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Bioelectrohydrogenesis and inhibition of methanogenic activity in microbial electrolysis cells - A review

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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 “bioelectrohydrogenation”. 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; Bioelectrohydrogenation

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References
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 (H₂, CH₄), 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 H₂, 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 (\(E^0 > 1.2 \text{ V}\)) 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:**

\[
\begin{align*}
\text{CH}_3\text{COO}^- + 4\text{H}_2\text{O} & \rightarrow 2\text{HCO}_3^- + 9\text{H}^+ + 8\text{e}^- & E_{\text{anode}} = -0.279 \text{ V} \\
2\text{H}^+ + 2\text{e}^- & \rightarrow \text{H}_2 & E_{\text{cathode}} = -0.414 \text{ V} \\
E^0 & = E_{\text{cathode}} - E_{\text{anode}} = -0.135 \text{ V}
\end{align*}
\]

**Water Electrolysis:**

\[
\begin{align*}
2\text{H}_2\text{O} & \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^- & E_{\text{anode}} = 0.82 \text{ V} \\
2\text{H}^+ + 2\text{e}^- & \rightarrow \text{H}_2 & E_{\text{cathode}} = -0.414 \text{ V} \\
E^0 & = E_{\text{cathode}} - E_{\text{anode}} = -1.22 \text{ V}
\end{align*}
\]

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\(^3\)H\(_2\) 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.
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...
catholyte, which provided high solution conductivity and hence lowered ohmic resistance (Nam and Logan 2011). The use of a membrane is considered an effective way to minimize hydrogen diffusion into the anode chamber, but it introduces complexity and cost to the process. Nonetheless, in most cases the use of two-chamber MECs only enabled hydrogen production rates ranging from 0.01 to 6.3 m³ m⁻³ d⁻¹ (Cheng and Logan 2011).

2.2. Single-chamber MECs

It is accepted that hydrogen evolution occurs due to the cathodic reduction reaction in MECs. The cathodic conversion efficiency (CCE) can be calculated from the ratio of e⁻ equivalent donated to hydrogen formation and e⁻ equivalent transferred from anode to cathode (Logan et al., 2008). A CCE of less than 100% could be attributed to the diffusion of hydrogen to the anode surface, or to biological oxidation. It was inferred that hydrogen diffusion would decrease the CCE by up to 33% in two-chamber MECs (Tartakovsky et al., 2008). To maximize the overall efficiency of a MEC for bioelectrohydrogenesis, the e⁻ equivalent liberated from the anodic substrate must first be efficiently captured by the bio-anode, and subsequently dissipated at the cathode exclusively as hydrogen gas for external collection. Indeed if the produced hydrogen gas could be rapidly harvested to avoid hydrogen diffusion to the anode, the use of membrane may be omitted.

In fact, the use of single-chamber MECs for bioelectrohydrogenesis has been the subject of many earlier studies (Rozendal et al., 2007; Call and Logan 2007; Hu et al., 2008; Tartakovsky et al., 2009). A key attractive feature of single chamber MECs is that both the anode and cathode are housed within one chamber. This single chamber MEC system could be more compact with a lower capital cost. Further, single chamber MECs often exhibit a lower internal resistance. Such systems generally have low ohmic loss and concentration overpotential due to the nonexistence of
detrimental pH gradient between the anolyte and catholyte. (Rozendal et al., 2007; Call and Logan
2007; Hu et al., 2008; Tartakovsky et al., 2009). Call et al., (2008) also found that the bio-hydrogen
production rate recorded from their single-chamber MEC was more than double (3.12 m³ m⁻³ d⁻¹ at
an applied voltage of 0.8V) as compared to that obtained from a two-chamber MEC under identical
operating conditions.

2.3. Continuous flow MECs

Like most other waste treatment bioprocesses, MECs are often characterized for their
ability to treat their feedstock in a continuous fashion (Fig. 3). When operated in continuous mode,
the organic stream is continuously loaded into the MEC at a defined flow rate. Often, the liquid
electrolyte within a continuous flow system is recirculated to maximize mass transfer. The
hydraulic turbulence created as such may help to minimize the accumulation of stagnant hydrogen
gas in the porous electrode matrix (e.g. granular graphite bed), which may help to avoid any
undesirable biological oxidation (loss) of hydrogen in the reactor.

Both organic loading rate (OLR) and applied potential are significant parameters to
determine the yield of hydrogen from continuous flow MECs, and so these parameters are often
selected for process optimization (Cusick et al., 2011; Escapa et al., 2012; Rader et al., 2010). For
instance, Escapa et al. (2012) reported a Monod-type relationship between OLR and hydrogen
production rate (0.3 m³ m⁻³ d⁻¹) in their continuous flow domestic waste water (DWW) fed MECs.
They found that the increase in hydrogen production rate reached a plateau, when the OLRs of
DWW were above 2000 mgCOD m⁻³ d⁻¹. In addition, the energy consumption for pumping the
solution should also be accounted. The produced H₂ and the energy consumption for pumping may
vary depending on the pumping flow rate. For instance, Kim and Logan (2011) noted that 4 x 10⁻
W was required for pumping flow rate at 0.8 ml min\(^{-1}\). This was however, negligible (1%) compared to the energy produced as H\(_2\) (3.8 x 10\(^{-3}\) W) (Kim and Logan 2011).

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

Further, to improve the hydrogen production efficiency from MEC reactors, a suitable electrode configuration should be adopted. The planar electrodes (plate type) and flow through or porous electrodes (3D type) are more common electrode types used in MEC reactors. The planar electrode (e.g. graphite plate) has advantages such as high conductivity, chemical stability, low cost and surface accessibility, and ease of placement (Zhou et al., 2011). However, it is difficult to 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\(\times\)) 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
catalysts (Rozendal et al., 2008). Alternatively, some of the metal catalysts such as Co/FeCo (Cheng and Logan 2008), NiMo/NiW (Hu et al., 2009), Fe/Fe₃C (Li et al., 2012), Nickel powder (Selembo et al., 2010), Pd nanoparticles (Huang et al., 2011), MoS₂ (Tokash and Logan 2011; Tenca et al., 2013), carbon nanotubes (MWCNT) (Wang et al., 2012), and WC (Tungsten carbide) (Harnisch et al., 2009) were investigated to replace Pt catalyst. Metal alloys such as NiFeMo/CoMo (Jeremiasse et al., 2011), Ni-W-P/Ni-Ce-P (Wang et al., 2011), NiFe, NiFeP and NiFeCoP (Mitov et al., 2012) were also investigated for HER in MECs under neutral/mild alkaline conditions. The alloy cathodes NiMo, NiFeMo or CoMo showed superior catalytic activity towards HER (at pH 7) compared with cathodes coated with only Ni (Mitov et al., 2012). These findings suggest that Ni-based cathodes or cathodes modified with nanomaterials are promising cathode materials for HER in MECs (Mitov et al., 2012). High surface area Ni foam cathodes (128 m² m⁻² projected area) were constructed to produce high volumetric hydrogen production (50 m³ m⁻³ d⁻¹ at 1.0 V) in continuous flow MEC using an anion exchange membrane. This effect was due to a lower cathode overpotential (Ni foam cathode) than for Pt-based cathode. However, the performance of the Ni foam cathode was unstable, and often associated with an increase of overpotentials over time (Jeremiasse et al., 2010). On the other hand, stainless steel is another widely used cathode material for MECs due to low cost, high current density and low cathodic overpotential (Zhang et al., 2010; Ambler and Logan 2011; Munoz et al., 2010; Selembo et al., 2009b). A high hydrogen production rate of up to 4.9 L h⁻¹ m⁻² (with 0.8 V applied voltage) was obtained from a MEC equipped with a stainless steel (AISI 316 L) cathode (Munoz et al., 2010).

Alternatively, biocathodes are increasingly being considered for HER in MECs due to low cost and high operational sustainability. Though the concept of a biocathode was discovered in the 1960s, it has not received much attention (He and Angenent 2008). It was found that
microorganisms that contain hydrogenase enzyme could catalyze hydrogen production in various environments (Schwartz and Friedrich 2006). In recent years, further research using biocathodes has shown that they have many advantages over chemical cathodes for HER in MECs (He and Angenent 2008). For instance, it was reported that a biocathode developed from a selected electrochemically active mixed microbial culture could efficiently drive HER in a cathodic half-cell. The biocathode was poised at a potential of -0.7 V vs. Ag/AgCl, and the corresponding hydrogen production rate was up to 0.6 m$^3$ m$^{-3}$ d$^{-1}$, which is 3.6 times higher than the abiotic control (0.08 m$^3$ m$^{-3}$ d$^{-1}$) (Rozendal et al., 2007). A similar finding was reported by Jeremiasses et al. (2010), who found that compared with an abiotic control, the biocathode increased HER by 21% (up to 0.11 L for 52 h). Microorganisms in the biocathode consisted of 46% Proteobacteria, 25% Firmicutes, 17% Bacteroidetes, and 12% related to other phyla (Croese et al., 2011). Considering that biocathodes could potentially be a low-cost substitute to metal-based catalysts, further understanding and development of biocathodes for HER is crucial.

3.3. Membrane options

In general, most MECs are equipped with a cation exchange membrane or proton exchange membrane (PEM) such as Nafion® 117 type PEM (Dhar and Lee 2013). The use of a membrane separator in a MEC helps to prevent substrate crossover between the two half-cells, thereby minimizing the loss of hydrogen (Logan et al., 2008). However, the membranes in wastewater-treating MECs often leads to the so-called pH splitting limitation due to the magnitudes higher concentration of other ions such as Na$^+$, K$^+$, NH$_4^+$, and Ca$^{2+}$ compared with H$^+$ in wastewater (nearly $10^5$ times higher than that of proton H$^+$) (Rozendal et al., 2006). As a result, the anolyte can easily become acidified, suppressing the microbial activity of substrate oxidation (Liu et al.,
and the catholyte to become more alkaline, which is unfavorable for the hydrogen evolution reaction. Recently, a sulfonated polyether ketone-based novel nanofiber reinforced PEM (NFR-PEM) was developed as a proton conductor for MECs, which showed lower gas and fuel crossovers with higher proton conductivity compared with Nafion® membrane (Chae et al., 2014). Membrane electrode assembly (MEA) cathode has also been developed to enhance hydrogen production efficiency (maximum hydrogen efficiency of 41% with an applied voltage of 1.2 V) in MECs (Jia et al., 2012). However, the use of membrane would incur significant capital cost. It has been estimated that the cost of ion exchange membrane accounted for 38% (400 € m⁻²) of the capital cost of a laboratory–scale H₂-MEC, suggesting that nearly half of the total cost of MEC was associated with the use of membrane (Rozendal et al., 2008).

On the other hand, avoiding the use of membranes could prevent the pH splitting limitation and reduce capital costs. This may also allow the design of simpler reactor configurations (Call and Logan 2008). However, the membrane free MECs were also found to be problematic due to diffusion of hydrogen from cathode to anode, where hydrogen may become available to hydrogenotrophic methanogens leading to methane production. It was found that at an applied voltage of 0.2 V, methane concentrations in the product gas increased up to 28% due to the long cycle time of the reactor. The high cathodic hydrogen recoveries (78±1% to 96±1%) and lower methane (1.9±1.3%) were achieved in a membrane free MEC with applied voltages ranging from 0.3 to 0.8 V, and with a solution conductivity of 7.5 mS cm⁻¹ (Call and Logan 2008).

3.4. Substrate versatility

MEC can produce hydrogen from a wide range of simple and complex organic substrates. Table 1 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/m²), applied voltage (V); overall H₂ recovery (R_H₂, %); and energy efficiency relative to electrical input (η_E, %). Particularly, the selection of substrate can remarkably affect the hydrogen production rate (Q, m³H₂/m³d) (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 m³m⁻³d⁻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 m³m⁻³d⁻¹), butyric acid (0.45 m³m⁻³d⁻¹), lactic acid (1.04 m³m⁻³d⁻¹), propionic acid (0.72 m³m⁻³d⁻¹), valeric acid (m³m⁻³d⁻¹) (Cheng and Logan 2007), P-glycerol (0.8 m³m⁻³d⁻¹) (Selembo et al., 2009b), B-glycerol (0.41 m³m⁻³d⁻¹) (Selembo et al., 2009b) and Trehalose (0.25 m³m⁻³d⁻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 m³m⁻³d⁻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-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-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,

\[ \text{Anode: } \text{CH}_3\text{COOH} + 2\text{H}_2\text{O} \rightarrow 2\text{CO}_2 + 8\text{H}^+ + 8\text{e}^- \]  

\[ \text{Cathode: } 8\text{H}^+ + 8\text{e}^- \rightarrow 4\text{H}_2 \]

Co-production of CH$_4$ by methanogens,
\[
\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2 \quad (5)
\]

\[
4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \quad (6)
\]

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
that halogenated hydrocarbons (e.g. CHCl$_3$ or CHX$_3$) can inhibit the production of methane from H$_2$/CO$_2$ and acetate. This is due to the complete blocking of corrinoid enzymes. To inhibit methyl-coenzyme M reductase in hydrogenotrophic and acetoclastic methanogens, 2-bromomethane sulfonate (2-BES) and Lumazine are often used as methanogenic inhibitors (Liu et al., 2011). 2-BES is a structural analog of CoM. Hence, it can block methane formation catalyzed by methyl-CoM reductase. Similarly, Lumazine is a structural analogue of methanopetrin and it can inhibit methanogenesis. Due to the specificity of these chemicals, they are considered specific inhibitors for methanogens.

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

The specific inhibitor sodium molybdate (5 mM) can be effectively used as to inhibit sulfate reducing bacteria (Scholten et al., 2000) to control hydrogen sulfide formation. Also, halogenated aliphatic hydrocarbon compounds (e.g. CHCl$_3$) can inhibit the activity of methanogenic archaea as well as of homoacetogenic bacteria and acetate/hydrogen-utilizing sulfate-reducing bacteria (Scholten et al., 2000; Liu et al., 2011).

Numerous reports have explored strategies to inhibit methanogens or suppress methane formation in H$_2$ producing MECs (Table 2). Typically, those strategies entail the manipulation of
the physiochemical conditions of the process, targeting the sensitive nature of methanogens to the imposed environmental stress. For example, Hu et al. (2008) examined three different suppression strategies, namely (i) lowering the electrolyte pH to 5.8 with phosphate buffer: NaH₂PO₄, 25.4 g/L; Na₂HPO₄, 4.25 g/L; (ii) exposing the cathode to air for 15 min when the methane was found to have accumulated in the MEC headspace; and (iii) boiling the anodes from MFCs at 100°C for 15 min before placing them in the MEC. Their results implied that lowering the pH in the MEC to 5.8 was immediately effective for suppressing methane production. However, methane production (up to 5.5%) resumed after two batch cycles, suggesting that the acidic shock could only be a short-term solution to the problem (Hu et al., 2008). Similar findings were reported by Kim et al. (2004) and Chae et al. (2010), who showed that acidification also led to inhibition of the exoelectrogen and hence a reduced efficiency of H₂ production. Hence, using acidification to suppress methanogenesis in MEC may not be suitable.

It has been demonstrated that a remarkable inhibition of methanogenesis was achieved by lowering the operating temperature to 15°C and 4-9°C (Liu et al., 2005; Lu et al., 2011). However, as most exoelectrogens and methanogens can tolerate a broad range of temperatures, lowering the temperature does not significantly contribute towards improving the hydrogen yield. Further, this method is not effective for suppressing methanogenic activity during long-term operation of H₂ producing MECs (Rader and Logan 2010).

Another effective strategy to suppress methane production is via optimization of applied voltage. In general, increasing the applied voltage of a MEC increases H₂ production and concentration. It was shown that methane production was higher than H₂ 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).
Recently, improved hydrogen production was demonstrated in single chamber MECs with
the addition of 5% chloroform to inhibit methanogens for up to 11 cycles (Zhang et al., 2016). The
maximum hydrogen production obtained was 8.4± 0.2 mol H₂ mol-glucose⁻¹ at a rate of 2.39 ± 0.3
m³ m⁻³ d⁻¹ with high energy efficiency (165 ±5%) (Zhang et al., 2016). Chloroform (CHCl₃) blocks
the activity of corrinoid enzymes and inhibits the activity of methyl-coenzyme M reductase in
methanogenic archaia (Table 2).

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

Catal et al., (2015) demonstrated that methanogenesis can be controlled effectively in long-
term by the addition of inhibitors in hydrogen producing MECs. The methanogenic inhibitors
namely neomycin sulfate, 8-aza-hypoxanthine, 2-bromoethanesulfonate and 2-chloroethane
sulfonate were used to examine the inhibition of methanogenesis. The application of antibiotics as
methanogenic inhibitors in this study provides a novel approach to inhibit methanogenesis in
MECs. Moreover, the methanogenic inhibition methods such as applied potential, rapid extraction
of H2, heat treated electrode, use of biocathode, addition of fatty acids, intermittent oxygen exposure, and use of microbial cultures enriched in the presence of the chemical inhibitor were only able to limit methane formation to a certain extent. In contrast, no methane was detected when methanogenic inhibitors were added directly into MECs (Table 2). Also, the methanogenic inhibitors specifically compete with MCR and inhibit methane generation in hydrogen producing MEC. The growth of methanogen in MECs is a known challenge and requires specific control strategies like methanogenic inhibitors (Table 2).

In general, methanogenic pathways use several cofactors, namely coenzyme M (CoM; HSCH2CH2SO3−), methanofuran (2-aminomethylfuran linked to phenoxy group), and methanopterin (H4MPT;5,6,7,8-tetrahydromethanopterin) (Fig. 5). These cofactors act as C1 carriers in methanogenesis (Liu et al., 2011) and they are used by all methanogens. The terminal step of the methanogenic pathway is methane formation, whereby the methyl group carried by CoM is reduced to methane by an enzyme known as methyl-coenzyme M reductase (MCR). This enzyme catalyzes the reaction of CH3-S-CoM (Methyl CoM) with CoB (CoenzymeB) to produce methane (CH4) and heterodisulfide CoM-S-S-CoB as presented in Fig. 6.

In the methanogenesis pathway, the terminal step is the reaction of CoM with N-7-mercaptoheptanoylthreonine phosphate (CoB). The main product of this terminal step is methane, although mixed disulfide (CoM-S-S-HTP) could also be formed (Ellermann et al., 1988). The MCR enzyme was isolated from methanogens and tested for the inhibition. Enzyme inhibitors that were selected had a terminal sulfonate (SO3−) and are structural analogues of CoM. Several inhibitors have been investigated such as 1-butanesulfonate, 1-propanesulfonate, 2-azidoethanesulfonate, 2-bromoethanesulfonate, 3-azidopropanesulfonate, 3-bromopropane sulfonate, 3-bromopropionate, 3-chloropropanesulfonyl chloride, 3-fluoropropanesulfonate, 3-
hydroxypropanesulfonate, 3-iodopropane sulfonate, 3-mercaptopropanesulfonate, 4-bromobutyrate, 4-bromobutyrate sulfonate, 7-bromoheptanoylthreonine phosphate (CoB analogue), 4-chlorobutyrate and chloromethanesulfonate (Table 3). These inhibitors compete with MCR and inhibit methane generation. It is known that MCR has cofactor 430 (F₄₃₀), which has Ni(I) in its active site. This Ni(I) reacts with inhibitors and changes to the inactive Ni(III) state (Kunz et al., 2006). The central nickel atom of F₄₃₀ is coordinated by four planar tetraarylporphyrine nitrogen atoms. For example, the methanogenic inhibitor, 1-bromoethane sulfonate (1-BES) can interact with Ni(I)-MCR_red and forms the inactive state of Ni(III)-MCR_sulfonate, while in the absence of inhibitor, Ni(I)-MCR_red interacts with CH₃-SCoM to form methane as depicted in the reaction scheme in Fig. 7. The use of inhibitors in H₂-MECs offers an advantage of long-term inhibition. However, the concentration of inhibitors can vary based on the field application and this can influence cost of operation of the MECs. To address this challenge for practical applications, the inhibitors can be added only when needed. Another option could be by adopting feedback inhibitor-dosing strategy based on the composition of biogas. Here, if H₂ partial pressure is lower than a certain threshold, dosing of an inhibitor is triggered.

5. Conclusion and future prospects

To achieve large-scale implementation of MECs for hydrogen production, methanogenesis has to be controlled. Other issues that can also influence H₂-MEC performance are those relating to the bioanode. These include the pH sensitivity of biofilms. Bioelectrohydrogenesis is a microbial process. Therefore, a better understanding of microbial electron transfer mechanisms will certainly be important from a process stability perspective. Reactor design also plays an important role for scaling up of MEC. For example, single chamber MECs that lack a membrane
always showed the production of methane with lower hydrogen yields. As discussed, most MEC studies were conducted with small-scale laboratory systems (Table 1). Only few pilot scale plants with capacities between 20 L and 1000 L were trialed, and the performance of these plants was affected by technical challenges such as influent flocculation, water leakage, electrochemical losses and production of unfavorable products (Wang et al., 2013). Cusik et al. (2011) developed the first pilot scale (1000 L) single chamber continuous flow membrane-less MECs for bioelectrohydrogenesis. However, their process failed to produce hydrogen due to formation of methane via hydrogenotrophic methanogenesis. It is now accepted that using membrane-less MECs for hydrogen production is practically challenging. To maximize the yield and purity of hydrogen, effective and implementable strategies should be identified to reduce the formation of methanogenic growth and to promote hydrogen formation. As reviewed here, it is feasible to select suitable inhibitor(s) to prevent methane formation (Fig. 8). Future research should be devoted towards developing robust, combinatorial and specific anti-microbial approaches to bring the technology towards practical application.

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Figure captions

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

Fig. 2 Operational principle of microbial electrolysis cell (a) and water electrolysis cell (b); Acetate - organic substrate for exoelectrogenic bacteria (Biofilm), Anode- positive terminal electrode that accept e⁻ from Exoelectrogenic bacteria, Cathode - negative terminal electrode that donate e⁻ for H₂ evolution; Potentiostat or power supply - Electrical device to control applied cell potential for hydrogen evolution reaction, and PEM- proton exchange membrane (optional)

Fig. 3. Hydrogen producing microbial electrolysis set up; (A) H - shaped two chamber MEC — 320 mL (Liu et al., 2005) (B) two chamber MEC - 32 mL (Cheng and Logan 2007), (C) single chamber MEC - 28 mL (Calland Logan 2008), (D) Single chamber MEC in round bottom flasks - 250 mL (Brown et al., 2014), (E) single chamber MEC in borosilicate glass serum vials -100 mL (Hu et al., 2008), F) single chamber MEC in borosilicate glass serum tubes - 28 mL (Hu et al., 2009), (G) continuous flow MEC with multi-electrodes - 2.4 L, 1.67 mL min⁻¹ (Rader and Logan 2010), (H) pilot-scale continuous flow MEC fed 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$_3$F, Na$_2$MoO$_4$, 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. (H$_4$MPT, tetrahydromethanopterin; CoA, Co enzyme A; CH$_3$COSCoA, 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.

<table>
<thead>
<tr>
<th>MEC configuration / Working volume</th>
<th>Anode</th>
<th>Cathode</th>
<th>Microbial inoculum/ Source</th>
<th>Substrate</th>
<th>Applied voltage (V)</th>
<th>H$_2$ rate or Yield (m$^3$H$_2$m$^{-3}$d$^{-1}$)</th>
<th>H$_2$ (%)</th>
<th>CH$_4$ (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two chamber continuous flow at 2.6 mL min$^{-1}$ / 200 mL</td>
<td>Graphite felt</td>
<td>Co-Mo alloy</td>
<td>Mixed cultures / Waste water effluent</td>
<td>Acetate / 2.72 g L$^{-1}$</td>
<td>1.0</td>
<td>50</td>
<td>NA</td>
<td>NA</td>
<td>Jeremiasse et al., 2011</td>
</tr>
<tr>
<td>Single chamber fed batch / 28 mL</td>
<td>Heat treated graphite brush</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / Pennsylvania State University WWP</td>
<td>Acetate / 1.5 g L$^{-1}$</td>
<td>0.6</td>
<td>3.6</td>
<td>68</td>
<td>35</td>
<td>(Nam et al., 2011)</td>
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<td>Single chamber fed batch / 28 mL</td>
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<td>Carbon cloth/Pt</td>
<td>Mixed cultures / enriched biofilm in MFC</td>
<td>Acetate / 1 g L$^{-1}$</td>
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<td>3.12</td>
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<td>1.9</td>
<td>(Call and Logan 2008)</td>
</tr>
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<td>Carbon cloth</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / Pennsylvania State University WWP</td>
<td>Acetate / 5 g L$^{-1}$</td>
<td>0.6</td>
<td>2.3</td>
<td>85</td>
<td>&gt;1%</td>
<td>(Hu et al., 2009)</td>
</tr>
<tr>
<td>Single chamber fed batch / 26 mL</td>
<td>Graphite brush</td>
<td>Carbon cloth</td>
<td>Mixed cultures / enriched biofilm in MFC</td>
<td>Fermentation effluent / 6.5 g L$^{-1}$</td>
<td>0.6</td>
<td>2.11</td>
<td>96</td>
<td>NA</td>
<td>(Lu et al., 2009)</td>
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<td>Single chamber fed batch / 28 mL</td>
<td>Ammonia treated graphite brush</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / WWP</td>
<td>P-Glycerol / 1 g L$^{-1}$</td>
<td>0.9</td>
<td>2.01</td>
<td>88</td>
<td>1.2</td>
<td>(Selembo et al., 2009b)</td>
</tr>
<tr>
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<td>Carbon cloth/NiMo</td>
<td>Mixed cultures / Pennsylvania State University WWP</td>
<td>Acetate / 5 g L$^{-1}$</td>
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<td>2.0</td>
<td>86</td>
<td>&lt;1</td>
<td>(Hu et al., 2009)</td>
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<td>Carbon cloth/Pt</td>
<td>Mixed cultures / enriched biofilm in MFC</td>
<td>Acetate / 1 g L$^{-1}$</td>
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<td>1.99</td>
<td>78</td>
<td>28</td>
<td>(Call and Logan 2008)</td>
</tr>
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<td>Ammonia treated graphite brush</td>
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<td>Mixed cultures / WWP</td>
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<td>Carbon cloth/Pt</td>
<td>Mixed cultures / enriched biofilm in MFC</td>
<td>Food processing waste water / 8.1 Kg m$^{-3}$</td>
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<td>1.8</td>
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<td>55</td>
<td>(Tenca et al., 2013)</td>
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<tr>
<td>Single chamber / 28 mL</td>
<td>graphite fiber brush</td>
<td>SS brush</td>
<td>Mixed cultures/ ARB biofilm from MFC</td>
<td>Acetate / 1 g L$^{-1}$</td>
<td>0.5</td>
<td>1.7</td>
<td>84</td>
<td>2.3</td>
<td>(Call et al., 2009)</td>
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<td>Single chamber batch / 400 mL</td>
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<td>Ti tube/Pt</td>
<td>Mixed cultures / enriched biofilm in MFC</td>
<td>Acetate / 0.5 g</td>
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<td>88</td>
<td>0.04</td>
<td>(Guo et al., 2010)</td>
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<tr>
<td>Experiment</td>
<td>Reactor</td>
<td>Media</td>
<td>Nutrient(s)</td>
<td>pH</td>
<td>Current Density</td>
<td>COD Removal (%)</td>
<td></td>
<td></td>
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<td>Carbon cloth/NiW</td>
<td>Mixed cultures / Pennsylvania State University WWP</td>
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<td>Mixed cultures / enriched biofilm in MFC</td>
<td>Buffered effluent / 6.5 g⁻¹</td>
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<td>Mixed cultures / Pennsylvania State University WWP</td>
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<td>91%</td>
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<td>Ambler and Logan 2011</td>
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<td>Glucose / 1 g L⁻¹</td>
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<td>NA</td>
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<td>(Cheng and Logan 2007)</td>
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<td>Clostridium thermocellum enriched biofilm in MFC</td>
<td>Synthetic effluent / 5 g L⁻¹</td>
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<td>120 mL g-COD⁻¹</td>
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<td>Mixed cultures / Swine farm WWP</td>
<td>Swine waste water / 2 g L⁻¹</td>
<td>0.55</td>
<td>1</td>
<td>77</td>
<td>13</td>
<td>(Wagner et al., 2009)</td>
</tr>
<tr>
<td>Two chamber fed batch / 14 mL</td>
<td>Ammonia treated graphite granule</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / WWP</td>
<td>Acetic acid / 1 g L⁻¹</td>
<td>0.6</td>
<td>1.1</td>
<td>91</td>
<td>NA</td>
<td>(Cheng and Logan 2007)</td>
</tr>
<tr>
<td>Two chamber fed batch / 14 mL</td>
<td>Ammonia treated graphite granule</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / WWP</td>
<td>Lactic acid / 1 g L⁻¹</td>
<td>1.04</td>
<td>91</td>
<td>NA</td>
<td></td>
<td>(Cheng and Logan 2007)</td>
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<tr>
<td>Single chamber fed batch / 28 mL</td>
<td>Graphite fiber brush</td>
<td>Carbon cloth/Pt</td>
<td>Clostridium thermocellum enriched biofilm in MFC</td>
<td>Cellobiose / 5 g L⁻¹</td>
<td>0.5</td>
<td>0.96</td>
<td>69</td>
<td>210 mL g-COD⁻¹</td>
<td>Lalaurette et al., 2009</td>
</tr>
<tr>
<td>Two chamber fed batch / 26 mL</td>
<td>Graphite brush</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / WAS</td>
<td>Alkaline WAS / 2.4 g L⁻¹</td>
<td>0.6</td>
<td>0.91</td>
<td>72</td>
<td>NA</td>
<td>(Lu et al., 2012c)</td>
</tr>
<tr>
<td>Single chamber fed batch / 28 mL</td>
<td>Ammonia treated graphite brush</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / WWP</td>
<td>Glucose / 1 g L⁻¹</td>
<td>0.5</td>
<td>0.83</td>
<td>81</td>
<td>9.5</td>
<td>(Selembo et al., 2009b)</td>
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<td>Single chamber fed batch / 28 mL</td>
<td>Ammonia treated graphite brush</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / WWP</td>
<td>P-Glycerol / 1 g L⁻¹</td>
<td>0.5</td>
<td>0.80</td>
<td>80</td>
<td>9.5</td>
<td>(Selembo et al., 2009b)</td>
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<tr>
<td>Single chamber fed batch / 28 mL</td>
<td>Ammonia treated graphite brush</td>
<td>Carbon cloth/Pt</td>
<td>Geobacter sp./ enriched biofilm in MFC</td>
<td>Potato waste water / 1.9-2.5 g L⁻¹</td>
<td>0.9</td>
<td>0.74</td>
<td>73</td>
<td>13</td>
<td>(Kiely et al., 2011)</td>
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<td>Configuration</td>
<td>Electrode Material</td>
<td>Microbial Community</td>
<td>Added Substrate</td>
<td>Voltage (V)</td>
<td>Current Density (mA cm$^{-2}$)</td>
<td>COD Removal (%)</td>
<td>Reference</td>
<td></td>
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<tr>
<td>Two chamber fed batch / 14 mL</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / WWP</td>
<td>Propionic acid / 1 g L$^{-1}$</td>
<td>0.72</td>
<td>89</td>
<td>NA</td>
<td>(Cheng and Logan 2007)</td>
<td></td>
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<tr>
<td>Single chamber fed batch / 28 mL</td>
<td>graphite fiber brush</td>
<td>Mixed cultures / enriched biofilm in MFC</td>
<td>Acetate / 1 g L$^{-1}$</td>
<td>0.59</td>
<td>77</td>
<td>NA</td>
<td>(Selembbo et al., 2009a)</td>
<td></td>
<td></td>
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<tr>
<td>Single chamber fed batch / 28 mL</td>
<td>SS420 graphite fiber brush</td>
<td>Mixed cultures / enriched biofilm in MFC</td>
<td>Acetate / 1 g L$^{-1}$</td>
<td>0.58</td>
<td>67</td>
<td>NA</td>
<td>(Selembbo et al., 2009a)</td>
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<tr>
<td>Single chamber continuous flow at 0.88 mL min$^{-1}$ / 140 mL</td>
<td>Graphite granules</td>
<td>Carbon felt</td>
<td>Mixed cultures / ARB biofilm from an acetate-fed MFC having a Geobacter-rich community</td>
<td>Acetate / 10 mM</td>
<td>0.57</td>
<td>59</td>
<td>2 (Lee et al., 2009)</td>
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<td>Two chamber fed batch / 14 mL</td>
<td>Ammonia treated graphite granule</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / WWP</td>
<td>0.45</td>
<td>80</td>
<td>NA</td>
<td>(Cheng and Logan 2007)</td>
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<td>Single chamber fed batch / 26 mL</td>
<td>Graphite brush</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / WWP</td>
<td>0.42</td>
<td>34</td>
<td>&lt;0.9 mM g-COD$^{-1}$</td>
<td>(Lu et al., 2010)</td>
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<tr>
<td>Single chamber fed batch / 28 mL</td>
<td>Ammonia treated graphite brush</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / WWP</td>
<td>0.41</td>
<td>87</td>
<td>1.2</td>
<td>(Selembbo et al., 2009b)</td>
<td></td>
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<td>Single chamber fed batch / 28 mL</td>
<td>graphite fiber brush</td>
<td>SS316 mixed cultures / enriched biofilm in MFC</td>
<td>Acetate / 1 g L$^{-1}$</td>
<td>0.35</td>
<td>55</td>
<td>NA</td>
<td>(Selembbo et al., 2009a)</td>
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<td>Single chamber fed batch / 38 mL</td>
<td>Graphite brush</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / WAS</td>
<td>0.25</td>
<td>80</td>
<td>NA</td>
<td>(Xu et al., 2014a)</td>
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<tr>
<td>Single chamber fed batch / 28 mL</td>
<td>Heat treated graphite brush</td>
<td>MoS$_2$</td>
<td>Mixed cultures / enriched biofilm in MFC</td>
<td>Industrial waste water 4.1 Kg m$^{-3}$</td>
<td>0.17</td>
<td>NA</td>
<td>70 (Tenca et al., 2013)</td>
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<td></td>
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<tr>
<td>Two chamber fed batch / 14 mL</td>
<td>Ammonia treated graphite granule</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / WWP</td>
<td>0.14</td>
<td>67</td>
<td>NA</td>
<td>(Cheng and Logan 2007)</td>
<td></td>
<td></td>
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<tr>
<td>Single chamber fed batch / 28 mL</td>
<td>Ammonia treated graphite brush</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / WWP</td>
<td>0.14</td>
<td>82</td>
<td>9.5</td>
<td>(Selembbo et al., 2009b)</td>
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<td></td>
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<td>Single chamber fed batch / 28 mL</td>
<td>Heat treated graphite brush</td>
<td>SS304 sheet</td>
<td>Mixed cultures / enriched biofilm in MFC</td>
<td>Industrial waste water 4.1 Kg m$^{-3}$</td>
<td>0.12</td>
<td>NA</td>
<td>62 (Tenca et al., 2013)</td>
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<tr>
<td>Two chamber fed batch / 14 mL</td>
<td>Ammonia treated graphite granule</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures / WWP</td>
<td>0.11</td>
<td>68</td>
<td>NA</td>
<td>(Cheng and Logan 2007)</td>
<td></td>
<td></td>
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<tr>
<td>Configuration</td>
<td>Electrode Material</td>
<td>Electrode Composition</td>
<td>Biofilm Type / Feedstock</td>
<td>COD Removal (%)</td>
<td>Volatile H2 (mol/mol substrate)</td>
<td>Reference</td>
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<tr>
<td>Single chamber fed batch / 28 mL</td>
<td>Graphite fiber brush</td>
<td>Carbon cloth/Pt</td>
<td><em>Clostridium thermocellum</em> enriched biofilm in MFC</td>
<td>0.5</td>
<td>0.11</td>
<td>68</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Lignocellulose / 5 g L⁻¹</td>
<td></td>
<td>120 mL g-COD⁻¹</td>
<td>Lalaurette et al., 2009</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Two chamber fed batch / 28 mL</td>
<td>Graphite felt</td>
<td>Ti plate/Pt</td>
<td><em>Pelobacter propionicus</em> / Anaerobic digested sludge</td>
<td>0.8</td>
<td>0.052</td>
<td>97</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Acetate / 2 mM</td>
<td></td>
<td>2.5</td>
<td>(Chae et al., 2008)</td>
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<tr>
<td>Two chamber fed batch / 120 mL</td>
<td>Carbon brush</td>
<td>Pt/C</td>
<td>Mixed cultures / anaerobic sludge from WWP</td>
<td>0.8</td>
<td>0.0231</td>
<td>32</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Acetate / 1 g L⁻¹</td>
<td></td>
<td>NA</td>
<td>(Xiao et al., 2012)</td>
<td></td>
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<tr>
<td>Two Chamber fed batch / 6.6 L</td>
<td>Graphite felt</td>
<td>Ti/Pt</td>
<td>Mixed cultures / sludge from UASB reactor</td>
<td>0.5</td>
<td>0.02</td>
<td>NA</td>
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<td></td>
<td></td>
<td>Acetate / 10 Mm</td>
<td></td>
<td>NA</td>
<td>(Rozendal et al., 2006)</td>
<td></td>
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<tr>
<td>Two chamber fed batch / 120 mL</td>
<td>Carbon brush</td>
<td>Fe/Fe3C @C</td>
<td>Mixed cultures / anaerobic sludge from WWP</td>
<td>0.8</td>
<td>0.0182</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acetate / 1 g L⁻¹</td>
<td></td>
<td>NA</td>
<td>(Xiao et al., 2012)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two chamber fed batch / 200 mL</td>
<td>Carbon felt</td>
<td>Ti/Pt</td>
<td>Mixed cultures / Gwangju sewage treatment plant</td>
<td>-</td>
<td>0.013</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acetate / 1.5 g L⁻¹</td>
<td></td>
<td>NA</td>
<td>(Lee et al., 2015)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Two chamber fed batch / 130 mL</td>
<td>Carbon brush</td>
<td>Carbon cloth/MoS₂/CNT-90</td>
<td>NA</td>
<td>0.8</td>
<td>0.01</td>
<td>12.7</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Acetate / 1 g L⁻¹</td>
<td></td>
<td>NA</td>
<td>(Yuan et al., 2014)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two chamber fed batch / 120 mL</td>
<td>Carbon brush</td>
<td>CNT</td>
<td>Mixed cultures / anaerobic sludge from WWP</td>
<td>0.8</td>
<td>0.0076</td>
<td>16</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Acetate / 1 g L⁻¹</td>
<td></td>
<td>NA</td>
<td>(Xiao et al., 2012)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two chamber fed batch / 120 mL</td>
<td>Carbon brush</td>
<td>CNT</td>
<td>Mixed cultures / anaerobic sludge from WWP</td>
<td>1.06</td>
<td>NA</td>
<td>31</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Acetate / 30 mM</td>
<td></td>
<td>32</td>
<td>(Lee et al., 2009)</td>
<td></td>
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<tr>
<td>Two chamber fed batch / 120 mL</td>
<td>Carbon brush</td>
<td>CNT</td>
<td>Mixed cultures / anaerobic sludge from WWP</td>
<td>1.06</td>
<td>NA</td>
<td>28</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Acetate / 80 mM</td>
<td></td>
<td>37</td>
<td>(Lee et al., 2009)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single chamber fed batch / 130 mL</td>
<td>Graphite fiber brush</td>
<td>Carbon cloth/Pt</td>
<td>Mixed cultures/ Liede WWP</td>
<td>0.8</td>
<td>3.7 mol H₂/mol acetate</td>
<td>95</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Acetate</td>
<td></td>
<td>&lt;0.6</td>
<td>(Hou et al., 2014)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two chamber fed batch / 28 mL</td>
<td>Heat treated graphite brush</td>
<td>SS/Pt</td>
<td>Mixed cultures/ Pennsylvania State University WWP</td>
<td>0.9</td>
<td>3.2 mol H₂ / mol acetate</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Two chamber fed batch / 200 mL</td>
<td>Carbon paper</td>
<td>Carbon paper/Pt</td>
<td>Mixed cultures / enriched biofilm in MFC</td>
<td>0.5</td>
<td>0.154 H₂ g-COD⁻¹</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Domestic waste water / 1 g L⁻¹</td>
<td></td>
<td>NA</td>
<td>(Ditzig et al., 2007)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Methanogenesis suppression method</th>
<th>Details</th>
<th>Hydrogen production rate (m$^3$H$_2$m$^{-3}$d$^{-1}$)</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied potential</td>
<td>0.8 V</td>
<td>-</td>
<td>Methane increased at below 0.8 V</td>
<td>Ding et al., 2016</td>
</tr>
<tr>
<td>Rapid H$_2$ extraction method</td>
<td>gas-permeable hydrophobic membrane and vacuum</td>
<td>1.58±0.5</td>
<td>No methane</td>
<td>Lu et al., 2016</td>
</tr>
<tr>
<td>Heat treated electrode</td>
<td>Bioanode boiled at 100°C for 15 min</td>
<td>0.69</td>
<td>1% methane detected in head space</td>
<td>Hu et al., 2008</td>
</tr>
<tr>
<td>Biocathode</td>
<td>Hydrogen producing bioelectrode developed at -0.65 V</td>
<td>10</td>
<td>Methane detected at start up time</td>
<td>Rozendal et al., 2008</td>
</tr>
<tr>
<td>Effect of fatty acids</td>
<td>Acetic acid and propionic acid mixture</td>
<td>0.265</td>
<td>No Methane detected.</td>
<td>Ruiz et al., 2014</td>
</tr>
<tr>
<td>Oxygen exposure</td>
<td>Bio-anode exposed to air for 24 h</td>
<td>-</td>
<td>No Methane production for 12 h</td>
<td>Ajayi et al., 2010</td>
</tr>
<tr>
<td>Specific culture</td>
<td>Heat treated Clostridium ljungdahlii isolated from anaerobic sludge treated with 2-bromoethanesulfonate</td>
<td>-</td>
<td>No methane detected over 300 days. Acetate along with hydrogen were produced from CO$_2$.</td>
<td>Bajracharya et al., 2017</td>
</tr>
<tr>
<td>Chemical inhibitor or methanogen</td>
<td>5% chloroform</td>
<td>2.39 ± 0.3</td>
<td>No methane was detected in fed batch cycle</td>
<td>Zhang et al., 2016</td>
</tr>
<tr>
<td></td>
<td>2-bromoethanesulfonate, 10 mM</td>
<td>1.08±0.1</td>
<td>No methane detected</td>
<td>Hari et al., 2016</td>
</tr>
<tr>
<td></td>
<td>2-bromoethanesulfonate (286 µM)</td>
<td>-</td>
<td>No methane detected</td>
<td>Chae et al., 2010</td>
</tr>
<tr>
<td>Compound</td>
<td>Concentration</td>
<td>Effect</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>---------------</td>
<td>---------------------------------------------</td>
<td>---------------------------</td>
<td></td>
</tr>
<tr>
<td>2-bromoethanesulfonate</td>
<td>50 mM</td>
<td>Methanogens were completed inhibited</td>
<td>Parameswaran et al., 2009</td>
<td></td>
</tr>
<tr>
<td>Alamethicin</td>
<td>13 µM</td>
<td>No methane detected</td>
<td>Zhu et al., 2015</td>
<td></td>
</tr>
<tr>
<td>2-chloroethane sulfonate</td>
<td>20 mM</td>
<td>Methane inhibited with increasing hydrogen production</td>
<td>Catal et al., 2015</td>
<td></td>
</tr>
<tr>
<td>2-bromoethane sulfonate</td>
<td>20 mM,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-aza-hypoxanthine</td>
<td>3.6 mM</td>
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Table 3. Inhibition of Methyl-Coenzyme M reductase (MCR) for different methanogens

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Apparent concentration (mM)</th>
<th>Organisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-butanesulfonate</td>
<td>70 mM</td>
<td><em>Methanothermobacter marburgensis</em></td>
<td>(Kunz et al., 2006)</td>
</tr>
<tr>
<td>1-propanesulfonate</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2-azidoethanesulfonate</td>
<td>0.001 mM</td>
<td>-</td>
<td>(Gunsalus et al., 1980)</td>
</tr>
<tr>
<td>2-bromoethanesulfonate</td>
<td>4 µM</td>
<td><em>Methanothermobacter thermautotrophicus, Methanothermobacter marburgensis</em></td>
<td></td>
</tr>
<tr>
<td>3-azidopropanesulfonate</td>
<td>1 µM</td>
<td><em>Methanothermobacter thermautotrophicus</em></td>
<td>(Ellermann et al., 1989)</td>
</tr>
<tr>
<td>2-bromoethanesulfonate</td>
<td>0.004 mM</td>
<td>-</td>
<td>(Ellermann et al., 1988)</td>
</tr>
<tr>
<td>3-azidopropanesulfonate</td>
<td>0.04 mM</td>
<td><em>Methanothermobacter thermautotrophicus</em></td>
<td>(Ellermann et al., 1989)</td>
</tr>
<tr>
<td>3-bromopropane sulfonate</td>
<td>0.00005 mM, irreversible, strong inhibitor and competitive substrate</td>
<td><em>Methanothermobacter marburgensis</em></td>
<td>(Goenrich et al., 2004)</td>
</tr>
<tr>
<td>3-Bromopropionate</td>
<td>irreversible</td>
<td><em>Methanothermobacter marburgensis</em></td>
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<td>3-chloropropanesulfonyl chloride</td>
<td>1mM</td>
<td><em>Methanothermobacter marburgensis</em></td>
<td>(Kunz et al., 2006)</td>
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<td>3-fluoropropanesulfonate</td>
<td>-</td>
<td><em>Methanothermobacter thermautotrophicus</em></td>
<td>(Rospert et al., 1992)</td>
</tr>
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<td>3-hydroxypropanesulfonate</td>
<td>-</td>
<td><em>Methanothermobacter thermautotrophicus</em></td>
<td>(Ellermann et al., 1989)</td>
</tr>
<tr>
<td>3-iodopropane sulfonate</td>
<td>-</td>
<td><em>Methanothermobacter marburgensis</em></td>
<td>(Goenrich et al., 2004)</td>
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<td>3-mercapto-1-propanesulfonate</td>
<td></td>
<td><em>Methanothermobacter marburgensis</em></td>
<td>(Kunz et al., 2006)</td>
</tr>
<tr>
<td>Compound</td>
<td>Concentration</td>
<td>Organism</td>
<td>References</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>---------------</td>
<td>----------------------------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>4-bromobutyrate</td>
<td>-</td>
<td><em>Methanothermobacter marburgensis</em></td>
<td>(Kunz et al., 2006; Goenrich et al., 2004)</td>
</tr>
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<td>4- bromobutanesulfonate</td>
<td>0.006 mM</td>
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<td>(Kunz et al., 2006)</td>
</tr>
<tr>
<td>7-bromoheptanoylthreonine phosphate</td>
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<td>(Gunsalus et al., 1980)</td>
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<td>4-Chlorobutyrate</td>
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<td><em>Methanothermobacter marburgensis</em></td>
<td>(Kunz et al., 2006)</td>
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<td>4-bromobutyrate sulfonate</td>
<td>-</td>
<td><em>Methanothermobacter marburgensis</em></td>
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<td>Chloromethanesulfonate</td>
<td>-</td>
<td><em>Methanothermobacter thermautotrophicus</em></td>
<td>(Ellermann et al., 1989)</td>
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</table>
Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.
Fig. 5.
Fig. 6
Ni(II)-MCR_{red} \xleftarrow{\text{CH}_3\text{-SCoM}} [Ni(II)-MCR_{red}][\text{CH}_3\text{-SCoM}] \rightarrow \text{CH}_4

Michaelis Complex

1-Bromoethane sulfonate (1-BES)

HBr

Ni(III)-MCR_{sulfonate} \xrightarrow{\text{CH}_3\text{-SCoM}} \text{No Methane}

Fig. 7
Fig. 8.