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ABSTRACT

Microbial electrolysis cells (MECs) are a promising technology for biological hydrogen production. Compared to abiotic water electrolysis, a much lower electrical voltage (~ 0.2 V) is required for hydrogen production in MECs. It is also an attractive waste treatment technology as a variety of biodegradable substances can be used as the process feedstock. Underpinning this technology is a recently discovered bioelectrochemical pathway known as "bioelectrohydrogenesis". However, little is known about the mechanism of this pathway, and numerous hurdles are yet to be addressed to maximize hydrogen yield and purity. Here, we review various aspects including reactor configurations, microorganisms, substrates, electrode materials, and inhibitors of methanogenesis in order to improve hydrogen generation in MECs.

Keywords: Microbial electrolysis cell; Hydrogen; Methane; Methanogenesis; Inhibitor; Bioelectrohydrogenesis

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Table of contents

1. Introduction

Hydrogen is an important chemical feedstock for many industries, such as the fertilizer industry for ammonia synthesis, and the oil industry for the conversion of crude oils into transportation fuels. It is a valuable energy carrier widely used to power hydrogen fuel cells (Logan 2004). However, most of the hydrogen is conventionally derived from fossil fuel-based resources, primarily natural gas, via chemical refinery processes (Milbrant et al., 2009). Hence, its production is generally considered as environmentally unsustainable. Biological production of hydrogen (bio-hydrogen) is a potentially more sustainable alternative, especially when organic wastes are used as the process feedstock (Hallenbeck and Benemann 2002).

One promising option for bio-hydrogen production is via "bioelectrohydrogenesis" which can be accomplished using an emerging technology platform known as bioelectrochemical systems (BESs) or microbial electrochemical technologies (METs) (Liu et al., 2005; Rozendal et al., 2006). BESs have been developed for a wide range of applications, including wastewater treatment, fuel gas production (H_2, CH_4), nutrient recovery, chemical synthesis, desalination and bioremediation (Sleutels et al., 2012). A key feature of this technology is that it employs microorganisms to catalyze redox reactions at conductive electrode surfaces. The most widely studied BESs are either microbial fuel cells (MFC), which aim to produce electricity; and microbial electrolysis cells (MECs), which aim to produce biogas or value added chemicals (Logan et al., 2008; Clauwaert et al., 2009; Chookaew et al., 2014). During the conversion of bio-waste into H_2, exoelectrogenic bacteria first oxidize (degrade) organic matter and transfer the electrons to a solid electrode (bioanode) (Fig.2a). The electrons then travel through an external circuit and combine with protons at an anaerobic cathode resulting in the generation of hydrogen (Logan et al., 2008). Typically, the reducing power attainable with a bioanode is insufficient to drive the hydrogen evolution reaction

(HER) at the cathode. However, by supplementing the process with a small voltage (normally ranging from 0.2 V to 1.0 V) the cathodic HER can be facilitated in a MEC (Reaction 1&2). Since a much higher voltage (E^0 > 1.2 V) is required in conventional water electrolysis (Fig. 2b) processes (Reaction 3&4), using MEC for bio-hydrogen production is considered as an energy-efficient option. Indeed, it has been reported that the energy requirement for MECs is only about 0.6 kWh m^{-3} (0.2 mol H_2 energy/mol-H_2 produced), whereas in water electrolysis 4.5-5 kWh m^{-3} is required (1.5-1.7 mol H_2 energy/mol-H_2 produced) (Logan et al., 2008, Cheng and Logan 2007).

Microbial Electrolysis:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad E_{anode} = -0.279\ V \qquad (1)$$

$$2H^+ + 2e^- \rightarrow H_2 \quad E_{cathode} = -0.414\ V \qquad (2)$$

$$E^0 = E_{cathode} - E_{anode} = -0.135\ V$$

Water Electrolysis:

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \quad E_{anode} = 0.82\ V \qquad (3)$$

$$2H^+ + 2e^- \rightarrow H_2 \quad E_{cathode} = -0.414\ V \qquad (4)$$

$$E^0 = E_{cathode} - E_{anode} = -1.22\ V$$

Further, waste materials other than fossil fuels are used as the feedstock to drive the HER, and the H_2 production rate can be more than 1 $m^3H_2\ m^{-3}\ d^{-1}$(11 mol H_2/mol glucose), which is three times higher than dark fermentation (Logan et al., 2008; Wang and Ren 2013).

These features collectively make MECs a promising topic for research and development across the world, as reflected by the expanding volume of research outputs over the past decade

(Fig. 1). Nonetheless, only a few review articles have discussed the use of MEC for hydrogen production and methanogenesis (Logan et al., 2008; Geelhoed et al., 2010; Kundu et al., 2013; Zhou et al., 2013; Zhang and Angelidaki 2014; Kadier et al., 2014; Jafary et al., 2015; Escapa et al., 2016). A notable challenge to maximize hydrogen yields from MECs is the side production of methane via methanogenesis. Herein we discuss the currently available methods for the inhibition of methanogenesis in MECs, and highlight the use of chemical methanogenic inhibitors with the focus on their mechanisms underpinning at the enzymatic level. We suggest options of using these methanogenic inhibitors to improve the purity of the produced hydrogen from MECs. We also discuss chemical inhibition strategies for other undesirable microbes such as sulfate reducers and acetogens.

2. Reactor configurations

2.1. Two-chamber MECs

The concept of bioelectrohydrogenesis was first demonstrated with a two-chamber MEC design in 2005 (Liu et al., 2005). In this conventional design, the anode and cathode chambers are separated by an ion (proton) exchange membrane (Fig. 2a). Liu et al. (2000) observed that over 90% of the organic substrate (acetate) in the anode chamber was degraded at the end of batch mode with 78% coulombic efficiency (Fig. 3). However, the overall hydrogen production efficiency was only 60-73%. This is largely due to losses of the produced hydrogen in unwanted processes within the MEC, such as biomass production, conversion of substrate to polymers, and methanogenesis from hydrogen and acetate. To increase the hydrogen production efficiency in MECs, preventing hydrogen diffusion into the anode chamber is critical. Also, the internal resistance of the MEC must be minimized by reducing the distance between the electrode pair. It was reported that a higher rate of hydrogen (1.6 $m^3 m^{-3} d^{-1}$) could be obtained from two-chamber MECs using saline

117 catholyte, which provided high solution conductivity and hence lowered ohmic resistance (Nam
118 and Logan 2011). The use of a membrane is considered an effective way to minimize hydrogen
119 diffusion into the anode chamber, but it introduces complexity and cost to the process.
120 Nonetheless, in most cases the use of two-chamber MECs only enabled hydrogen production rates
121 ranging from 0.01 to 6.3 $m^3 m^{-3} d^{-1}$(Cheng and Logan 2011).
122
123 *2.2. Single-chamber MECs*
124 It is accepted that hydrogen evolution occurs due to the cathodic reduction reaction in MECs. The
125 cathodic conversion efficiency (CCE) can be calculated from the ratio of e^- equivalent donated to
126 hydrogen formation and e^- equivalent transferred from anode to cathode (Logan et al., 2008). A
127 CCE of less than 100% could be attributed to the diffusion of hydrogen to the anode surface, or to
128 biological oxidation. It was inferred that hydrogen diffusion would decrease the CCE by up to 33%
129 in two-chamber MECs (Tartakovsky et al., 2008). To maximize the overall efficiency of a MEC
130 for bioelectrohydrogenesis, the e^- equivalent liberated from the anodic substrate must first be
131 efficiently captured by the bio-anode, and subsequently dissipated at the cathode exclusively as
132 hydrogen gas for external collection. Indeed if the produced hydrogen gas could be rapidly
133 harvested to avoid hydrogen diffusion to the anode, the use of membrane may be omitted..
134 In fact, the use of single-chamber MECs for bioelectrohydrogenesis has been the subject
135 of many earlier studies (Rozendal et al., 2007; Call and Logan 2007; Hu et al., 2008; Tartakovsky
136 et al., 2009). A key attractive feature of single chamber MECs is that both the anode and cathode
137 are housed within one chamber. This single chamber MEC system could be more compact with a
138 lower capital cost. Further, single chamber MECs often exhibit a lower internal resistance. Such
139 systems generally have low ohmic loss and concentration overpotential due to the nonexistence of

140 detrimental pH gradient between the anolyte and catholyte.(Rozendal et al., 2007; Call and Logan
141 2007; Hu et al., 2008; Tartakovsky et al., 2009). Call et al., (2008) also found that the bio-hydrogen
142 production rate recorded from their single-chamber MEC was more than double (3.12 $m^3 m^{-3} d^{-1}$ at
143 an applied voltage of 0.8V) as compared to that obtained from a two-chamber MEC under identical
144 operating conditions.
145
146 *2.3. Continuous flow MECs*
147 Like most other waste treatment bioprocesses, MECs are often characterized for their
148 ability to treat their feedstock in a continuous fashion (Fig. 3). When operated in continuous mode,
149 the organic stream is continuously loaded into the MEC at a defined flow rate. Often, the liquid
150 electrolyte within a continuous flow system is recirculated to maximize mass transfer. The
151 hydraulic turbulence created as such may help to minimize the accumulation of stagnant hydrogen
152 gas in the porous electrode matrix (e.g. granular graphite bed), which may help to avoid any
153 undesirable biological oxidation (loss) of hydrogen in the reactor.
154 Both organic loading rate (OLR) and applied potential are significant parameters to
155 determine the yield of hydrogen from continuous flow MECs, and so these parameters are often
156 selected for process optimization (Cusick et al., 2011; Escapa et al., 2012; Rader et al., 2010). For
157 instance, Escapa et al. (2012) reported a Monod-type relationship between OLR and hydrogen
158 production rate (0.3 $m^3 m^{-3} d^{-1}$) in their continuous flow domestic waste water (DWW) fed MECs.
159 They found that the increase in hydrogen production rate reached a plateau, when the OLRs of
160 DWW were above 2000 mgCOD m^{-3} d^{-1}. In addition, the energy consumption for pumping the
161 solution should also be accounted. The produced H_2 and the energy consumption for pumping may
162 vary depending on the pumping flow rate. For instance, Kim and Logan (2011) noted that 4 x 10^{-}

163 ^{5} W was required for pumping flow rate at 0.8 ml min^{-1}. This was however, negligible (1%)
164 compared to the energy produced as H_2 (3.8 x 10^{-3} W) (Kim and Logan 2011).

165 Most of the MECs were operated with a single pair of electrodes, and only rarely multi-
166 electrode pair equipped MECs were used (Rader et al., 2010). Rader et al. (2010) evaluated a multi-
167 electrode MEC equipped with eight separate pairs of graphite fiber anodes and stainless steel
168 cathodes (with a working capacity of 2.5 L) for bioelectrohydrogenesis. They found that similar
169 to single pair systems, the hydrogen production rate in their multi-electrode system was also
170 directly proportional to the cathode surface area, yielding a hydrogen production rate of up to 0.53
171 $m^3 m^{-3} d^{-1}$ (Rader et al., 2010). The first pilot scale (1000 L) bio-hydrogen producing MEC was also
172 operated with the use of multiple electrode pairs in continuous mode for about 100 days using
173 winery wastewater as the feedstock (Cusick et al., 2011). Although the gas production of the pilot
174 system could reach up to 0.19 $m^3 m^{-3} d^{-1}$, the main component of the produced gas was methane
175 (86%) suggesting that most of the cathodically produced hydrogen was consumed by the
176 methanogens. Hence, to increase hydrogen yield, an effective method to prevent methanogenesis,
177 and to efficiently extract the hydrogen from the cathode is required. Other factors such as
178 enrichment of exoelectrogenic biofilms, optimization of electrolyte pH and electrode arrangements
179 are also paramount at a pilot scale level.

180 Further, to improve the hydrogen production efficiency from MEC reactors, a suitable
181 electrode configuration should be adopted. The planar electrodes (plate type) and flow through or
182 porous electrodes (3D type) are more common electrode types used in MEC reactors. The planar
183 electrode (e.g. graphite plate) has advantages such as high conductivity, chemical stability, low
184 cost and surface accessibility, and ease of placement (Zhou et al., 2011). However, it is difficult to
185 increase the surface area of the planar electrode. Gil-Carrera et al., (2011) increased the surface

area of the planar electrode by sandwiching the anode between a pair of cathodes. They found that the sandwich electrode only increased the current density rather than hydrogen production due to the activity of hydrogenotrophic methanogens. 3D type electrodes (e.g. graphite granules, graphite fiber brush, and reticulated vitreous carbon) have also been shown to have increased surface area as well as large relative porosity, and good electrical conductivity. Their major limitations are relatively high cost, clogging and biofouling that leads to large resistivity. Also, the main disadvantage of 3D electrode configuration in the MEC is the mass transport limitation at the anode matrix (Zhou et al., 2011; Escapa et al., 2016)

3. MEC components

Understanding the role of various components of a MEC system is critical to optimize the bio-hydrogen production rate. Table 1 summarizes the bio-hydrogen production performances and characteristics of some key components such as applied potential, substrates, microorganisms, and electrode materials in various MEC studies.

3.1. Effect of anode materials

The anode materials for MECs must be chosen based on several features such as - i. non-corrosive nature with electrolytes, ii. good electrical conductivity, iii. lack of toxicity to microorganisms, iv. ability to support the adherence and proliferation of microorganisms, v. high surface to volume ratio, vi. feasible electron transfer from a microorganism, vii. low overpotential, viii. ease of fabrication, and ix. low cost and scalability (Logan et al., 2008; Logan 2008). The anode materials can be broadly classified as carbon or non-carbon based materials. Typically, carbon-based materials such as carbon cloth and carbon paper are more widely used in MEC systems (Liu et al., 2005; Cheng and Logan 2007; Rozendal et al., 2007; Call and Logan 2008; Hu

et al., 2008). High current densities (0.05 mA cm^{-2}) were obtained with graphite granules (Cheng and Logan 2007; Ditzig et al., 2007; Freguia et al., 2007), graphite felt (Rozendal et al., 2006; Rozendal et al., 2007), and graphite brushes (Call and Logan 2008) based MECs due to the large porosity and surface specificity of these materials (Sleutels et al., 2011). Therefore, graphite is considered a good material of choice for anodes. Using granular graphite bed (528 cm^2), hydrogen production has been reported to reach 3.5 mol H_2 per mol acetate with a coulombic efficiency (CE) of 88% (Cheng and Logan 2007). Further improvement of the CE (92%) could be achieved by modifying the electrode with a positively charged ammoniacal compound as reported by Call and Logan (2008), who observed that with their modified anode, there was more bacterial adhesion, a faster start-up period and an overall more efficient electron transfer during the MEC process. The application of conducting polymers and metal nanoparticles (Fe, Au, Pd) for electrode modification has also been attempted to improve substrate oxidation, and electron transfer efficiency in MEC (Xu et al., 2012; Fan et al., 2011). The structural strength of the electrode also appeared to be important. For instance, it was found that using a more structurally robust carbon material (activated carbon) resulted in higher (3×) current density than with a relatively fragile material (carbon cloth) (Wang et al., 2010; Li et al., 2009).

3.2. Effect of cathode materials

Cathodic hydrogen production on plain carbon materials is often associated with a high over-potential, which could limit the hydrogen production efficiency of a MEC. To address this issue, metal-based catalysts could be used for catalyzing the HER. Platinum (Pt) has been a commonly used noble-metal based catalyst in MECs (Logan et al., 2008). However, it has been suggested that about 47% of the capital cost of a MEC was associated with the use of noble-metal based cathodic

232 catalysts (Rozendal et al., 2008). Alternatively, some of the metal catalysts such as Co/FeCo
233 (Cheng and Logan 2008), NiMo/NiW (Hu et al., 2009), Fe/Fe_3C (Li et al., 2012), Nickel powder
234 (Selembo et al., 2010), Pd nanoparticles (Huang et al., 2011), MoS_2 (Tokash and Logan 2011;
235 Tenca et al., 2013), carbon nanotubes (MWCNT) (Wang et al., 2012), and WC (Tungsten carbide)
236 (Harnisch et al., 2009) were investigated to replace Pt catalyst. Metal alloys such as
237 NiFeMo/CoMo (Jeremiasse et al., 2011), Ni-W-P/Ni-Ce-P (Wang et al., 2011), NiFe, NiFeP and
238 NiFeCoP (Mitov et al., 2012) were also investigated for HER in MECs under neutral/mild alkaline
239 conditions. The alloy cathodes NiMo, NiFeMo or CoMo showed superior catalytic activity
240 towards HER (at pH 7) compared with cathodes coated with only Ni (Mitov et al., 2012). These
241 findings suggest that Ni-based cathodes or cathodes modified with nanomaterials are promising
242 cathode materials for HER in MECs (Mitov et al., 2012). High surface area Ni foam cathodes (128
243 $m^2\ m^{-2}$ projected area) were constructed to produce high volumetric hydrogen production (50 m^3
244 $m^{-3}\ d^{-1}$ at 1.0 V) in continuous flow MEC using an anion exchange membrane. This effect was due
245 to a lower cathode overpotential (Ni foam cathode) than for Pt-based cathode. However, the
246 performance of the Ni foam cathode was unstable, and often associated with an increase of
247 overpotentials over time (Jeremiasse et al., 2010). On the other hand, stainless steel is another
248 widely used cathode material for MECs due to low cost, high current density and low cathodic
249 overpotential (Zhang et al., 2010; Ambler and Logan 2011; Munoz et al., 2010; Selembo et al.,
250 2009b). A high hydrogen production rate of up to 4.9 L $h^{-1}\ m^{-2}$ (with 0.8 V applied voltage) was
251 obtained from a MEC equipped with a stainless steel (AISI 316 L) cathode (Munoz et al., 2010).
252 Alternatively, biocathodes are increasingly being considered for HER in MECs due to low
253 cost and high operational sustainability. Though the concept of a biocathode was discovered in the
254 1960s, it has not received much attention (He and Angenent 2008). It was found that

255 microorganisms that contain hydrogenase enzyme could catalyze hydrogen production in various
256 environments (Schwartz and Friedrich 2006). In recent years, further research using biocathodes
257 has shown that they have many advantages over chemical cathodes for HER in MECs (He and
258 Angenent 2008). For instance, it was reported that a biocathode developed from a selected
259 electrochemically active mixed microbial culture could efficiently drive HER in a cathodic half-
260 cell. The biocathode was poised at a potential of -0.7 V vs. Ag/AgCl, and the corresponding
261 hydrogen production rate was up to 0.6 $m^3 m^{-3} d^{-1}$, which is 3.6 times higher than the abiotic control
262 (0.08 $m^3 m^{-3} d^{-1}$) (Rozendal et al., 2007). A similar finding was reported by Jeremiasses et al. (2010),
263 who found that compared with an abiotic control, the biocathode increased HER by 21% (up to
264 0.11 L for 52 h). Microorganisms in the biocathode consisted of 46% *Proteobacteria*, 25%
265 *Firmicutes*, 17% *Bacteroidetes*, and 12% related to other phyla (Croese et al., 2011). Considering
266 that biocathodes could potentially be a low-cost substitute to metal-based catalysts, further
267 understanding and development of biocathodes for HER is crucial.
268
269 *3.3. Membrane options*
270 In general, most MECs are equipped with a cation exchange membrane or proton exchange
271 membrane (PEM) such as Nafion® 117 type PEM (Dhar and Lee 2013). The use of a membrane
272 separator in a MEC helps to prevent substrate crossover between the two half-cells, thereby
273 minimizing the loss of hydrogen (Logan et al., 2008). However, the membranes in wastewater-
274 treating MECs often leads to the so-called pH splitting limitation due to the magnitudes higher
275 concentration of other ions such as Na^+, K^+, NH_4^+, and Ca^{2+} compared with H^+ in wastewater
276 (nearly 10^5 times higher than that of proton H^+) (Rozendal et al., 2006). As a result, the anolyte
277 can easily become acidified, suppressing the microbial activity of substrate oxidation (Liu et al.,

278 2005), and the catholyte to become more alkaline, which is unfavorable for the hydrogen evolution
279 reaction. Recently, a sulfonated polyether ketone-based novel nanofiber reinforced PEM (NFR-
280 PEM) was developed as a proton conductor for MECs, which showed lower gas and fuel
281 crossovers with higher proton conductivity compared with Nafion® membrane (Chae et al., 2014).
282 Membrane electrode assembly (MEA) cathode has also been developed to enhance hydrogen
283 production efficiency (maximum hydrogen efficiency of 41% with an applied voltage of 1.2 V) in
284 MECs (Jia et al., 2012). However, the use of membrane would incur significant capital cost. It has
285 been estimated that the cost of ion exchange membrane accounted for 38% (400 € m^{-2}) of the
286 capital cost of a laboratory –scale H_2-MEC, suggesting that nearly half of the total cost of MEC
287 was associated with the use of membrane (Rozendal et al., 2008).
288 On the other hand, avoiding the use of membranes could prevent the pH splitting limitation
289 and reduce capital costs. This may also allow the design of simpler reactor configurations (Call
290 and Logan 2008). However, the membrane free MECs were also found to be problematic due to
291 diffusion of hydrogen from cathode to anode, where hydrogen may become available to
292 hydrogenotrophic methanogens leading to methane production. It was found that at an applied
293 voltage of 0.2 V, methane concentrations in the product gas increased up to 28% due to the long
294 cycle time of the reactor. The high cathodic hydrogen recoveries (78± 1% to 96 ± 1%) and lower
295 methane (1.9±1.3%) were achieved in a membrane free MEC with applied voltages ranging from
296 0.3 to 0.8 V, and with a solution conductivity of 7.5 mS cm^{-1}(Call and Logan 2008).
297
298 *3.4. Substrate versatility*
299 MEC can produce hydrogen from a wide range of simple and complex organic substrates. Table 1
300 summarizes hydrogen production rate (in decreasing order) with different amounts of substrate

(mM or g/L) such as acetate, glucose, trehalose, glycerol, bovine serum lignocellulose and different mixed waste stream from domestic and industrial sources. Indeed, the selection of substrates used in MEC can influence many process parameters such as current density (I, A/m^3), applied voltage (V); overall H_2 recovery (R_{H_2}, %); and energy efficiency relative to electrical input (ηE, %). Particularly, the selection of substrate can remarkably affect the hydrogen production rate (Q, m^3H_2/m^3d) (Kadier et al., 2014). Typically, fermentation end products such as acetate have most commonly been used as MEC feedstocks. In fact, the most efficient MEC (hydrogen production rate of 50 $m^3\ m^{-3}\ d^{-1}$) reported thus far were fed with acetate (Jeremiasse et al., 2011). Many other substrates have also been used for bioelectrohydrogenesis, including glucose (1.23 $m^3\ m^{-3}\ d^{-1}$), butyric acid (0.45 $m^3\ m^{-3}\ d^{-1}$), lactic acid (1.04 $m^3\ m^{-3}\ d^{-1}$), propionic acid (0.72 $m^3\ m^{-3}\ d^{-1}$), valeric acid ($m^3\ m^{-3}\ d^{-1}$) (Cheng and Logan 2007), P-glycerol (0.8 $m^3\ m^{-3}\ d^{-1}$) (Selembo et al., 2009b), B-glycerol (0.41 $m^3\ m^{-3}\ d^{-1}$) (Selembo et al., 2009b) and Trehalose (0.25 $m^3\ m^{-3}\ d^{-1}$)(Xu et al., 2014a). However, it should be noted that because the anodic substrate oxidation and cathodic hydrogen production take place at different locations within a MEC, bioelectrohydrogenesis rates of MECs can vary remarkably, even when the systems are loaded with the same substrate. For example, hydrogen production rates ranging from 0.01 to 50 $m^3\ m^{-3}\ d^{-1}$ were recorded from various acetate-fed MECs. Therefore, other operational factors such as substrate concentration, applied voltage, electrode materials, microbes and reactor configuration should also be considered (Kadier et al., 2014).

Using particulate, complex substrates such as sewage sludge directly as the feedstock for bioelectrohydrogenesis is uncommon due to the low concentration of soluble organic carbon (Ntaikou et al., 2010). To facilitate the treatment of these substrates, feedstock pretreatment could be an effective option. For instance, the bioelectrohydrogenesis rate of a MEC fed with an alkaline-

pretreated waste activated sludge (WAS) was 16-fold higher than the control without pretreatment (0.91 vs. 0.056 $m^3\ m^{-3}\ d^{-1}$) (Lu et al., 2012c). It was also found that bifrequency ultrasonic solubilization pretreatment could significantly increase the solubilization of carbon (mainly as short chain fatty acids) from WAS, leading to an improved bio-hydrogen yield (Liu et al., 2012). Their results showed that >90% of acetate and ~90% of propionate were effectively converted to hydrogen, followed by the utilization of n-butyrate and n-valerate. This finding suggested that cascade utilization of fermentative products occur during bioelectrohydrogenesis in a MEC.

Lu et al., (2010) examined the possibilities of using proteins as the substrate for bioelectrohydrogenesis in MECs. Using bovine serum albumin (BSA), they found that hydrogen was produced at a rate of 0.42 $m^3\ m^{-3}\ d^{-1}$ with a yield of 21 mmol H_2 $g\text{-}COD^{-1}$ (applied voltage 0.6 V) in single chamber MECs. However, with the same operational condition a substantially lower performance (0.05 $m^3\ m^{-3}\ d^{-1}$ and 2.6 mmol H_2 $g\text{-}COD^{-1}$) was obtained when a more complex protein (peptone) was used as the substrate. Lignocellulose waste biomass such as corn stover, sugarcane bagasse, straw, sawmill and paper mill discards could be a promising feedstock for the bio-hydrogen production in MECs (Lalaurette et al., 2009). Lalaurette et al., (2009) investigated a two-stage process by combining dark-fermentation and electrohydrogenesis process that produces the overall hydrogen yield of 9.95 mol-H_2/mol-glucose using cellobiose. Similarly, the integrated hydrogen production process from cellulose by combining dark fermentation, MFC, and MEC yielded a higher maximum of 14.3 mmol H_2/g cellulose with a rate of 0.24 $m^3\ m^{-3}\ d^{-1}$ (Wang et al., 2011).

4. Interference of methanogens in H_2-MEC

A vast diversity of microbes can be co-enriched within a MEC. These microbes include extracellular electron transferring bacteria such as *Geobacter sulfurreducens, Shewanella putrefaciens, Rhodoferax ferrireducens, Rhodopseudomonas palustris DX-1,* and *Ochrobactrum anthropi YZ-1* (Fedorovich et al., 2009). Additionally, methanogenic archaea, e.g. hydrogenotrophic methanogen orders *Methanobacteriales* (MBT) and *Methanomicrobiales* (MMB), and acetoclastic methanogen families *Methanosarcinaceae* (MSC) and *Methanosaetaceae* (MST) within the order *Methanosarcinales* may also be present in these MECs (Lu et al., 2012b). These microorganisms were generally found in most of the mixed inoculums of bioelectrochemical systems (MEC/MFC). The activity of methanogens in H_2 producing MECs severely suppresses hydrogen yield and the purity of the produced hydrogen (Tice et al., 2014).

The co-production of methane with hydrogen has been observed in MECs fed with acetate, glucose and complex organic matter (Call and Logan 2008; Chae et al., 2010; Hou et al., 2014; Chae et al., 2008; Wagner et al., 2009). Because most MEC processes are operated under fully anaerobic conditions, methanogenesis can also take place when acetate or H_2 are available as substrates. Acetoclastic methanogens convert acetate to methane (reaction 5) whereas hydrogenotrophic methanogens can utilize carbon dioxide and hydrogen to form methane (reaction 6) (Wang et al., 2009; Chae et al., 2010). In H_2 producing MECs, the processes that lead to hydrogen and methane production are shown below,

Hydrogen production by ARB,

$$\textit{Anode:}\quad CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^- \qquad (3)$$

$$\textit{Cathode:}\quad 8H^+ + 8e^- \rightarrow 4H_2 \qquad (4)$$

Co-production of CH_4 by methanogens,

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (5)$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (6)$$

Hence, the production of hydrogen at the cathode would be tremendously hampered by methanogenic activity due to the consumption of acetate or hydrogen for methane production (Lu et al., 2012a). Ultimately, acetoclastic methanogens would decrease the efficiency of electron transfer from the substrate (electron donor) to the anode (reaction 5). In other words, acetoclastic methanogens would compete with exoelectrogens (ARB) for substrates such as acetate thus reducing the columbic efficiency of bioelectrohydrogenesis. Hydrogenotrophic methanogens directly consume H_2 produced on the cathode (reaction 6), decreasing the cathodic hydrogen recovery (Lu et al., 2011). Thus, to maximize the electron efficiency and cathodic hydrogen recovery, it is critical to suppress methanogenic activity in H_2 producing MECs.

4.1. Methanogenesis control methods and inhibition of methanogenesis by targeting Methyl Coenzyme M reductase (MCR)

To improve hydrogen yields in the MEC reactor we need to inhibit acetate and hydrogen utilizing methanogens, sulfate reducers and homoacetogens. The use of chemical inhibitors targeting specific groups of microbes may potentially address the challenge of low H_2 yields, as well as methane and sulfide contamination in H_2 producing MECs. To control the activity of methanogens for undesirable biological metabolisms in H_2 producing MECs, specific inhibitors should be used for acetate utilizing sulfate reducers, acetoclastic methanogens, hydrogen utilizing sulfate reducers, hydrogenotrophic methanogens, and homoacetogens (Fig. 4).

In general, anti-microbial compounds compete with the target enzymes involved in the biochemical pathways for methane formation (Chae et al., 2010; Catal et al., 2015). It is understood

390 that halogenated hydrocarbons (e.g. $CHCl_3$ or CHX_3) can inhibit the production of methane from
391 H_2/CO_2 and acetate. This is due to the complete blocking of corrinoid enzymes. To inhibit methyl-
392 coenzyme M reductase in hydrogenotrophic and acetoclastic methanogens, 2-bromomethane
393 sulfonate (2-BES) and Lumazine are often used as methanogenic inhibitors (Liu et al., 2011). 2-
394 BES is a structural analog of CoM. Hence, it can block methane formation catalyzed by methyl-
395 CoM reductase. Similarly, Lumazine is a structural analogue of methanopetrin and it can inhibit
396 methanogenesis. Due to the specificity of these chemicals, they are considered specific inhibitors
397 for methanogens.

398 For example, it has been reported that for complete inhibition of methanogenesis in a
399 thermophilic anaerobic digestion process, a very high concentration (50 mM) of 2-BES is required
400 (Zinder et al., 1984). In a separate study, a much lower concentration of 2-BES (10 mM) was found
401 to be effective at suppressing methanogenesis in a similar anaerobic digestion system
402 (Siriwongrungson et al., 2007). In soil systems, the effective inhibitory concentrations of 2-BES
403 were reported to range from 5 to 20 mM, whereas <1 mM 2-BES was required to inhibit rumen
404 methanogens (Wüst et al., 2009; Ungerfeld et al., 2004).

405 The specific inhibitor sodium molybdate (5 mM) can be effectively used as to inhibit
406 sulfate reducing bacteria (Scholten et al., 2000) to control hydrogen sulfide formation. Also,
407 halogenated aliphatic hydrocarbon compounds (e.g. $CHCl_3$) can inhibit the activity of
408 methanogenic archaea as well as of homoacetogenic bacteria and acetate/hydrogen-utilizing
409 sulfate-reducing bacteria (Scholten et al., 2000; Liu et al., 2011).

410 Numerous reports have explored strategies to inhibit methanogens or suppress methane
411 formation in H_2 producing MECs (Table 2). Typically, those strategies entail the manipulation of

412 the physiochemical conditions of the process, targeting the sensitive nature of methanogens to the
413 imposed environmental stress. For example, Hu et al. (2008) examined three different suppression
414 strategies, namely (i) lowering the electrolyte pH to 5.8 with phosphate buffer: NaH_2PO_4, 25.4
415 g/L; Na_2HPO_4, 4.25 g/L; (ii) exposing the cathode to air for 15 min when the methane was found
416 to have accumulated in the MEC headspace; and (iii) boiling the anodes from MFCs at 100°C for
417 15 min before placing them in the MEC. Their results implied that lowering the pH in the MEC to
418 5.8 was immediately effective for suppressing methane production. However, methane production
419 (up to 5.5%) resumed after two batch cycles, suggesting that the acidic shock could only be a short-
420 term solution to the problem (Hu et al., 2008). Similar findings were reported by Kim et al. (2004)
421 and Chae et al. (2010), who showed that acidification also led to inhibition of the exoelectrogen
422 and hence a reduced efficiency of H_2 production. Hence, using acidification to suppress
423 methanogenesis in MEC may not be suitable.

424 It has been demonstrated that a remarkable inhibition of methanogenesis was achieved by
425 lowering the operating temperature to 15°C and 4-9°C (Liu et al., 2005; Lu et al., 2011). However,
426 as most exoelectrogens and methanogens can tolerant a broad range of temperatures, lowering the
427 temperature does not significantly contribute towards improving the hydrogen yield. Further, this
428 method is not effective for suppressing methanogenic activity during long-term operation of H_2
429 producing MECs (Rader and Logan 2010).

430

431 Another effective strategy to suppress methane production is via optimization of applied
432 voltage. In general, increasing the applied voltage of a MEC increases H_2 production and
433 concentration. It was shown that methane production was higher than H_2 production with a

relatively low applied voltage of 0.4 V (22% H_2 and 68% CH_4), whereas with a higher applied voltage of 0.7 V, methane production decreased to <4% (Wang et al., 2009). However, increasing the applied voltage (at a given current density) would increase energy consumption, resulting in a "trade-off" between H_2 production and energy consumption. In single chamber MECs inoculated with mixed cultures from wastewater, the combination of short operation cycles and higher applied voltages could further reduce the methane production to 3%, albeit the methane production was not completely eliminated (Wang et al., 2009). Nam et al. (Nam et al., 2011) reported that there was lower methane production at the anode set potential of -0.2V (vs. Ag/AgCl) compared with other set potentials (-0.4 V, 0 V and 0.2 V vs. Ag/AgCl). However, the improved hydrogen yield (68% H_2 and 21% CH_4) was only transient (i.e. during the initial 38 days), and the composition of the produced biogas after 39 days became significantly enriched with methane (55% H_2 and 34% CH_4) (Nam et al., 2011).

The use of methanogenic inhibitors in MECs may offer several advantages over other physicochemical methods. The use of 2-bromoethane sulfonate (2-BES) to inhibit methane generation in MECs has been well studied. For example, it was reported that the addition of 2-BES (286 μM) reduced methane generation from 145.8 ± 17.4 μmol-CH_4 to 10.2 ± 1.2 μmol-CH_4, reducing the electron loss (as CH_4) from 36 ± 4.4 % to 2.5 ± 0.3 % in a mixed culture H_2 producing MECs (Chae et al., 2010). The acetate-fed MEC achieved an overall hydrogen efficiency from 56 ± 5.7 % to 80.1 ± 6.5 % (equal to 3.2 mol-H_2/mol-acetate). Also, it was found that in an MFC, a significant fraction (35-56 %) of removed soluble chemical oxygen demand (sCOD) was used by methanogenesis or other undesired biological processes leading to low coulombic efficiency (0.7-8 %). However, after adding 6 mM 2-BES to the MFC bioreactor, no methane was detected and the power density of the MFC increased by 25% (He et al., 2005).

457 Recently, improved hydrogen production was demonstrated in single chamber MECs with
458 the addition of 5% chloroform to inhibit methanogens for up to 11 cycles (Zhang et al., 2016). The
459 maximum hydrogen production obtained was 8.4± 0.2 mol H_2 mol-glucose^{-1} at a rate of 2.39 ± 0.3
460 $m^3 m^{-3} d^{-1}$ with high energy efficiency (165 ±5%) (Zhang et al., 2016). Chloroform ($CHCl_3$) blocks
461 the activity of corrinoid enzymes and inhibits the activity of methyl-coenzyme M reductase in
462 methanogenic archaea (Table 2).

463 Hari et al., (2016) examined that the chemical inhibitor 2-BES (10 mM) can effectively
464 suppress methanogenesis in MEC for bioenergy production using fermentable substrates like
465 propionate (Hari et al., 2016). The inhibition of methanogenesis increased coulombic efficiency to
466 about 84 % by encouraging new microbial interactions, which eventually diverted more electrons
467 to current conversion (Parameswaran et al., 2009 and 2010). Addition of Alamethicin (13 µM) can
468 also be used to suppress methanogenesis and promote acetogenesis in bioelectrochemical systems.
469 Alamethicin selectively suppressed the growth of methanogens in mixed-culture
470 bioelectrochemical systems. Also, no methane was detected in the mixed-culture reactors treated
471 with alamethicin, and methane was detected without alamethicin at nearly 100% coulombic
472 efficiency. This indicates that alamethicin can effectively suppress methanogens and inhibit
473 methanogenesis in MECs (Zhu et al., 2015).

474 Catal et al., (2015) demonstrated that methanogenesis can be controlled effectively in long-
475 term by the addition of inhibitors in hydrogen producing MECs. The methanogenic inhibitors
476 namely neomycin sulfate, 8-aza-hypoxanthine, 2-bromoethanesulfonate and 2-chloroethane
477 sulfonate were used to examine the inhibition of methanogenesis. The application of antibiotics as
478 methanogenic inhibitors in this study provides a novel approach to inhibit methanogenesis in
479 MECs. Moreover, the methanogenic inhibition methods such as applied potential, rapid extraction

480 of H2, heat treated electrode, use of biocathode, addition of fatty acids, intermittent oxygen
481 exposure, and use of microbial cultures enriched in the presence of the chemical inhibitor were
482 only able to limit methane formation to a certain extent. In contrast, no methane was detected when
483 methanogenic inhibitors were added directly into MECs (Table 2). Also, the methanogenic
484 inhibitors specifically compete with MCR and inhibit methane generation in hydrogen producing
485 MEC. The growth of methanogen in MECs is a known challenge and requires specific control
486 strategies like methanogenic inhibitors (Table 2).

487 In general, methanogenic pathways use several cofactors, namely coenzyme M (CoM;
488 $HSCH_2CH_2SO_3^-$), methanofuran (2-aminomethylfuran linked to phenoxy group), and
489 methanopterin (H_4MPT;5,6,7,8-tetrahydromethanopterin) (Fig. 5). These cofactors act as C1
490 carriers in methanogenesis (Liu et al., 2011) and they are used by all methanogens. The terminal
491 step of the methanogenic pathway is methane formation, whereby the methyl group carried by
492 CoM is reduced to methane by an enzyme known as methyl-coenzyme M reductase (MCR). This
493 enzyme catalyzes the reaction of CH_3-S-CoM (Methyl CoM) with CoB (CoenzymeB) to produce
494 methane (CH_4) and heterodisulfide CoM-S-S-CoB as presented in Fig. 6.

495 In the methanogenesis pathway, the terminal step is the reaction of CoM with N-7-
496 mercaptoheptanoylthreonine phosphate (CoB). The main product of this terminal step is methane,
497 although mixed disulfide (CoM-S-S-HTP) could also be formed (Ellermann et al., 1988). The
498 MCR enzyme was isolated from methanogens and tested for the inhibition. Enzyme inhibitors that
499 were selected had a terminal sulfonate (SO_3^-) and are structural analogues of CoM. Several
500 inhibitors have been investigated such as 1-butanesulfonate, 1-propanesulfonate, 2-
501 azidoethanesulfonate, 2-bromoethanesulfonate, 3-azidopropanesulfonate, 3-bromopropane
502 sulfonate, 3-bromopropionate, 3-chloropropanesulfonyl chloride, 3-fluoropropanesulfonate, 3-

503 hydroxypropanesulfonate, 3-iodopropane sulfonate, 3-mercapto-1-propanesulfonate, 4-
504 bromobutyrate, 4-bromobutyrate sulfonate, 7-bromoheptanoylthreonine phosphate (CoB
505 analogue), 4-chlorobutyrate and chloromethanesulfonate (Table 3). These inhibitors compete with
506 MCR and inhibit methane generation. It is known that MCR has cofactor 430 (F_{430}), which has
507 Ni(I) in its active site. This Ni(I) reacts with inhibitors and changes to the inactive Ni(III) state
508 (Kunz et al., 2006). The central nickel atom of F_{430} is coordinated by four planar tetrapyrrole
509 nitrogen atoms. For example, the methanogenic inhibitor, 1-bromoethane sulfonate (1-BES) can
510 interact with Ni(I)-MCR_{red} and forms the inactive state of Ni(III)-$MCR_{sulfonate}$, while in the absence
511 of inhibitor, Ni(I)-MCR_{red} interacts with CH_3-SCoM to form methane as depicted in the reaction
512 scheme in Fig. 7. The use of inhibitors in H_2-MECs offers an advantage of long-term inhibition.
513 However, the concentration of inhibitors can vary based on the field application and this can
514 influence cost of operation of the MECs. To address this challenge for practical applications, the
515 inhibitors can be added only when needed. Another option could be by adopting feedback
516 inhibitor-dosing strategy based on the composition of biogas. Here, if H_2 partial pressure is lower
517 than a certain threshold, dosing of an inhibitor is triggered.
518
519 **5. Conclusion and future prospects**
520 To achieve large-scale implementation of MECs for hydrogen production, methanogenesis
521 has to be controlled. Other issues that can also influence H_2-MEC performance are those relating
522 to the bioanode. These include the pH sensitivity of biofilms. Bioelectrohydrogenesis is a
523 microbial process. Therefore, a better understanding of microbial electron transfer mechanisms
524 will certainly be important from a process stability perspective. Reactor design also plays an
525 important role for scaling up of MEC. For example, single chamber MECs that lack a membrane

526 always showed the production of methane with lower hydrogen yields. As discussed, most MEC
527 studies were conducted with small-scale laboratory systems (Table 1). Only few pilot scale plants
528 with capacities between 20 L and 1000 L were trialed, and the performance of these plants was
529 affected by technical challenges such as influent flocculation, water leakage, electrochemical
530 losses and production of unfavorable products (Wang et al., 2013). Cusik et al. (2011) developed
531 the first pilot scale (1000 L) single chamber continuous flow membrane-less MECs for
532 bioelectrohydrogenesis. However, their process failed to produce hydrogen due to formation of
533 methane via hydrogenotrophic methanogenesis. It is now accepted that using membrane-less
534 MECs for hydrogen production is practically challenging. To maximize the yield and purity of
535 hydrogen, effective and implementable strategies should be identified to reduce the formation of
536 methanogenic growth and to promote hydrogen formation. As reviewed here, it is feasible to select
537 suitable inhibitor(s) to prevent methane formation (Fig. 8). Future research should be devoted
538 towards developing robust, combinatorial and specific anti-microbial approaches to bring the
539 technology towards practical application.
540

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875 **Figure captions**

876 **Fig. 1.** (A) Year-wise publication of journal papers on MECs and (B) country wise distribution
877 of publications on MECs. Source: "Web of Science" search with "Microbial electrolysis
878 cell" as the research paper topic as in June 2017. (others- Saudi Arabia, Germany,
879 Sweden, Mexico, Denmark, Taiwan, Iran, Wales, Switzerland, Malaysia, Hungary,
880 Greece, Finland, Turkey, Singapore, Qatar, Israel, Ireland, Bulgaria, U Arab Emirates,
881 Thailand, South Africa, Scotland, Russia, Poland, Nigeria, New Zealand, Ecuador,
882 Austria, Vietnam, Romania, Portugal, Morocco, Lebanon, Kuwait, Indonesia, Czech
883 Republic, Chile, Brazil, and Argentina)

884 **Fig.2** Operational principle of microbial electrolysis cell (a) and water electrolysis cell (b);
885 Acetate - organic substrate for exoelectrogenic bacteria (Biofilm), Anode- positive
886 terminal electrode that accept e^- from Exoelectrogenic bacteria, Cathode - negative
887 terminal electrode that donate e^- for H_2 evolution; Potentiostat or power supply -
888 Electrical device to control applied cell potential for hydrogen evolution reaction, and
889 PEM- proton exchange membrane (optional)

890 **Fig. 3.** Hydrogen producing microbial electrolysis set up; (A) H - shaped two chamber MEC —
891 320 mL (Liu et al., 2005) (B) two chamber MEC - 32 mL (Cheng and Logan 2007), (C)
892 single chamber MEC - 28 mL (Calland Logan 2008), (D) Single chamber MEC in round
893 bottom flasks - 250 mL (Brown et al., 2014), (E) single chamber MEC in borosilicate
894 glass serum vials -100 mL (Hu et al., 2008), F) single chamber MEC in borosilicate glass
895 serum tubes - 28 mL (Hu et al., 2009), (G) continuous flow MEC with multi-electrodes -
896 2.4 L, 1.67 mL min^{-1} (Rader and Logan 2010), (H) pilot-scale continuous flow MEC fed
897 with winery wastewater — 1000 L, 1 L d^{-1} (Cusik et al., 2011).

Fig. 4. Inhibition of undesirable biological metabolisms in H_2 producing MECs by selective methanogenic inhibitors ($CHCl_3$, 2-BES, CH_3F, Na_2MoO_4, etc.,) additions to augment electrohydrogenesis in MECs.

Fig. 5. Hydrogenotrophic methanogenesis and acetoclastic methanogenesis pathways. Hydrogenotrophic methanogenesis starts with stepwise (1-7) reduction of CO_2 to methane via coenzyme-bound intermediates. Acetoclastic methanogenesis starts with the activation of acetate to acetyl-CoA. (H4MPT, tetrahydromethanopterin; CoA, Co enzyme A; $CH_3COSCoA$, acetyl-CoA)

Fig. 6. Terminal step of methanogenesis for methane generation.

Fig. 7. The mechanism of inhibition of the methanogenic enzyme, Methyl –Coenzyme M Reductase (Mcr) by bromoethanesulfonic acid (BES).

Fig. 8. Perspective of single-chamber H_2 producing MECs with the addition of suitable inhibitors.

Table 1. Summary of hydrogen production rate in various MEC systems.

MEC configuration / Working volume	Anode	Cathode	Microbial inoculum/ Source	Substrate	Applied voltage (V)	H_2 rate or Yield ($m^3H_2m^{-3}d^{-1}$)	H_2 (%)	CH_4 (%)	Ref.
Two chamber continuous flow at 2.6 mL min^{-1} / 200 mL	Graphite felt	Co-Mo alloy	Mixed cultures / Waste water effluent	Acetate / 2.72 g L^{-1}	1.0	50	NA	NA	Jeremiasse et al., 2011
Single chamber fed batch / 28 mL	Heat treated Graphite brush	Carbon cloth/Pt	Mixed cultures / Pennsylvania State University WWP	Acetate / 1.5 g L^{-1}	0.6	3.6	68	35	(Nam et al., 2011)
Single chamber fed batch / 28 mL	graphite brush	Carbon cloth/Pt	Mixed cultures / enriched biofilm in MFC	Acetate / 1 g L^{-1}	0.8	3.12	96	1.9	(Call and Logan 2008)
Single chamber fed batch / 28 mL	Carbon cloth	Carbon cloth/Pt	Mixed cultures / Pennsylvania State University WWP	Acetate / 5 g L^{-1}	0.6	2.3	85	>1%	(Hu et al., 2009)
Single chamber fed batch / 26 mL	Graphite brush	Carbon cloth	Mixed cultures / enriched biofilm in MFC	Fermentation effluent / 6.5 g L^{-1}	0.6	2.11	96	NA	(Lu et al., 2009)
Single chamber fed batch / 28 mL	Ammonia treated graphite brush	Carbon cloth/Pt	Mixed cultures / WWP	P-Glycerol / 1 g L^{-1}	0.9	2.01	88	1.2	(Selembo et al., 2009b)
Single chamber fed batch / 28 mL	Carbon cloth	Carbon cloth/NiMo	Mixed cultures / Pennsylvania State University WWP	Acetate / 5 g L^{-1}	0.6	2.0	86	<1	(Hu et al., 2009)
Single chamber fed batch / 28 mL	graphite brush	Carbon cloth/Pt	Mixed cultures / enriched biofilm in MFC	Acetate / 1 g L^{-1}	0.6	1.99	78	28	(Call and Logan 2008)
Single chamber fed batch / 28 mL	Ammonia treated graphite brush	Carbon cloth/Pt	Mixed cultures / WWP	Glucose / 1 g L^{-1}	0.9	1.87	87	1.2	(Selembo et al., 2009b)
Single chamber fed batch / 28 mL	Heat treated graphite brush	Carbon cloth/Pt	Mixed cultures / enriched biofilm in MFC	Food processing waste water / 8.1 Kg m^{-3}	0.9	1.8	32	55	(Tenca et al., 2013)
Single chamber / 28 mL	graphite fiber brush	SS brush	Mixed cultures/ ARB biofilm from MFC	Acetate / 1 g L^{-1}	0.5	1.7	84	2.3	(Call et al.,2009)
Single chamber batch / 400 mL	Graphite granules	Ti tube/Pt	Mixed cultures / enriched biofilm in MFC	Acetate / 0.5 g	1.0	1.58	88	0.04	(Guo et al., 2010)

Single chamber fed batch / 28 mL	Carbon cloth	Carbon cloth/NiW	Mixed cultures / Pennsylvania State University WWP	Acetate / 5 g L^{-1}	0.6	1.5	75	<1%	(Hu et al., 2009)
Single chamber fed batch / 28 mL	graphite fiber brush	SS A286	Mixed cultures / enriched biofilm in MFC	Acetate / 1 g L^{-1}	0.9	1.5	80	NA	(Selembo et al., 2009a)
Single chamber fed batch / 26 mL	Graphite brush	Carbon cloth	Mixed cultures / enriched biofilm in MFC	Buffered effluent / 6.5 g $^{-1}$		1.41	83	NA	(Lu et al., 2009)
Single chamber fed batch / 28 mL	Ammonia treated Graphite brush	SS	Mixed cultures / Pennsylvania State University WWP	Acetate / 1 g L^{-1}	0.9	1.4	91%	<1	Ambler and Logan 2011
Two chamber fed batch / 14 mL	Ammonia treated graphite granule	Carbon cloth/Pt	Mixed cultures / WWP	Glucose / 1 g L^{-1}		1.23	71	NA	(Cheng and Logan 2007)
Single chamber fed batch / 28 mL	Graphite fiber brush	Carbon cloth/Pt	*Clostridium thermocellum* enriched biofilm in MFC	Synthetic effluent / 5 g L^{-1}	0.5	1.11	63	120 mL g-COD^{-1}	Lalaurette et al., 2009
Single chamber fed batch / 28 mL	Graphite fiber brush	Pt	Mixed cultures/ Swine farm WWP	Swine waste water/ 2g L^{-1}	0.55	1	77	13	(wagner et al., 2009)
Two chamber fed batch / 14 mL	Ammonia treated graphite granule	Carbon cloth/Pt	Mixed cultures / WWP	Acetic acid / 1 g L^{-1}	0.6	1.1	91	NA	(Cheng and Logan 2007)
Two chamber fed batch / 14 mL	Ammonia treated graphite granule	Carbon cloth/Pt	Mixed cultures / WWP	Lactic acid / 1 g L^{-1}		1.04	91	NA	(Cheng and Logan 2007)
Single chamber fed batch / 28 mL	Graphite fiber brush	Carbon cloth/Pt	*Clostridium thermocellum* enriched biofilm in MFC	Cellobiose / 5 g L^{-1}	0.5	0.96	69	210 mL g-COD^{-1}	Lalaurette et al., 2009
Two chamber fed batch / 26 mL	Graphite brush	Carbon cloth/Pt	Mixed cultures / WAS	Alkaline WAS / 2.4 g L^{-1}	0.6	0.91	72	NA	(Lu et al., 2012c)
Single chamber fed batch / 28 mL	Ammonia treated graphite brush	Carbon cloth/Pt	Mixed cultures / WWP	Glucose / 1 g L^{-1}	0.5	0.83	81	9.5	(Selembo et al., 2009b)
Single chamber fed batch / 28 mL	Ammonia treated graphite brush	Carbon cloth/Pt	Mixed cultures / WWP	P-Glycerol / 1 g L^{-1}	0.5	0.80	80	9.5	(Selembo et al., 2009b)
Single chamber fed batch / 28 mL	Ammonia treated graphite brush	Carbon cloth/Pt	*Geobacter sp.,/* enriched biofilm in MFC	Potato waste water / 1.9-2.5 g L^{-1}	0.9	0.74	73	13	(Kiely et al., 2011)

Two chamber fed batch / 14 mL	Ammonia treated graphite granule	Carbon cloth/Pt	Mixed cultures / WWP	Propionic acid / 1 g L^{-1}		0.72	89	NA	(Cheng and Logan 2007)
Single chamber fed batch / 28 mL	graphite fiber brush	SS 304	Mixed cultures / enriched biofilm in MFC	Acetate / 1 g L^{-1}	0.9	0.59	77	NA	(Selembo et al., 2009a)
Single chamber fed batch / 28 mL	graphite fiber brush	SS420	Mixed cultures / enriched biofilm in MFC	Acetate / 1 g L^{-1}	0.9	0.58	67	NA	(Selembo et al., 2009a)
Single chamber continuous flow at 0.88 mL min^{-1} / 140 mL	Graphite granules	Carbon felt	Mixed cultures / ARB biofilm from an acetate-fed MFC having a *Geobacter*-rich community	Acetate / 10 mM	1.06	0.57	59	2	(Lee et al., 2009)
Two chamber fed batch / 14 mL	Ammonia treated graphite granule	Carbon cloth/Pt	Mixed cultures / WWP	Butyric acid / 1 g L^{-1}		0.45	80	NA	(Cheng and Logan 2007)
Single chamber fed batch / 26 mL	Graphite brush	Carbon cloth/Pt	Mixed cultures / enriched biofilm of the Harbin Wenchang WWP in MFC	Bovine serum albumin / 0.7 g L^{-1}	0.6	0.42	34	<0.9 mM g-COD^{-1}	(Lu et al., 2010)
Single chamber fed batch / 28 mL	Ammonia treated graphite brush	Carbon cloth/Pt	Mixed cultures / WWP	B-Glycerol / 1 g L^{-1}	0.9	0.41	87	1.2	(Selembo et al., 2009b)
Single chamber fed batch / 28 mL	graphite fiber brush	SS316	Mixed cultures / enriched biofilm in MFC	Acetate / 1 g L^{-1}	0.9	0.35	55	NA	(Selembo et al., 2009a)
Single chamber fed batch / 38 mL	Graphite brush	Carbon cloth/Pt	Mixed cultures / WAS	Trehalose / 50 mM	0.8	0.25	80	NA	(Xu et al., 2014a)
Single chamber fed batch / 28 mL	Heat treated graphite brush	MoS_2	Mixed cultures / enriched biofilm in MFC	Industrial waste water 4.1 Kg m^{-3}	0.7	0.17	NA	70	(Tenca et al., 2013)
Two chamber fed batch / 14 mL	Ammonia treated graphite granule	Carbon cloth/Pt	Mixed cultures / WWP	Valeric acid / 1 g L^{-1}	0.6	0.14	67	NA	(Cheng and Logan 2007)
Single chamber fed batch / 28 mL	Ammonia treated graphite brush	Carbon cloth/Pt	Mixed cultures / WWP	B-Glycerol / 1 g L^{-1}	0.5	0.14	82	9.5	(Selembo et al., 2009b)
Single chamber fed batch / 28 mL	Heat treated graphite brush	SS304 sheet	Mixed cultures / enriched biofilm in MFC	Industrial waste water 4.1 Kg m^{-3}	0.7	0.12	NA	62	(Tenca et al., 2013)
Two chamber fed batch / 14 mL	Ammonia treated graphite granule	Carbon cloth/Pt	Mixed cultures / WWP	Cellulose / 1 g L^{-1}	0.6	0.11	68	NA	(Cheng and Logan 2007)

Single chamber fed batch / 28 mL	Graphite fiber brush	Carbon cloth/Pt	*Clostridium thermocellum* enriched biofilm in MFC	Lignocellulose / 5 g L^{-1}	0.5	0.11	68	120 mL g-COD^{-1}	Lalaurette et al., 2009
Two chamber fed batch / 28 mL	Graphite felt	Ti plate/Pt	*Pelobacter propionicus*/ Anaerobic digested sludge	Acetate / 2 mM	0.8	0.052	97	2.5	(Chae et al., 2008)
Two chamber fed batch / 120 mL	Carbon brush	Pt/C	Mixed cultures / anaerobic sludge from WWP	Acetate / 1 g L^{-1}	0.8	0.0231	32	NA	(Xiao et al., 2012)
Two Chamber fed batch / 6.6 L	Graphite felt	Ti/Pt	Mixed cultures / sludge from UASB reactor	Acetate / 10 Mm	0.5	0.02	NA	NA	(Rozendal et al., 2006)
Two chamber fed batch / 120 mL	Carbon brush	Fe/Fe3C @C	Mixed cultures / anaerobic sludge from WWP	Acetate / 1 g L^{-1}	0.8	0.0182	35	NA	(Xiao et al., 2012)
Two chamber fed batch / 200 mL	Carbon felt	Ti/Pt	Mixed cultures / Gwangju sewage treatment plant	Acetate / 1.5 g L^{-1}	-	0.013	44	NA	(Lee et al., 2015)
Two chamber fed batch / 130 mL	Carbon brush	Carbon cloth/MoS_2/CNT-90	NA	Acetate / 1 g L^{-1}	0.8	0.01	12.7	NA	(Yuan et al., 2014)
Two chamber fed batch / 120 mL	Carbon brush	CNT	Mixed cultures / anaerobic sludge from WWP	Acetate / 1 g L^{-1}	0.8	0.0076	16	NA	(Xiao et al., 2012)
Two chamber fed batch / 120 mL	Carbon brush	CNT	Mixed cultures / anaerobic sludge from WWP	Acetate / 30 mM	1.06	NA	31	32	(Lee et al., 2009)
Two chamber fed batch / 120 mL	Carbon brush	CNT	Mixed cultures / anaerobic sludge from WWP	Acetate / 80 mM	1.06	NA	28	37	(Lee et al., 2009)
Single chamber fed batch / 130 mL	Graphite fiber brush	Carbon cloth/Pt	Mixed cultures/ Liede WWP	Acetate	0.8	3.7 mol H_2/mol acetate	95	<0.6	(Hou et al., 2014)
Two chamber fed batch / 28 mL	Heat treated Graphite brush	SS/Pt	Mixed cultures/ Pennsylvania State University WWP	Acetate/ 1.5 g L^{-1}	0.9	3.2 mol H_2 / mol acetate	90	NA	(Nam and Logan 2011) (Nam and Logan 2011)
Two chamber continuous flow at 0.368 g L^{-1} / 292 mL	Carbon paper	Carbon paper/Pt	Mixed cultures / enriched biofilm in MFC	Domestic waste water/ 1 g L^{-1}	0.5	0.154 H_2 g-COD^{-1}	42	NA	(Ditzig et al., 2007)

Note: WAS- waste activated sludge; WWP- waste water treatment plant; MFC – Microbial fuel cell; NA- data not available

Table **2.** Methods used for the suppression of methanogens in microbial electrolysis cell for high yield hydrogen production

Methanogenesis suppression method	**Details**	**Hydrogen production rate** ($m^3H_2m^{-3}d^{-1}$)	**Remarks**	**Reference**
Applied potential	0.8 V	-	Methane increased at below 0.8 V	Ding et al., 2016
Rapid H_2 extraction method	gas-permeable hydrophobic membrane and vacuum	1.58± 0.5	No methane	Lu et al., 2016
Heat treated electrode	Bioanode boiled at 100°C for 15 min	0.69	1% methane detected in head space	Hu et al., 2008
Biocathode	Hydrogen producing bioelectrode developed at -0.65 V	10	Methane detected at start up time	Rozendal etal., 2008
Effect of fatty acids	Acetic acid and propionic acid mixture	0.265	No Methane detected.	Ruiz et al., 2014
Oxygen exposure	Bio-anode exposed to air for 24 h	-	No Methane production for 12 h	Ajayi et al., 2010
Specific culture	Heat treated Clostridium *ljungdahlii* isolated from anerobic sludge treated with 2-bromoethanesulfonate	-	No methane detected over 300 days. Acetate along with hydrogen were produced from CO_2	Bajracharya et al., 2017
Chemical inhibitor or methanogen	5% chloroform	2.39 ± 0.3	No methane was detected in fed batch cycle	Zhang et al., 2016
	2-bromoethanesulfonate, 10 mM	1.08± 0.1	No methane detected	Hari et al., 2016
	2-bromoethanesulfonate (286 µM)	-	No methane detected	Chae et al., 2010

	2-bromoethanesulfonate (50 mM)	-	Methanogens were completed inhibited	Parameswaran et al., 2009
	Alamethicin (13 µM)	-	No methane detected	Zhu et al., 2015
	2-chloroethane sulfonate (20 mM), 2-bromoethane sulfonate (20 mM), 8-aza-hypoxanthine (3.6 mM)	-	Methane inhibited with increasing hydrogen production	Catal et al., 2015

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Table 3. Inhibition of Methyl-Coenzyme M reductase (MCR) for different methanogens

Inhibitors	Apparent concentration (mM)	Organisms	References
1-butanesulfonate	70 mM	*Methanothermobacter marburgensis*	(Kunz et al., 2006)
1-propanesulfonate		-	
2-azidoethanesulfonate	0.001 mM	-	(Gunsalus et al., 1980)
2-bromoethanesulfonate	4 µM	*Methanothermobacter thermautotrophicus, Methanothermobacter marburgensis*	
3-azidopropanesulfonate	1 µM	*Methanothermobacter thermautotrophicus*	(Ellermann et al., 1989)
2-bromoethanesulfonate	0.004 mM	-	(Ellermann et al., 1988)
3-azidopropanesulfonate	0.04 mM competitive, reversible	*Methanothermobacter thermautotrophicus*	(Ellermann et al., 1989)
3-bromopropane sulfonate	0.00005 mM, irreversible, strong inhibitor and competitive substrate	*Methanothermobacter marburgensis*	(Goenrich et al., 2004)
3-Bromopropionate	irreversible	*Methanothermobacter marburgensis*	
3-chloropropanesulfonyl chloride	1mM	*Methanothermobacter marburgensis*	(Kunz et al., 2006)
3-fluoropropanesulfonate	-	*Methanothermobacter thermautotrophicus*	(Rospert et al., 1992)
3-hydroxypropanesulfonate	-	*Methanothermobacter thermautotrophicus*	(Ellermann et al., 1989)
3-iodopropane sulfonate	-	*Methanothermobacter marburgensis*	(Goenrich et al., 2004)
3-mercapto-1-propanesulfonate		*Methanothermobacter marburgensis*	(Kunz et al., 2006)

4-bromobutyrate	-	*Methanothermobacter marburgensis*	(Kunz et al., 2006; Goenrich et al., 2004)
4- bromobutanesulfonate	0.006 mM	*Methanothermobacter marburgensis*	(Kunz et al., 2006)
7-bromoheptanoylthreonine phosphate	-	*Methanothermobacter thermautotrophicus*	(Gunsalus et al., 1980)
4-Chlorobutyrate	-	*Methanothermobacter marburgensis*	(Kunz et al., 2006)
4-bromobutyrate sulfonate	-	*Methanothermobacter marburgensis*	(Dey et al., 2007)
Chloromethanesulfonate	-	*Methanothermobacter thermautotrophicus*	(Ellermann et al., 1989)

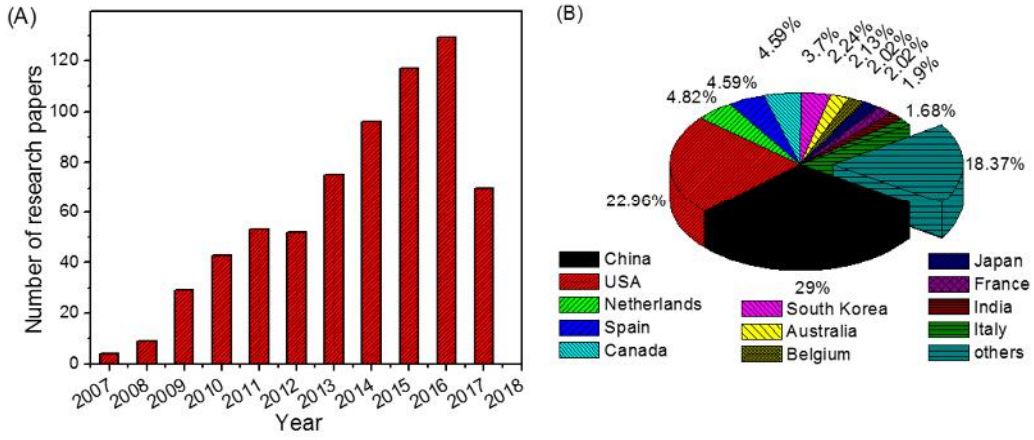
(A)
Number of research papers
0
20
40
60
80
100
120
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
Year
(B)
4.59%
3.7%
2.24%
2.13%
2.02%
2.02%
1.9%
4.59%
4.82%
1.68%
18.37%
22.96%
29%
China
USA
Netherlands
Spain
Canada
South Korea
Australia
Belgium
Japan
France
India
Italy
others

Fig. 1.

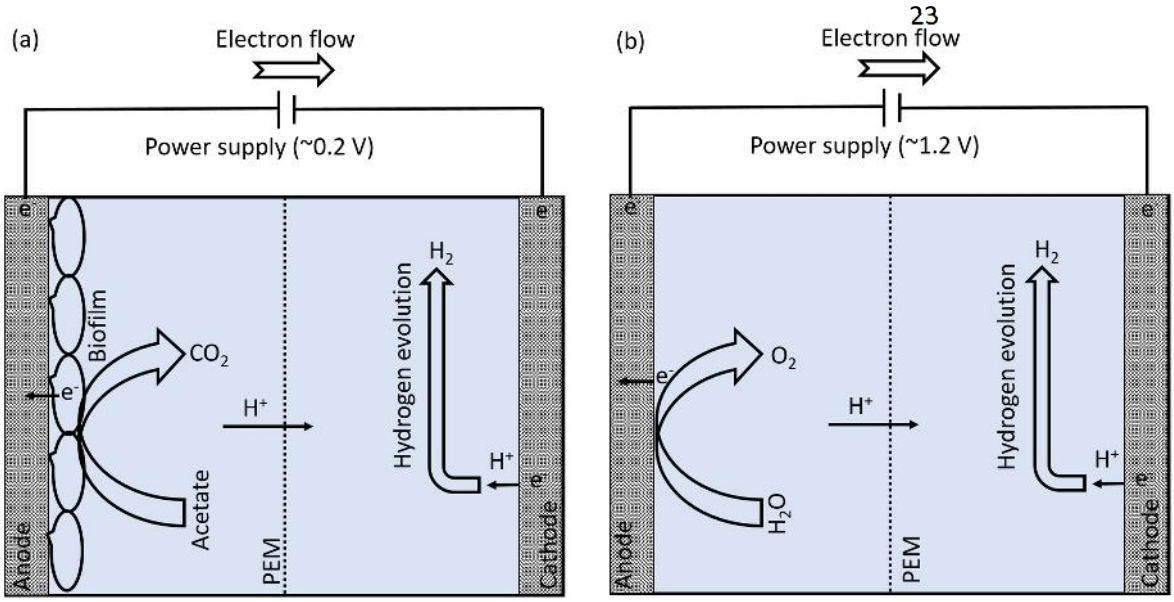
(a)
Electron flow
Power supply (~0.2 V)
e
e
Biofilm
CO_2
e^-
H^+
Acetate
PEM
H_2
Hydrogen evolution
H^+
e
Anode
Cathode
(b)
Electron flow
Power supply (~1.2 V)
e
e
e^-
O_2
H^+
H_2O
PEM
H_2
Hydrogen evolution
H^+
e
Anode
Cathode

Fig. 2.

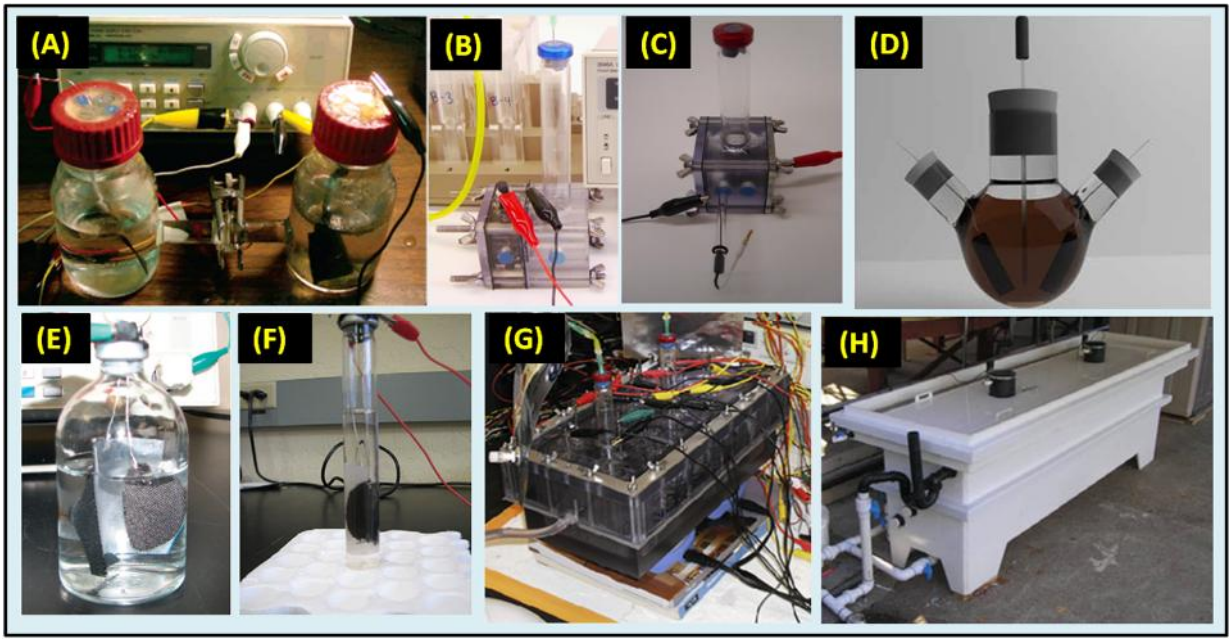

Fig. 3.

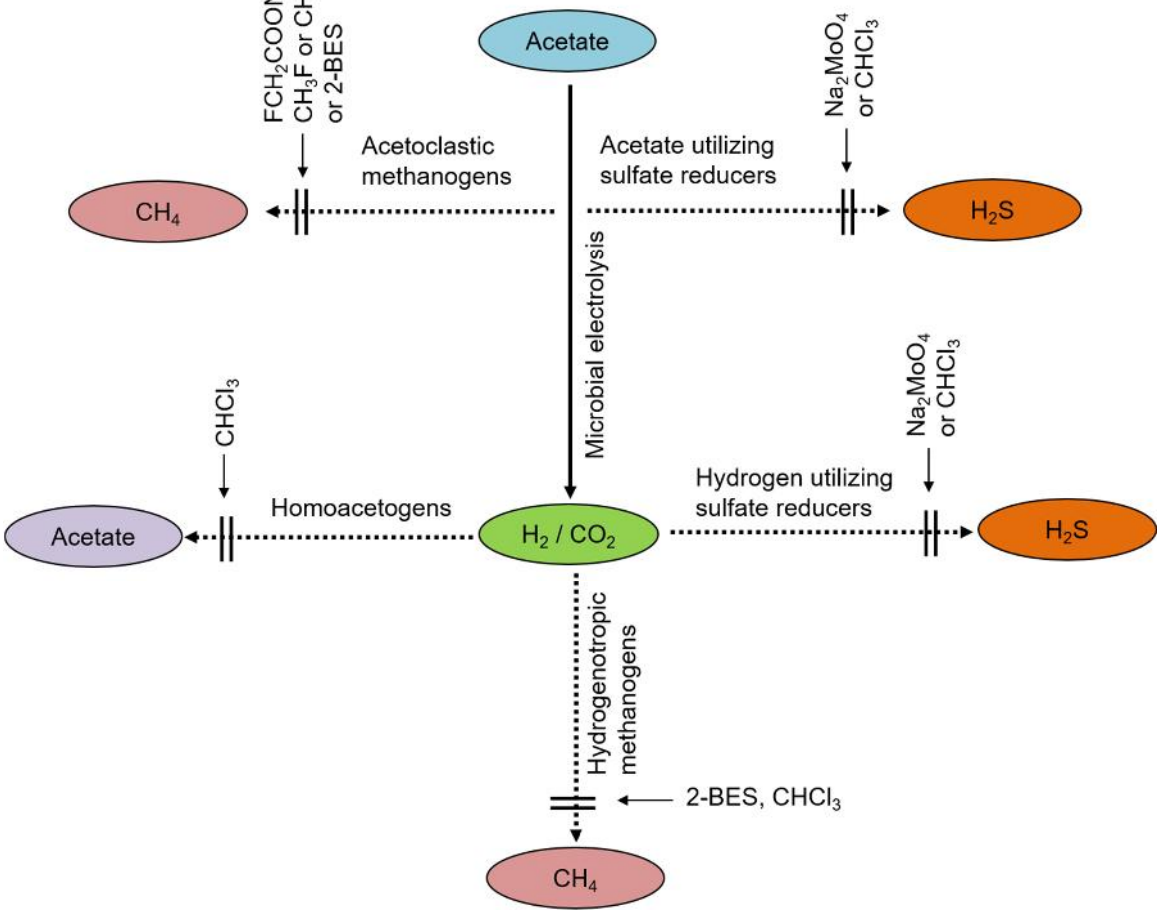
Acetate
FCH2COONa or CH3F or CHCl3 or 2-BES
Acetoclastic methanogens
CH4
Acetate utilizing sulfate reducers
Na2MoO4 or CHCl3
H2S
Microbial electrolysis
CHCl3
Homoacetogens
Acetate
H2 / CO2
Hydrogen utilizing sulfate reducers
Na2MoO4 or CHCl3
H2S
Hydrogenotropic methanogens
2-BES, CHCl3
CH4

Fig. 4.

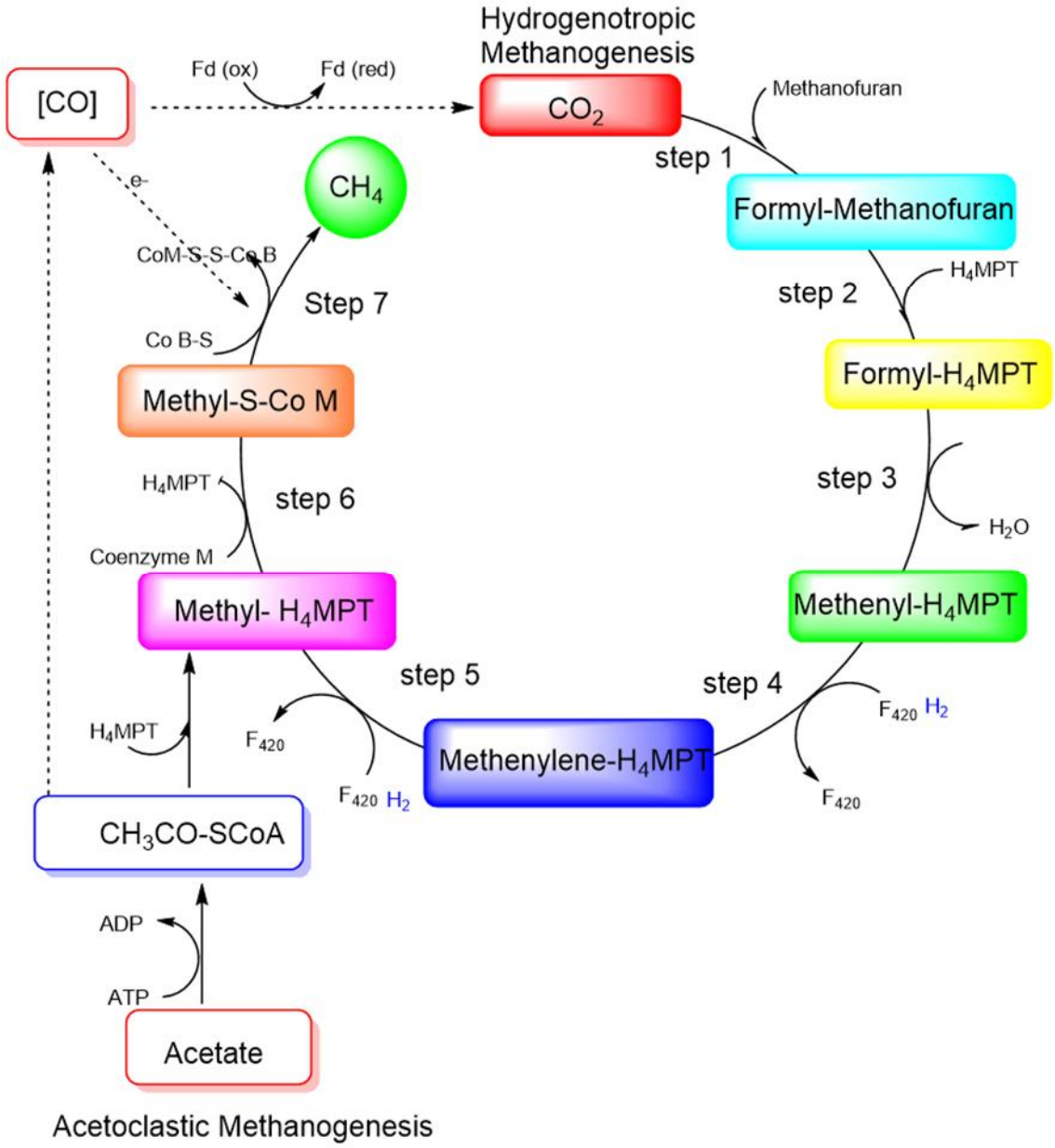
Hydrogenotropic
Methanogenesis
[CO]
Fd (ox)
Fd (red)
CO_2
Methanofuran
step 1
Formyl-Methanofuran
step 2
H_4MPT
Formyl-H_4MPT
step 3
H_2O
Methenyl-H_4MPT
step 4
F_{420} H_2
F_{420}
Methenylene-H_4MPT
step 5
F_{420}
F_{420} H_2
Methyl- H_4MPT
step 6
H_4MPT
Coenzyme M
Methyl-S-Co M
Step 7
CoM-S-S-Co B
Co B-S
CH_4
e-
H_4MPT
CH_3CO-SCoA
ADP
ATP
Acetate
Acetoclastic Methanogenesis

Fig. 5.

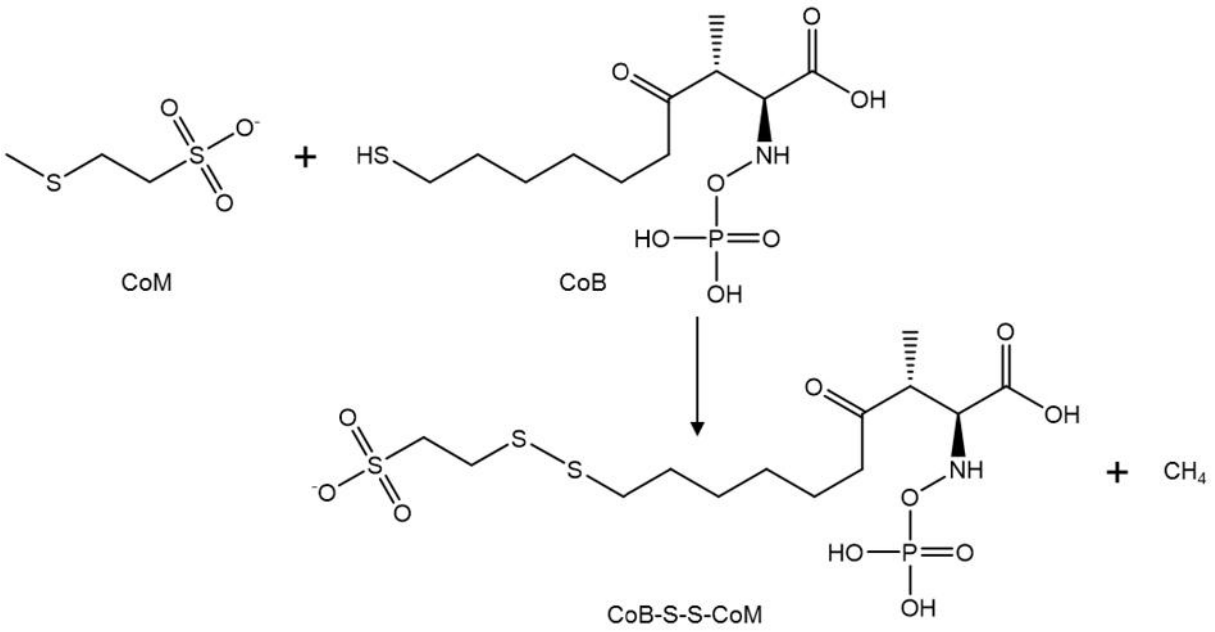

Fig. 6

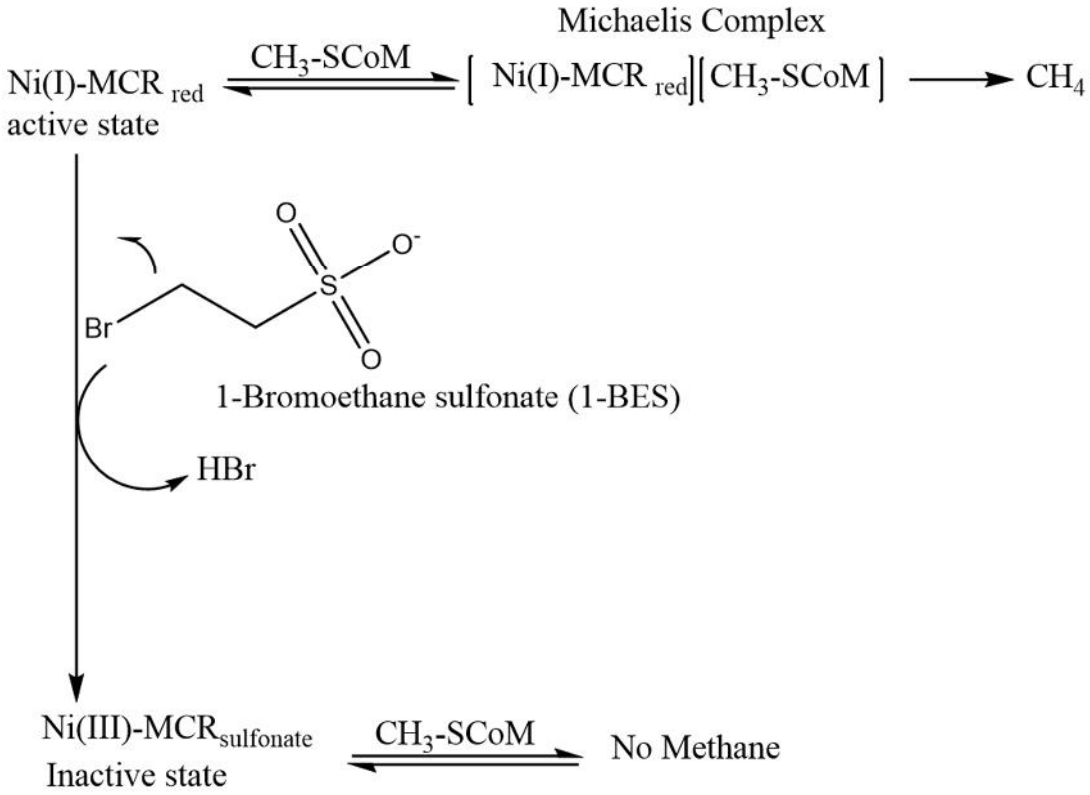
Michaelis Complex
Ni(I)-MCR red
active state
CH3-SCoM
[Ni(I)-MCR red][CH3-SCoM]
CH4
O
O-
S
O
Br
1-Bromoethane sulfonate (1-BES)
HBr
Ni(III)-MCRsulfonate
Inactive state
CH3-SCoM
No Methane

Fig. 7

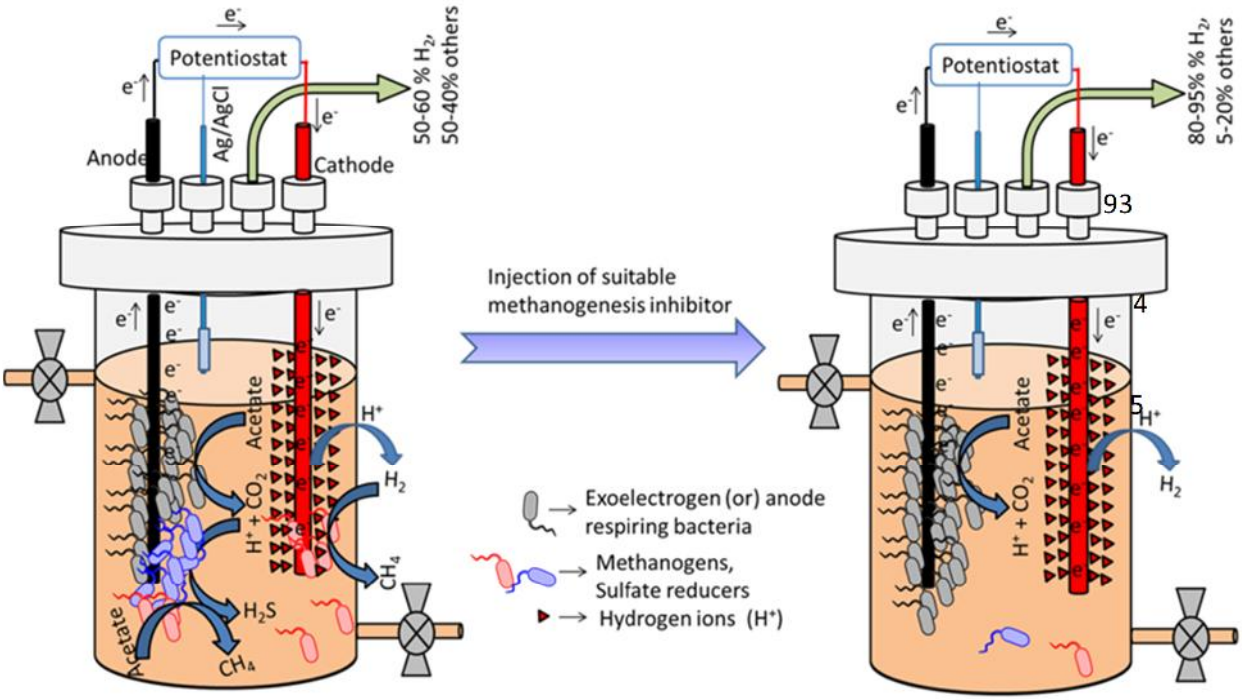
Potentiostat
Anode
Ag/AgCl
Cathode
50-60 % H2,
50-40% others
Injection of suitable
methanogenesis inhibitor
Potentiostat
80-95% % H2,
5-20% others
Acetate
H+ + CO2
H2
CH4
H2S
Acetate
CH4
Exoelectrogen (or) anode
respiring bacteria
Methanogens,
Sulfate reducers
Hydrogen ions (H+)

Fig. 8.