂ into CH₄ and inadequate CO₂ removal from biogas, while excess H₂ supply could lead to high residual H₂ 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.

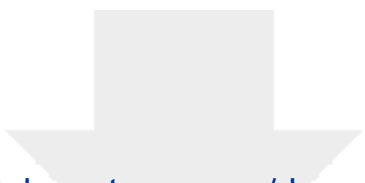
12. *There are also some works about ex-situ upgrading. It is better to give some suggestions for at which conditions the ex-situ should be pursued. Or ex-situ upgrading could be an option to overcome some present challenges such as pH issues.*

Response: Ex-situ biogas upgrading means the biogas upgrading is conducted in a separate reactor, where biogas and a suitable amount of H₂ 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* CO₂-to-CH₄ bioconversion could capture the endogenous CO₂ 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* CO₂-to-CH₄ 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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