Impact factors and novel strategies for improving biohydrogen production in microbial electrolysis cells

Dongle Cheng[a], Huu Hao Ngo[a,f,*], Wenshan Guo[a], Soon Woong Chang[b], Dinh Duc Nguyen[b], Shicheng Zhang[c], Shihai Deng[d], Ding An[e], Ngoc Bich Hoang[f]

[a]*Center for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Sydney, NSW 2007, Australia*
[b]*Department of Environmental Energy Engineering, Kyonggi University, 442-760, Republic of Korea*
[c]*Department of Environmental Science and Engineering, Fudan University, Shanghai 200438, China*
[d]*Department of Environmental Science and Engineering, Xi'an Jiaotong University, Xi'an 710049, China*
[e]*School of Environment, Harbin Institute of Technology, Harbin Institute of Technology, Nangang District, Harbin, 150090, China*
[f]*Institute of Environmental Sciences, Nguyen Tat Thanh University, Ho Chi Minh City, Viet Nam*

*Corresponding author: Email: ngohuuhao121@gmail.com

Abstract

Microbial electrolysis cell (MEC) system is an environmentally friendly method for clean biohydrogen production from a wide range of biowastes owing to low greenhouse gas emissions. This approach has relatively higher yields and lower energy costs for biohydrogen production compared to conventional biological technologies and direct water electrolysis, respectively. However, biohydrogen production efficiency and operating costs of MEC still need further optimization to realize its large-scale application. This paper provides a unique review of impact factors influencing biohydrogen production in MECs, such as microorganisms and electrodes. Novel strategies, including inhibition of methanogens, development of novel cathode catalyst, advanced reactor design and integrated systems, to enhance low-cost biohydrogen production, are discussed based on recent publications in terms of their opportunities, bottlenecks and future directions. In addition, the current challenges, and effective future perspectives towards the practical application of MECs are described in this review.

Key words: Microbial electrolysis cells, biohydrogen, biowastes, influencing factors, novel strategy

1. Introduction

Global energy demand increased nearly every year at the last half century as the growth of worldwide population, which is set to increase by 4.6% in 2021 (Rode et al., 2021). Fossil fuels, including coal, oil, and natural gas, remain the primary global energy sources, and account for almost 84% of primary energy supplies (Aydin et al., 2021). Burning of fossil fuel releases a large amount of CO_2 that is 57% of global greenhouse gases (GHGs) causing climate change (Yoro & Daramola, 2020). Therefore, it is essential to develop green and renewable energy to reduce fossil fuel consumption, thereby mitigating climate change. Hydrogen (H_2) is renewable and environment-friendly alternative to traditional fossil fuels, because it is clean burning with zero or near-zero emissions, and has a high calorific value (120–142 MJ/kg) compared to other gaseous fuels like CH_4 (50 MJ/kg), ethanol (26.8 MJ/kg) and gasoline (44 MJ/kg) (Kadier et al., 2016). However, up to 96% of current hydrogen is produced from non-renewable fossil fuels using steam reforming, electrolysis, thermo-chemical conversion (pyrolysis) and gasification, which not only require high energy input, but also aggravate the emissions of greenhouse gases (Aydin et al., 2021).

For long-term sustainable green hydrogen production, biohydrogen production from renewable biowastes via biological technologies is now gaining great attention in recent years. Biowastes, such as various wastewaters, agricultural wastes, sewage sludge and other biodegradable wastes, will cause negative impact to the environment if treated unproperly. Therefore, the use of biowastes as substrates for biohydrogen

production can achieve dual-benefits for the treatment of biowastes and bioenergy recovery (Hu et al., 2008).

Biological technologies for biohydrogen production from biowastes include dark fermentation (DF), photo-fermentation (PF) and microbial electrolysis cell (MEC). MEC system is a novel and encouraging technology for biohydrogen production using renewable biowastes and various wastewaters as feedstock (Liu et al., 2016). Electrochemically active bacteria in the MEC reactor oxidize organic contents to produce CO_2, electrons and protons. Electrons are transferred to the anode surface and then to the cathode through an external circuit. The hydrogen can be produced at the cathode via the combination of electrons and protons under a low applied voltage (>0.2 V) that is much lower than the voltage needed for water electrolysis (2.3V) (Kadier et al., 2016). In comparison with DF and PF, the use of electrochemically active bacteria to break down organic matter, combined with the addition of a small voltage (>0.2 V in practice) in the MEC system has advantages of 1) breaking through the "fermentation barrier" bottleneck, 2) providing high yields of biohydrogen production, and 3) achieving the depth use of carbon source (67-91%) (Cheng & Logan, 2011; Logan et al., 2008).

The design of the MEC reactor can be either in single- or double-chamber, as shown in Fig. 1. Ion exchange membranes (IEMs) are usually used in the double-chamber MEC to isolate the anode and cathode chambers. In the anode chamber of MEC, organic substrates are degraded by bacteria with the release of electrons and protons. The electrons are transferred to the anode through possible mechanisms of 1)

direct electron transfer, 2) transfer through electron shuttle or 3) transfer through conductive biofilm and conductive pili, and then pass to the cathode via an external circuit. The reduction of H^+ to molecular H_2 gas takes place at the cathode with the supply of an external voltage, practically at least 0.2–0.25 V must be supplemented to make it happen (Kumar et al., 2017). To reduce the potential loss associated with the membrane and increase energy recovery of this process, single-chamber MECs can be operated without membrane. Although the membraneless MECs have simple architecture and low cost, methanogens can cause the loss of biohydrogen production by completing with electrochemically active bacteria (EAB) for both substrate and hydrogen (Xing et al., 2020). In addition, widespread applications of MECs also have challenges of external voltage requirement and high cost of cathode catalyst (Kadier et al., 2016). To date, there have been extensive studies, which aim to develop novel strategies to enhance biohydrogen production in MECs.

Insert Fig. 1

In recent years, a considerable amount of review articles and book chapters have been published to indicate thermodynamic basics, advantages compared to DF, PF and water electrolysis, impacts of operating parameters, as well as challenges in biohydrogen production of MEC systems (Ferraren et al., 2021; Osman et al., 2020; Saravanan et al., 2020; Varanasi et al., 2019). Accordingly, the performance of biohydrogen production in MEC systems depends on various factors, mainly including biological factors, anode materials, and cathode and catalysts (Jayabalan et al., 2021). On the basis of previous studies, influencing factors and corresponding

optimization strategies for biohydrogen production in MEC systems are overviewed in this paper to improve the biohydrogen production yield and realize their practical application. Moreover, cutting-edge strategies for improving biohydrogen production cost effectively to achieve successful real-world application of this technology are also critically discussed.

2. Impact factors and strategies for improving biohydrogen production in MECs

2.1 Impacts of microorganisms and promoting strategies

Microorganisms in MECs are important parameters that convert organic matters to biohydrogen. Electro-active microorganisms or exoelectrogens in MECs are considered as extracellular electron transferors (EETs), due to their capacity to catalyze electron transfer from the substrate to electrodes. The microbial structure is not consistent throughout all MEC systems due to different substrates, environmental and operating conditions. Both pure- and mixed cultures can be applied in MECs. Detailed information about pure and mixed microbial culture used in MEC systems and their H_2 production rates have been given in a previous review article (Saratale et al., 2017). Pure cultures can provide well-controlled systems and produce methane-free gases in MECs. It is important to use pure cultures to conduct principle studies in MECs. For example, pure electrogenic species like *Pseudomonas* spp. and S*hewanella* spp., are easy to identify the strain characteristics, behaviours and function (Kadier et al., 2018).

Comparatively, mixed cultures are more feasible to inoculate MECs for practical application, considering that they have advantages of better stability, potential

versatility, and flexibility in the face of real situations and non-sterile applications (Kumar et al., 2017). At the phyla levels, *Proteobacteria*, *Firmicutes* and *Bacteroidetes* phyla are the most common electroactive microbes in the MEC anodic biofilm (Kumar et al., 2017). At the genera level, *Desulfovibrio*, *Acidaminococcus*, *Desulfocapsa* and *Acetobacterium* were dominant in anodes of MECs (Almatouq et al., 2020). Croese et al. (2013) indicated that methanogenic populations in the anode chamber were similar in the different MECs.

However, the competition of various microbial groups for substrate may occur in mixed culture MECs (Kumar et al., 2017). The coexistence of non-exoelectrogens can restrict the performance of MECs, although exoelectrogens gradually dominate the anode surface after a certain period of time (Manuel et al., 2010). Therefore, it is necessary to transfer the biofilm from one MEC or MFC system to another to eliminate or wash off non-exoelectrogens microorganisms and enrich active species.

In addition to the extracellular electron transferring bacteria, methanogens are also present in the mixed-culture MECs (Lu et al., 2012). In MECs fed with wastewater or other organic wastes, the hydrogen production yields could be reduced or even completely inhibited by the consumption of acetate and hydrogen by acetoclastic methanogen and hydrogenotrophic methanogen, respectively (Jadhav et al., 2019). This problem is more considerable in single-chamber devices, and the purity of hydrogen is contaminated by methane, which makes the downstream more complicated. Nonetheless, even in double-chamber MECs, methane could diffuse over time through the membrane from the anode to the cathode compartments, which

can also contaminate the hydrogen gas recovered at the cathode. Therefore, it is significant to inhibit the undesirable methanogens grow to maximize the biohydrogen production, and the development of strategies for suppressing methanogenesis in MECs have become a key research point (Karthikeyan et al., 2017).

According to recent review reports, methods used to inhibit the activity of methanogen in MECs mainly include the addition of chemical inhibitors, use of pure culture, modification of MEC design, and application of specific operating conditions like low pH, low temperature, shorter operating cycle, pretreatment of electrodes and substrates, and exposure of electrodes to air (Kadier et al., 2018; Karthikeyan et al., 2017). However, there are still a series of challenges and shortcomings of these methods. For example, although the continuous addition of specific chemicals, such as acetylene (5 and 10%), 2-bromoethanesulfonate (BESA) (20-286 mM), 2-chloroethanesulfonate (CES) (20mM), 8-aza-hypoxanthine (AHX) (3.6 mM), to inoculation medium is effective to inhibit methane generation and in turn improve the overall hydrogen production, high costs of continuous addition, toxic effects of chemicals and unsustainable for practical application are main challenges for this strategy (Catal et al., 2015; Chae et al., 2010; Kadier et al., 2018). Some advanced methods for inhibiting the growth of methanogens and promoting the performance of MECs have been summarized in Table 1.

Insert Table 1

The growth of methanogens has strict requirements for operating and environmental conditions. Methanogens are sensitive to variation of pH and

temperature. The fastest growth of methanogens mainly occurs within a pH range of 6.5–7.5, a temperature range of 30-38℃. Therefore, it is reported that the suppression of methanogens growth could be achieved by controlling operating conditions under low-pH and temperature (Hu et al., 2008; Logan et al., 2008; Lu et al., 2011). For example, the study by Lu et al. (2011) and Zhao et al. (2014) indicated that the operation of a single-chamber MEC at 4 °C or 9 °C and the reduce of influent pH from 7.0 to 5.5 could inhibit the growth of hydrogenotrophic methanogens and reduce the methane production. On the contrary, no effect on methanogenesis was observed by changing the pH from 7 to 4.9 and decreasing the temperature from 30 °C to 20 °C (Chae et al., 2010; Hu et al., 2008). Although controlling other operating parameters, such as shorting operation cycles, exposing reactor or electrodes to air and applying high eternal potential, also exhibited an inhibitory effect on the methane production in MECs, they only showed successful in a short-term applications (Jobin et al., 2018; Zhao et al., 2020). Additionally, methanogen growth can not be completely inhibited in MECs, and changing these operating parameters may also damage the electrochemically active biocatalyst (Kadier et al., 2018). Other technologies, like ultraviolet (UV) irradiation of the reactor, or quick H_2 harvesting from the MECs system by using gas-permeable membranes and a vacuum, also have been considered as effective strategies to inhibit methanogenesis, but they are restricted by high costs for practical applications (Hou et al., 2014; Lu et al., 2016).

Comparatively, biological strategies utilized in bioelectrodes could be alternative technologies for inhibiting the growth of hydrogenotrophic methanogens and

improving hydrogen production in MECs. It has been found that introducing or enriching denitrifying bacteria in the MECs systems can effectively suppressing methanogens growth, mainly due to the inhibitory effect of metabolites produced by denitrifier (Hardhi et al., 2018). Hardhi et al. (2018) reported that the hydrogen production increased 100% after introducing isolates of denitrifying bacteria in a single-chamber MEC compared with the control. Pratiwi et al. (2020) also found that the addition of *Pseudomonas stutzeri* as biological control of methanogenesis could reduce the methane up to 76.28% and increase the H_2 produced 128% higher compared to the control reactor.

Supplement of carbon nanomaterials in the MEC system have also been found to inhibit the methane production. Yadav et al. (2016) reported that carbon nanotubes exhibit antimicrobial activities, due to their damage to cell walls/membranes of microbe. Fujinawa et al. (2019) examined the hydrogen production in cathode chambers of MECs by supplying conductive carbon nanoparticles (2%), and found that the hydrogen could be stably produced by effectively suppressing hydrogen consumption and methane production. The study indicated that the presence of conductive carbon nanoparticles in the system could seriously damage methanogens and promote their cell lysis. The author found that absolute abundances of *Methanobacteriaceae* and *Methanosarcinaceae* were decreased in response to carbon nanoparticles. In comparison with other chemical inhibitors, carbon nanomaterials are attractive for practical applications in MECs considering their low costs and persistent effects.

The application of a relative high voltage (until reaching a threshold value) also has been considered as a promising method to reduce methane production. Guo et al. (2010) investigated the influence of applied voltages to the biohydrogen production in a single-chamber MEC, and found that the hydrogen production rates increased from 0.03 L/L/d to 1.58 L/L/d by increasing the voltage from 0.2 V to 1.0 V. The maximum hydrogen recovery efficiency (87.73%) was achieved at the applied voltage of 0.6 V, due to the reduced methane production at a higher voltage (>0.6 V). Ding et al. (2016) also found that the methane production was inhibited when the applied voltage was higher than 0.8 V. The study indicated that the cell membrane could be destroyed under higher applied voltages, resulting in lower growth rate and activity of methanogen. The optimal applied voltage was proved to be 0.8 V for anaerobic wastewater treatment and biohydrogen production. However, the application of higher voltage is hindered by the challenge of energy intensive. Alternative energy sources need to be explored to replace the direct current (DC) power supply in the future. The development of advanced self-sustainable MEC system by combining conventional MECs with microbial fuel cell, solar cell or other technologies should be a potential solution (Yang et al., 2020).

A new approach developed by Lu et al. (2016) indicated that rapid hydrogen extraction using a gas-permeable hydrophobic membrane and vacuum could prevent methane production and achieve a much high yields (3.3 – 4.4 times higher than the control system) and high purity biohydrogen, but more research is required for the development of membrane, the understanding of membrane fouling and hydrogen

purification. The operation of MEC under negative pressure also has been considered as a promising method to dramatically enhance the efficiency of biohydrogen production by controlling the diffusion of hydrogen and the growth of methanogens (Feng et al., 2018). It is reported that the maximum hydrogen production rate of a single chamber MEC operated under negative pressure (40.52 kPa) was four times higher than that operated under normal pressure. The negative pressure operation of the MEC also archived 13 times enhancement for hydrogen recovery and 1.5 times improvement for energy recovery. Another study by Huang et al. (2020) also obtained high biohydrogen recovery from food waste by running the combined anaerobic digestion and single chamber MEC system under negative pressure. It is notable that the methane production cannot be completely inhibited by operating MEC under negative pressure, more and long-term studies about this strategy are required in the future (Feng et al., 2018).

Additionally, hydrophilic porous membranes were also reported to be effective in improving hydrogen production rate in MEC in comparison with the conventional proton exchange membrane, due to its effective inhibition of methanogenesis and lower pH gradient and ion transport resistance. Zhao et al. (2021) indicated that the hydrogen production rate in MEC with low-cost hydrophilic non-woven cloth (NWC) was around 1.14 times higher than in MEC with proton exchange membrane.

2.2 Impacts of electrode materials and improving strategies

2.2.1 Anode materials

Anode materials also play important roles in MECs and its robustness to maintain its catalytic activity affects the performance of the MEC system. The anode colonised by exoelectrogens should be able to transfer electrons to the cathode of MEC, which is also a limiting fator in hydrogen production (Lim et al., 2017). Carbon-based materials are the most widely used anode in MECs, due to their characteristics of good conductivity, biocompatibility, chemically stable, morphological diversity, low overpotential and relatively low cost. As reviewed previously, the common carbon-based anode in lab-scale MECs mainly includes carbon paper, carbon cloth, carbon felt, carbon brush, carbon fiber, carbon mesh, graphite granules, graphite felt, etc. (Kadier et al., 2016). Feng et al. (2018) compared the performance of a two-chamber MEC using felt and brush anodes and demonstrated that higher hydrogen production rate in the MEC with brush anodes was 1.2 times higher than that of felt anodes, might due to the substrate-limited mass transfer to the felt anodes or high surface area of the brush anodes.

To improve the anode performance, the carbon-based materials could be pretreated to change the porosity and interface conductivity of the anode material for promoting bacterial attachment to the anode (Kadier et al., 2016). An increase in the porosity of the anode surface usually leads to an increase in the number of biocatalytic sites in the pores, thereby increasing the electrochemically active surface area (Dhand et al., 2014; Sarathi et al., 2013; Sleutels et al., 2011). Moreover, the increase in catalytic activity at the anode interface was considered to occur through changes in the conductivity of the electrode interface by modifying the anode surface with

particles such as iron or conductive polymers (Yasri et al., 2019). For example, Kang et al. (2015) stated that the carbon felt anode coated with a conductive poly (3,4-ethylenedioxythiophene (PEDOT) film showed superior biocatalytic performance, but the role of the conductivity of the anode surface in promoting the growth and performance of the anode biofilm still requires further research. It is reported that several methods including electrochemical oxidation, high temperature ammonia gas or N_2 gas treatment, heat treatment, and combinations of the above methods have been used to modify the surface of anode (Kadier et al., 2016). Plasma-pretreated carbon cloth and stainless steel anodes also led to higher biofilm viability and hydrogen production rate compared to the untreated anodes in single-chamber MECs (Rozenfeld et al., 2019). In one recent study, recycled carbon fibre anodes, derived from the waste of carbon product manufacture in the automotive and aerospace industry, have been considered as an inexpensive alternative anode material to conventional graphite felt in commercially viable MECs (Carlotta et al., 2020). The author found that the hydrogen production in recycled carbon fibre anode MECs treating real wastewater was 21.52 L/ d/m^3 while graphite felt anode MECs was 3.65 L/d/m^3, and the cost of recycled carbon fibre anode MECs was 93% less expensive than similar graphite anodes.

2.2.2 Cathode and catalysts

Cathode plays an important role in the MECs where hydrogen as well as other value-added chemical compounds are produced (Ghasemi et al., 2020). Hydrogen generation in MECs usually occurs at cathode, but the hydrogen evolution reaction on

plain carbon electrode is very slow and requires a high overpotential to produce. Expensive metal catalysts, such as platinum (Pt), are often used as a cathode catalyst in MECs to reduce the reaction overpotential (Jayabalan et al., 2020). However, high cost, negative environmental effects in the production process, and the possibility of deactivation by chemicals present in wastewater (such as sulfide) are major draw backs of using Pt as catalysts (Logan et al., 2008). As reported earlier, the cathode and the loaded metal catalyst account for about 47% of the total cost of MECs (Rozendal et al., 2008a). For the commercialization of the MEC system, several alternatives to Pt cathode, such as stainless steel or Ni-based material have been extensively researched (Kundu et al., 2013). It is found that relatively inexpensive stainless steel cathodes and Ni-based materials, including Ni foam and Ni alloys like nickel–molybdenum (NiMo), nickel-tungsten (NiW), nickel–iron–molybdenum (NiFeMo), are effective for hydrogen production in MECs (Kadier et al., 2016). Nanostructured materials such as electrodeposited palladium (Pd) nanoparticles and Ni-based nanomodified materials like Nickel–iron (NiFe), nickel–iron–phosphorous (NiFeP) and nickel–iron–cobalt–phosphorous (NiFeCoP), have been studied as cathode catalysts and proven to have good electrocatalytic activity. However, these metal electrocatalyst materials and nanostructured particles may have adverse effects on biofilms and reduce the MEC performance.

Biocathode has been considered as a promising alternative to the expensive metal catalysts. It has been proposed that the hydrogenase existing in various microorganisms was responsible to catalyze the reaction of $2\,H^+ + 2e^- \leftrightarrow H_2$ for

hydrogen production in the biocathode MEC system. Although purified hydrogenases can be used to enhance hydrogen production on a carbon electrode, the enzymes are unstable and usually lose their catalytic activity over time (Vincent et al., 2007). Therefore, electrochemically-active microbes harboring hydrogenases attached on the cathode are used as catalysts to reduce protons to biohydrogen MECs. Compared to common abiotic catalyst, the biocathode has advantages of low costs, self-renewable, sustainable and resistant to sulfide poisoning (Fu et al., 2013). According to the review paper by Jafary et al. (2015), the biocathode can be (I) half biological start-up by reversing the polarity of a bioanode to function as a biocathode, and/or transferring the active culture or bioeletrode of the anode chamber to the cathode chamber in a dual chamber MEC without polarity reversal, and (II) full biological start-up with electrochemically active microorganisms in dual- or single- chamber MEC, as shown in Fig. 2(a). It has been confirmed that biocathode was comparable with the Pt and carbon cathode in terms of current density, energy efficiency, hydrogen production rate and hydrogen recovery (Dai et al., 2019; Hasany et al., 2016).

Insert Fig. 2

For the first time, Rozendal et al. (2008b) developed a half cell biocathode for hydrogen production by reversing the polarity of a bioanode in a microbial fuel cell (MFC) and found that the current density (1.1 A/m2) and biohydrogen production rate (about 0.63 m3/m3d) in the biocathode MEC at a controlled potential of − 0.7 V were around four and eight times higher than that of the control electrode (without biofilm), but it requires the use of ferrocyanide at the anode. One study by Pisciotta et al.

(2012) pointed out that there was no requirement of chemicals like ferricyanide for the enrichment of MEC biocathode from the bioanode of a sediment MFC, as the sediment MFC could provide more anaerobic conditions in comparison with the typical electrode suspension in the electrolyte. According to the report of Jeremiasse et al. (2010), the biocathode in a full biological double chamber MECs also had a higher current density (1.9 - 3.3 A/m^2) than a control cathode without biofilm (0.3 A/m^2) in an electrochemical half-cell MEC. Jafary et al. (2019) demonstrated that the enrichment of the bioanode and biocathode for a full double chamber biological MEC in half biological systems was more efficient than that simultaneously enriched in one system. A membrane-less single-chamber biocathoe MEC was developed by Xu et al. (2014) to study its performance for simultaneous hydrogen production and wastewater treatment. The author stated that the biocathode achieved comparable performance to that of the platinum cathode, with hydrogen production rate, current density, chemical oxygen demand (COD) removal efficiency, coulombic efficiency, and cathodic hydrogen recovery were 0.39 $m^3/m^3/$ d, 134 Am^{-3}, 90%, 37%, and 63%, respectively, at an applied voltage of 0.9 V.

The mixed culture inoculum from wastewater and anaerobic sludge is commonly used to start up the biocathodes in MECs (Saheb-Alam et al., 2018). Croese et al. (2014) and Liang et al. (2014) indicated that bacteria in *Firmicutes*, *Proteobacteria* and *Bacteroidetes* phyla in the mixed culture might play key roles for the formation of biocathodes in MECs. Whereas, the enrichment of biocathode through the mixed culture take a long time. Pre-enrichment of a suitable

microbial community and use it as inoculum has been considered as a promising method to speed up the startup of biochathodes (Saheb-Alam et al., 2019). Jafary et al. (2017) indicated that the origin biocathode MEC developed by introducing the pre-enriched sulphate-reducing bacteria (SRB) culture and the autotrophic medium into the cathode chamber showed higher hydrogen production rate compared to the MFC-MEC biocathode system. The hydrogen production was improved significantly by about 6 times from 0.31 to 1.85 m3 $H_2/m^3/d$ while the methane production was totally hindered by using the origin biocathode MEC. Pure sulphate-reducing bacteria (SRB) species like *Geobacter sulfurreducens* and *Desulfovibrio* have been reported to catalyze the formation of biohydrogen (Geelhoed et al., 2011). For instance, Geelhoed et al. (2011) indicated that the biohydrogen was produced from acetate by *Geobacter sulfurreducens* at electrode potentials ranging from −0.8 to −1.0 V. The study by Aulenta et al. (2012) found that the attachment of *Desulfovribio* sp. on a graphite electrode catalyzed the biohydrogen production with the highest amount of 8 mmol/L per day, the cathode potential was lower than -900 mV, and the coulombic efficiency was close to 100%. Call et al. (2009) compared the hydrogen production rates and hydrogen recoveries of pure *Geobacter sulfurreducens* and mixed cultures in MECs, and concluded that the pure *Geobacter sulfurreducens* could achieve similar current densities (160 A/m^3) and hydrogen production rates (1.9 m3 $H_2/m^3/day$) with the mixed culture at an applied voltage of 0.7 V. Although the overall energy recovery in the MEC with mixed culture (82 ± 8%) was slightly higher than that in the *Geobacter sulfurreducens MEC* (77 ± 2%) before methane was produced in the mixed-culture

MEC, the result was reversed when methane was detected in the mixed-culture MEC. *Geobacter sulfurreducens* has been found as the dominant specie in the mixed-culture MEC.

3. Advanced MEC systems for improving hydrogen production

3.1 MFC-MEC coupled systems

Microbial fuel cells (MFCs) can be used as an alternative to the DC power supplier to supply voltage and drive MECs reactions for biohydrogen production in a combined MFC-MEC system (see Fig. 2(b)) (Sun et al., 2008). Sun et al. (2008) investigated the potential of biohydrogen production from acetate by a MEC-MFC coupled system without the external voltage supply, and concluded that the hydrogen production rate, hydrogen yield, cathodic hydrogen recovery could reach 2.2 ± 0.2 mL/L/d, 1.21 mol H_2/mol acetate and 88∼96%, respectively. As mentioned previously, a relatively higher voltage supply (0.6 - 1 V) showed a positive influence on the biohydrogen production in MECs. The study by Sun et al. (2009) indicated that the hydrogen production rate in the MEC–MFC-coupled system rose from 0.2 ± 0.0 to 2.9 ± 0.2 mL/ L/d with a decline in the loading resistor from 10 kΩ to 10 Ω. Furthermore, the authors found that the biohydrogen production could be significantly better by connecting several electricity-assisting MFCs in a series to achieve a higher power input.

Wang et al. (2011) discovered that the voltage obtained by connecting two MFCs (0.435 V) in a series was higher than that of the single MFC (0.330 V). Furthermore the total biohydrogen production from cellulose (14.3 mmol H_2/g

cellulose) in the integrated system of DF, MEC and MFC was enhanced by 41% compared with the DF process alone. However, biohydrogen production in the combined MFC and MEC system was unstable, which decreased significantly with a small reduction of the voltage generated in MFC. The use of capacitor-based energy storage circuits is promising for providing stable and high voltages to MECs (Kim et al., 2011). The capacitors could be charged by multiple MFCs in parallel and then discharged in a series to enhance the biohydrogen production in MECs. Hatzell et al. (2013) stated that the biohydrogen production rate and energy recovery in a MFC and MEC coupled system could be increased from 0.31 to 0.72 m^3 /m^3/d and 9 to 13%, respectively, by using the capacitor charging system.

3.2 Solar-assisted MECs

Dye-sensitized solar cells (DSSCs), which convert sunlight into electricity, also can be used as a power supplier to MECs for biohydrogen production, as displayed in Fig. 2(c) (Chae et al., 2009). Chae et al. (2009) found that the biohydrogen conversion efficiency in a MEC without a Pt catalyst on the cathode was 71.3-77.0% by using a DSSC voltage supplier at 0.7 V, which was comparable with the value of the Pt-loaded carbon felt (79.3 - 82.0%). The DSSC could supply a stable voltage during DSSC-MEC systems, and the average cathode hydrogen recovery efficiency was 78 ± 2.5% (Ajayi et al., 2009). Thus, the combination of DSSCs with MECs might be a successful and cost-effective way to produce biohydrogen, and more research is necessary.

The application of photocathode in MECs is a promising cost-effective method for biohydrogen production, and it can be achieved by obtaining energy from solar energy. The photocathode absorbs photon from sunlight to produce electrons at its conduction-band (CB) and holes at its valence-band (VB). Electrons generated at the photocathode CB and the bio-anode are used to: firstly, drive the reaction of hydrogen reduction; and secondly, fill the holes at the photocathode VB to suppress the reoxidation of the hydrogen (Fig. 2 (d)) (He et al., 2014). Oxide semiconductors like TiO_2, Cu_2O, are promising photocatalysts. Chen et al. (2013) found that using a microbial anode and a TiO_2 photocathode system was feasible for continuous biohydrogen production at a rate of 3.5 μmol/h by under UV-irradiation, although the efficiency and cost of the system needs to be further optimized. Cu_2O is another promising p-type semiconductor with higher visible light capture capacity than TiO_2 and excellent photocatalytic potential of hydrogen release reaction (HER). However, its instability in the photoelectrochemical process limits its long-term application as a photocathode (Jang & Lee, 2019).

To enhance the stability of the p-type Cu_2O semiconductor, MoS_2 and NiOx were coated on the Cu_2O photocathode as cocatalyst (Jeon et al., 2018; Liang et al., 2016). Jeon et al. (2018) indicated that the MoS_2-coated p-type Cu_2O with high stability has been employed as a photocathode in photo-assisted MEC and demonstrated exclusive biohydrogen production at a rate of 2.72 m^3H_2 /m^3 /d from acetate under visible light illumination and 0.8 V external voltage supply. The coating of NiO_x on Cu_2O photocathode at a layer of 240 nm also could achieve a stable

biohydrogen generation at a rate of 5.09 μL /h/cm^2 under continuous light illumination with 0.2 V external bias. Moreover, novel MoS_3 modified p-type Si nanowire, the MoS_2/PDA/TiO_2, the polyaniline nanofibers, and g-C_3N_4/BiOBr heterojunction photocathode all showed high stability and they are promising alternatives for obtaining a significant improvement in biohydrogen production (Yang et al., 2020).

3.3 Integration of dark fermentation and MECs process

In recent years, the integration of dark fermentation (DF) and microbial electrolysis cell (MEC) (DF-MEC) has been proposed to achieve higher yields of biohydrogen and improve the treatment efficiency of complex wastes (Bakonyi et al., 2018). The problem of the bottleneck in the DF process about the low conversion efficiency of biowastes can be solved in the DF-MEC system (Kadier et al., 2016). In the DF-MEC system, the complex substrates could be converted to biohydrogen, VFAs and other metabolites by the DF process, and the effluent from DF could be further oxidized by the exoelectrogens in MECs thereby improving biohydrogen production (Lu & Ren, 2016). The DF effluent with high levels of VFAs has been regarded as a promising feed solution of MECs to improve biohydrogen production compared with other feed solution containing complex organic matters or glucose, due to the slow degradation of the complex organic matter reduced the total biohydrogen yield (Rivera et al., 2015).

The integrated DF-MEC system can be operated as a two-stage process in a series and a single-stage process in one reactor. In a two-stage DF-MEC process,

biohydrogen could be firstly produced in the first fermentative reactor from refractory biowastes like wastewaters and lignocellulosic wastes, and then produced in the second MEC reactor from the fermentative effluent. Accordingly, the two-stage DF-MEC process has been commonly used by other researchers to enhance biohydrogen production and wastes treatment. For example, Lalaurette et al. (2009) demonstrated that high hydrogen yields (9.95 mol H_2/mol glucose) and production rates can be achieved from cellobiose using a two-stage DF-MEC process. Li et al. (2014) remarked that biohydrogen production from corn stalk could be enhanced by combining DF and a single chamber MEC with double anodes. It resulted in the maximum biohydrogen yield of 387.1 mL/g corn stalk (129.8 mL H_2/g corn stalk in DF stage and 257.3 mL H_2/g corn stalk in MEC stage).

The energy efficiency and acetate conversion efficiency were 166 ± 10% and 90 ± 2%, respectively, during the MEC process under the external voltage of 0.8 V. An analysis of biohydrogen produced from palm oil mill effluent utilizing two-stage DF-MEC was conducted by Khongkliang et al. (2019). The authors observed a 3-fold higher biohydrogen production (236 ml H_2/g COD) from the combined process in comparison with the single DF system. *Thermoanaerobacterium* sp. dominated the dark fermentation stage while *Geobacter* sp. and *Desulfovibrio* sp. dominated the microbial electrolysis cell stage. However, in the two-stage DF-MEC process, the DF effluent is quite acidic with the pH as low as 4-4.5, which is not suitable for growing electrochemically-active bacteria in MECs (Bakonyi et al., 2018). Thus, additional

operating costs are required for the pretreatment of the DF effluent for pH adjustment (Moreno et al., 2015).

The simultaneous biohydrogen production by DF and MEC in a single reactor has been developed recently by Nguyen et al. (2020). In the single DF-MEC reactor, the produced VFAs by DF could be simultaneously used by the exoelectrogens in the MEC to produce biohydrogen, thereby minimizing the accumulation of VFAs and balancing the pH value in the reactor. Nguyen et al. (2020) indicated that the production of biohydrogen from Saccharina Japonica by a single stage DF-MEC process was around 8 times and 1.1 times higher than that in the DF process alone and the two-stage DF-MEC process, respectively. In order to enhance biohydrogen recovery and suppress methane production in the single stage DF-MEC, higher external voltages should be supplied (Wang et al., 2011). With the development of self-drive MEC technology, further research on the single-stage DF-MFC system is necessary to achieve higher biohydrogen loads being produced.

4. Future perspectives

Although many lab-scale studies have proved the feasibility of MECs for hydrogen production from various biowastes, there are still considerable challenges to improve biohydrogen production and promote the industrial application of MECs to meet the increasing energy demand worldwide. For example, low hydrogen purity and loss due to the presence of methanogens in mixed-culture MECs are one of cardinal challenges. Although strategies like the addition of chemical inhibitors, modification of MEC design, and application of specific operating conditions like low pH, low

temperature, shorter operating cycle, pretreatment of electrodes and substrates, and exposure of electrodes to air, have been extensively studied to inhibit the activity of methanogens in MECs, their application was restricted due to their shortcomings as discussed in section 2.1. Comparatively, several novel technologies could be alternatives for inhibiting the growth of methanogens and improving hydrogen production in MECs, such as introducing denitrifying bacteria and carbon nanomaterials in the MECs, operating the MECs under a relative high voltage or negative pressure, using gas-permeable hydrophobic membrane and vacuum to extract hydrogen rapidly, or using hydrophilic porous membranes to replace conventional proton exchange membrane in MECS, but more researches are required in the future.

Highs cost and unsustainable of expensive metal catalysts are key factors limiting the practical application of MECs. Operating MECs under the application of biocathode has been considered a potential alternative to noble metals as cathode catalysts. However, more studies are required to investigate the formation of biocathode, the detailed mechanism of biohydrogen production via biocathode, microorganisms on the biocathode responsible for biocatalytic biohydrogen production. The effectiveness of biocathode in large-scale operation is also unknown and requires further studies in the future.

MFC and solar-assisted MECs are alternative energy sources for supplying power to MECs to reduce the costs involved in operating the MEC process. However, these technologies are still in their infancy. Their long-term performance, stability and practical feasibility should be further accessed. Integrating different processes could

enhance biohydrogen production from various biowastes by overcoming the disadvantages in the individual process. The integration of DF system and MEC system can be a promising technology for improving hydrogen production in MECs from various complex substrates. Both two-stage and single-stage DF-MEC systems have their advantages and disadvantages, thereby requiring further researches to optimize conditions for biohydrogen production.

Moreover, further assessment of MEC systems in terms of economic feasibility and environmental sustainability is also significant for the scale-up of these technologies. Even though the MEC technology has a highly positive overall net environmental benefit considering crisis of biowaste treatment, global warming, and non-renewable energy, its operational and maintenance cost could be higher than conventional technologies. Novel strategies and advanced technologies as discussed in Sections 3 and 4 could assist to reduce costs of the MEC systems, but techno-economic assessments and life cycle analysis of these technologies require to be conducted in the future to achieve the goal of circular bioeconomy.

5. Conclusion

To improve biohydrogen production in MECs, a comprehensive discussion of major impact factors, novel strategies, and advanced MEC systems has been demonstrated in this review. Novel strategies and advanced MEC systems, including the inhibition of methanogenic activity, the application of effective anodes and biocathodes, MFC and solar assistance in MECs, as well as the integration of DF and MEC, hold great promise for improving biohydrogen production cost-effectively.

More research is required to optimize these adopted strategies and integrated systems to realize the practical application of MEC in hydrogen production, thereby achieving the goal of meeting the world's growing energy demand.

Acknowledgement

This research was supported by University of Technology Sydney, Australia (UTS, RIA NGO; UTS, 2021 SRS) and the Korea Institute of Energy Technology Evaluation and Planning (KETEP) and the Ministry of Trade, Industry & Energy (MOTIE), Republic of Korea (No. 20183020141270 and No. 20194110300040).

References

1. Ajayi, F.F., Kim, K.-Y., Chae, K.-J., Choi, M.-J., Kim, S.-Y., Chang, I.-S., Kim, 2009. Study of hydrogen production in light assisted microbial electrolysis cell operated with dye sensitized solar cell. Int. J. Hydrog. Energy 34(23), 9297-9304.

2. Almatouq, A., Babatunde, A.O., Khajah, M., Webster, G. and Alfodari, M., 2020. Microbial community structure of anode electrodes in microbial fuel cells and microbial electrolysis cells. J. Water Process. Eng. 34, p.101140.

3. Aulenta, F., Catapano, L., Snip, L., Villano, M., Majone, M., 2012. Linking Bacterial Metabolism to Graphite Cathodes: Electrochemical Insights into the H_2-Producing Capability of Desulfovibrio sp. ChemSusChem 5(6), 1080-1085.

4. Aydin, M.I., Karaca, A.E., Qureshy, A.M., Dincer, I., 2021. A comparative review on clean hydrogen production from wastewaters. J. Environ. Manage. 279, 111793.

5. Bakonyi, P., Kumar, G., Koók, L., Tóth, G., Rózsenberszki, T., Bélafi-Bakó,

K., Nemestóthy, N., 2018. Microbial electrohydrogenesis linked to dark fermentation as integrated application for enhanced biohydrogen production: A review on process characteristics, experiences and lessons. Bioresour. Technol. 251, 381-389.

6. Call, D.F., Wagner, R.C., Logan, B.E., 2009. Hydrogen production by Geobacter species and a mixed consortium in a microbial electrolysis cell. Appl. Environ. Microbiol. 75(24), 7579-7587.

7. Carlotta-Jones, D.I., Purdy, K., Kirwan, K., Stratford, J., Coles, S.R., 2020. Improved hydrogen gas production in microbial electrolysis cells using inexpensive recycled carbon fibre fabrics. Bioresour. Technol. 304, 122983.

8. Catal, T., Lesnik, K.L., Liu, H., 2015. Suppression of methanogenesis for hydrogen production in single-chamber microbial electrolysis cells using various antibiotics. Bioresour. Technol. 187, 77-83.

9. Chae, K.-J., Choi, M.-J., Kim, K.-Y., Ajayi, F.F., Park, W., Chang, I.-S., Kim, I.S.,, 2010. Methanogenesis control by employing various environmental stress conditions in two-chambered microbial fuel cells. Bioresour. Technol. 101(14), 5350-5357.

10. Chae, K.-J., Choi, M.-J., Kim, K.-Y., Ajayi, F.F., Chang, I.-S., Kim, I.S., 2009. A solar-powered microbial electrolysis cell with a platinum catalyst-free cathode to produce hydrogen. Environ. Sci. Technol. 43(24), 9525-9530.

11. Chen, Q.-Y., Liu, J.-S., Liu, Y., Wang, Y.-H.J., 2013. Hydrogen production on TiO2 nanorod arrays cathode coupling with bio-anode with

additional electricity generation. J. Power Sources 238, 345-349.

12. Cheng, S., Logan, B.E., 2011. High hydrogen production rate of microbial electrolysis cell (MEC) with reduced electrode spacing. Bioresour. Technol. 102(3), 3571-3574.

13. Croese, E., Jeremiasse, A.W., Marshall, I.P., Spormann, A.M., Euverink, G.-J.W., Geelhoed, J.S., Stams, A.J., Plugge, C.M., 2014. Influence of setup and carbon source on the bacterial community of biocathodes in microbial electrolysis cells. Enzyme Microb. Technol. 61, 67-75.

14. Croese, E., Keesman, K.J., Geelhoed, J.S., Plugge, C.M., Sleutels, T.H., Stams, A.J. and Euverink, G.J.W., 2013. Relating MEC population dynamics to anode performance from DGGE and electrical data. Syst. Appl. Microbiol. 36(6), 408-416.

15. Dai, H.-Y., Yang, H.-M., Liu, X., Song, X.-L., Liang, Z.-H., 2019. Hydrogen Production Using "Direct-Starting" Biocathode Microbial Electrolysis Cell and the Analysis of Microbial Communities. Acta Metall. Sin. 32(3), 297-304.

16. Dhand, V., Rao, M.V., Prasad, J., Mittal, G., Rhee, K.Y., Kim, H.J., Jung, D.H., 2014. Carbon nanospheres synthesized via solution combustion method: their application as an anode material and catalyst for hydrogen production. Carbon Lett. 15(3), 198-202.

17. Ding, A., Yang, Y., Sun, G., Wu, D., 2016. Impact of applied voltage on methane generation and microbial activities in an anaerobic microbial

electrolysis cell (MEC). Chem. Eng. J. 283, 260-265.

18. Feng, H., Huang, L., Wang, M., Xu, Y., Shen, D., Li, N., Chen, T., Guo, K., 2018. An effective method for hydrogen production in a single-chamber microbial electrolysis by negative pressure control. Int. J. Hydrogen. Energ. 43(37), 17556-17561.

19. Ferraren-De Cagalitan, D.D.T. and Abundo, M.L.S., 2021. A review of biohydrogen production technology for application towards hydrogen fuel cells. Renew. Sust. Energ. Rev. 151, p.111413.

20. Fischer, F., 2018. Photoelectrode, photovoltaic and photosynthetic microbial fuel cells. Renew. Sust. Energ. Rev. 90, 16-27.

21. Fu, Q., Kobayashi, H., Kuramochi, Y., Xu, J., Wakayama, T., Maeda, H., Sato, K., 2013. Bioelectrochemical analyses of a thermophilic biocathode catalyzing sustainable hydrogen production. Int. J. Hydrogen. Energ. 38(35), 15638-15645.

22. Fujinawa, K., Nagoya, M., Kouzuma, A., Watanabe, K., 2019. Conductive carbon nanoparticles inhibit methanogens and stabilize hydrogen production in microbial electrolysis cells. Appl. Microbiol. Biotechnol. 103(15), 6385-6392.

23. Geelhoed, J.S., Stams, A.J., 2011. Electricity-assisted biological hydrogen production from acetate by Geobacter sulfurreducens. Environ. Sci. Technol. 45(2), 815-820.

24. Ghasemi, B., Yaghmaei, S., Abdi, K., Mardanpour, M.M., Haddadi,

S.A.J., bioengineering, 2020. Introducing an affordable catalyst for biohydrogen production in microbial electrolysis cells. J. Biosci. Bioeng. 129(1), 67-76.

25. Guo, K., Tang, X., Du, Z., Li, H., 2010. Hydrogen production from acetate in a cathode-on-top single-chamber microbial electrolysis cell with a mipor cathode. Biochem. Eng. J. 51(1-2), 48-52.

26. Hardhi, M., Ekadewi, P., Arbianti, R., Utami, T.S., Hermansyah, H. 2018. Improvement of biohydrogen production in microbial electrolysis cell (MEC) system by tackling methanogenesis. *E3S Web of Conferences*. EDP Sciences. pp. 02008.

27. Hasany, M., Mardanpour, M.M., Yaghmaei, S., 2016. Biocatalysts in microbial electrolysis cells: a review. Int. J. Hydrogen. Energ. 41(3), 1477-1493.

28. Hatzell, M.C., Kim, Y., Logan, B.E.J., 2013. Powering microbial electrolysis cells by capacitor circuits charged using microbial fuel cell. J. Power Sources 229, 198-202.

29. He, Y.-R., Yan, F.-F., Yu, H.-Q., Yuan, S.-J., Tong, Z.-H., Sheng, G.-P., 2014. Hydrogen production in a light-driven photoelectrochemical cell. Appl. Energy 113, 164-168.

30. Hou, Y., Luo, H., Liu, G., Zhang, R., Li, J., Fu, S., 2014. Improved hydrogen production in the microbial electrolysis cell by inhibiting methanogenesis using ultraviolet irradiation. Environ. Sci. Technol. 48(17), 10482-10488.

31. Hu, H., Fan, Y., Liu, H., 2008. Hydrogen production using single-

chamber membrane-free microbial electrolysis cells. Water Res. 42(15), 4172-4178.

32. Huang, J., Feng, H., Huang, L., Ying, X., Shen, D., Chen, T., Shen, X., Zhou, Y., Xu, Y., 2020. Continuous hydrogen production from food waste by anaerobic digestion (AD) coupled single-chamber microbial electrolysis cell (MEC) under negative pressure. Waste Manage. 103, 61-66.

33. Jadhav, D.A., Chendake, A.D., Schievano, A., Pant, D., 2019. Suppressing methanogens and enriching electrogens in bioelectrochemical systems. Bioresour. Technol. 277, 148-156.

34. Jafary, T., Daud, W.R.W., Ghasemi, M., Bakar, M.H.A., Sedighi, M., Kim, B.H., Carmona-Martínez, A.A., Jahim, J.M., Ismail, M., 2019. Clean hydrogen production in a full biological microbial electrolysis cell. Int. J. Hydrogen. Energ. 44(58), 30524-30531.

35. Jafary, T., Daud, W.R.W., Ghasemi, M., Kim, B.H., Carmona-Martínez, A.A., Bakar, M.H.A., Jahim, J.M., Ismail, M.J., 2017. A comprehensive study on development of a biocathode for cleaner production of hydrogen in a microbial electrolysis cell. J. Clean. Prod. 164, 1135-1144.

36. Jafary, T., Daud, W.R.W., Ghasemi, M., Kim, B.H., Jahim, J.M., Ismail, M., Lim, S.S., 2015. Biocathode in microbial electrolysis cell; present status and future prospects. Renew. Sust. Energ. Rev. 47, 23-33.

37. Jang, Y.J., Lee, J.S., 2019. Photoelectrochemical Water Splitting with p-Type Metal Oxide Semiconductor Photocathodes. ChemSusChem 12(9), 1835-

1845.

38. Jayabalan, T., Matheswaran, M., Preethi, V., Mohamed, S.N., 2020. Enhancing biohydrogen production from sugar industry wastewater using metal oxide/graphene nanocomposite catalysts in microbial electrolysis cell. Int. J. Hydrogen. Energ. 45(13), 7647-7655.

39. Jayabalan, T., Matheswaran, M., Radhakrishnan, T., Mohamed, S.N., 2021. Influence of Nickel molybdate nanocatalyst for enhancing biohydrogen production in microbial electrolysis cell utilizing sugar industrial effluent. Bioresour. Technol. 320, 124284.

40. Jeon, Y., Kim, J.H., Koo, K., Kim, S.J., 2018. A photo-assisted microbial electrolysis cell for the exclusive biohydrogen production using a MoS2-coated p-type copper oxide. J. Power Sources 373, 79-84.

41. Jeremiasse, A.W., Hamelers, H.V., Buisman, C.J., 2010. Microbial electrolysis cell with a microbial biocathode. Bioelectrochemistry 78(1), 39-43.

42. Jobin, L., Jose, C., Pages, C., Raffin, G., Saupin, X., Jame, P., Jaffrezic-Renault, N., Namour, P.J., 2018. Methanogenesis control in bioelectrochemical systems: A carbon footprint reduction assessment. J. Environ. Chem. Eng. 6(1), 803-810.

43. Kadier, A., Kalil, M.S., Abdeshahian, P., Chandrasekhar, K., Mohamed, A., Azman, N.F., Logroño, W., Simayi, Y., Hamid, A.A., 2016. Recent advances and emerging challenges in microbial electrolysis cells (MECs) for microbial production of hydrogen and value-added chemicals. Renew. Sust.

Energ. Rev. 61, 501-525.

44. Kadier, A., Kalil, M.S., Chandrasekhar, K., Mohanakrishna, G., Saratale, G.D., Saratale, R.G., Kumar, G., Pugazhendhi, A., Sivagurunathan, P., 2018. Surpassing the current limitations of high purity H_2 production in microbial electrolysis cell (MECs): Strategies for inhibiting growth of methanogens. Bioelectrochemistry 119, 211-219.

45. Kang, Y.L., Ibrahim, S., Pichiah, S., 2015. Synergetic effect of conductive polymer poly (3, 4-ethylenedioxythiophene) with different structural configuration of anode for microbial fuel cell application. Bioresour. Technol. 189, 364-369.

46. Karthikeyan, R., Cheng, K.Y., Selvam, A., Bose, A., Wong, J.W., 2017. Bioelectrohydrogenesis and inhibition of methanogenic activity in microbial electrolysis cells-A review. Biotechnol. Adv. 35(6), 758-771.

47. Khongkliang, P., Jehlee, A., Kongjan, P., Reungsang, A., Sompong, O., 2019. High efficient biohydrogen production from palm oil mill effluent by two-stage dark fermentation and microbial electrolysis under thermophilic condition. Int. J. Hydrogen. Energ. 44(60), 31841-31852.

48. Kim, Y., Hatzell, M.C., Hutchinson, A.J., Logan, B.E., 2011. Capturing power at higher voltages from arrays of microbial fuel cells without voltage reversal. Energy Sci. Eng. 4(11), 4662-4667.

49. Kumar, G., Bakonyi, P., Zhen, G., Sivagurunathan, P., Koók, L., Kim, S.-H., Tóth, G., Nemestóthy, N., Bélafi-Bakó, K., 2017. Microbial

electrochemical systems for sustainable biohydrogen production: surveying the experiences from a start-up viewpoint. Renew. Sust. Energ. Rev. 70, 589-597.

50. Kundu, A., Sahu, J.N., Redzwan, G., Hashim, M., 2013. An overview of cathode material and catalysts suitable for generating hydrogen in microbial electrolysis cell. Int. J. Hydrogen. Energ. 38(4), 1745-1757.

51. Lalaurette, E., Thammannagowda, S., Mohagheghi, A., Maness, P.-C., Logan, B.E., 2009. Hydrogen production from cellulose in a two-stage process combining fermentation and electrohydrogenesis. Int. J. Hydrogen. Energ. 34(15), 6201-6210.

52. Li, X.-H., Liang, D.-W., Bai, Y.-X., Fan, Y.-T., Hou, H.-W., 2014. Enhanced H_2 production from corn stalk by integrating dark fermentation and single chamber microbial electrolysis cells with double anode arrangement. Int. J. Hydrogen. Energ. 39(17), 8977-8982.

53. Liang, B., Cheng, H., Van Nostrand, J.D., Ma, J., Yu, H., Kong, D., Liu, W., Ren, N., Wu, L., Wang, A., 2014. Microbial community structure and function of nitrobenzene reduction biocathode in response to carbon source switchover. Water Res. 54, 137-148.

54. Liang, D., Han, G., Zhang, Y., Rao, S., Lu, S., Wang, H., Xiang, Y., 2016. Efficient H_2 production in a microbial photoelectrochemical cell with a composite Cu_2O/NiOx photocathode under visible light. Appl. Energy 168, 544-549.

55. Lim, S.S., Yu, E.H., Daud, W.R.W., Kim, B.H., Scott, K., 2017.

Bioanode as a limiting factor to biocathode performance in microbial electrolysis cells. Bioresour. Technol. 238, 313-324.

56. Liu, Q., Ren, Z.J., Huang, C., Liu, B., Ren, N., Xing, D., 2016. Multiple syntrophic interactions drive biohythane production from waste sludge in microbial electrolysis cells. Biotechnol. Biofuels 9(1), 1-10.

57. Logan, B.E., Call, D., Cheng, S., Hamelers, H.V., Sleutels, T.H., Jeremiasse, A.W., Rozendal, R.A., 2008. Microbial electrolysis cells for high yield hydrogen gas production from organic matter. Environ. Sci. Technol. 42(23), 8630-8640.

58. Lu, L., Hou, D., Wang, X., Jassby, D., Ren, Z.J., 2016. Active H_2 harvesting prevents methanogenesis in microbial electrolysis cells. Environ. Sci. Technol. Lett. 3(8), 286-290.

59. Lu, L., Ren, N., Zhao, X., Wang, H., Wu, D., Xing, D., 2011. Hydrogen production, methanogen inhibition and microbial community structures in psychrophilic single-chamber microbial electrolysis cells. Energy Sci. Eng. 4(4), 1329-1336.

60. Lu, L., Ren, Z.J.., 2016. Microbial electrolysis cells for waste biorefinery: A state of the art review. Bioresour. Technol. 215, 254-264.

61. Lu, L., Xing, D., Ren, N., 2012. Bioreactor performance and quantitative analysis of methanogenic and bacterial community dynamics in microbial electrolysis cells during large temperature fluctuations. Environ. Sci. Technol. 46(12), 6874-6881.

62. Manuel, M.-F., Neburchilov, V., Wang, H., Guiot, S., Tartakovsky, B.J.., 2010. Hydrogen production in a microbial electrolysis cell with nickel-based gas diffusion cathodes. J. Power Sources 195(17), 5514-5519.

63. Moreno, R., Escapa, A., Cara, J., Carracedo, B., Gómez, X., 2015. A two-stage process for hydrogen production from cheese whey: Integration of dark fermentation and biocatalyzed electrolysis. Int. J. Hydrogen. Energ. 40(1), 168-175.

64. Nguyen, P.K.T., Das, G., Kim, J., Yoon, H.H., 2020. Hydrogen production from macroalgae by simultaneous dark fermentation and microbial electrolysis cell. Bioresour. Technol. 315, 123795.

65. Osman, A.I., Deka, T.J., Baruah, D.C. and Rooney, D.W., 2020. Critical challenges in biohydrogen production processes from the organic feedstocks. Biomass Convers. Biorefin., 1-19.

66. Pisciotta, J.M., Zaybak, Z., Call, D.F., Nam, J.-Y., Logan, B.E., 2012. Enrichment of microbial electrolysis cell biocathodes from sediment microbial fuel cell bioanodes. Appl. Environ. Microbiol. 78(15), 5212-5219.

67. Pratiwi, R.D., Putri, K.L., Arbianti, R., Utami, T.S., Hermansyah, H. 2020. Improvement of biohydrogen production by reduction of methanogenesis at optimum electrode spacing in microbial electrolysis cell system. *AIP Conference Proceedings*. AIP Publishing LLC. pp. 040012.

68. Rivera, I., Buitrón, G., Bakonyi, P., Nemestóthy, N., Bélafi-Bakó, K., 2015. Hydrogen production in a microbial electrolysis cell fed with a dark

fermentation effluent. J. Appl. Electrochem. 45(11), 1223-1229.

69. Rode, A., Carleton, T., Delgado, M., Greenstone, M., Houser, T., Hsiang, S., Hultgren, A., Jina, A., Kopp, R.E., McCusker, K.E.J.N., 2021. Estimating a social cost of carbon for global energy consumption. Nature 598(7880), 308-314.

70. Rozendal, R.A., Hamelers, H.V., Rabaey, K., Keller, J., Buisman, C.J., 2008a. Towards practical implementation of bioelectrochemical wastewater treatment. Trends Biotechnol. 26(8), 450-459.

71. Rozendal, R.A., Jeremiasse, A.W., Hamelers, H.V., Buisman, C.J, 2008b. Hydrogen production with a microbial biocathode. Environ. Sci. Technol. 42(2), 629-634.

72. Rozenfeld, S., Ouaknin Hirsch, L., Gandu, B., Farber, R., Schechter, A., Cahan, R., 2019. Improvement of microbial electrolysis cell activity by using anode based on combined plasma-pretreated carbon cloth and stainless steel. Energies 12(10), 1968.

73. Saheb-Alam, S., Persson, F., Wilén, B.-M., Hermansson, M., Modin, O., 2019. A variety of hydrogenotrophic enrichment cultures catalyse cathodic reactions. Sci. Rep. 9(1), 1-13.

74. Saheb-Alam, S., Singh, A., Hermansson, M., Persson, F., Schnürer, A., Wilén, B.-M., Modin, O., 2018. Effect of start-up strategies and electrode materials on carbon dioxide reduction on biocathodes. Appl. Environ. Microbiol. 84(4), e02242-17.

75. Saratale, R.G., Saratale, G.D., Pugazhendhi, A., Zhen, G., Kumar, G., Kadier, A. and Sivagurunathan, P., 2017. Microbiome involved in microbial electrochemical systems (MESs): a review. Chemosphere. 177, 176-188.

76. Sarathi, V.S., Nahm, K.S., 2013. Recent advances and challenges in the anode architecture and their modifications for the applications of microbial fuel cells. Biosens. Bioelectron. 43, 461-475.

77. Saravanan, A., Karishma, S., Kumar, P.S., Yaashikaa, P.R., Jeevanantham, S. and Gayathri, B., 2020. Microbial electrolysis cells and microbial fuel cells for biohydrogen production: current advances and emerging challenges. Biomass Convers. Biorefin., 1-21.

78.

79. Sleutels, T.H., Hamelers, H.V., Buisman, C.J., 2011. Effect of mass and charge transport speed and direction in porous anodes on microbial electrolysis cell performance. Bioresour. Technol. 102(1), 399-403.

80. Sun, M., Sheng, G.-P., Mu, Z.-X., Liu, X.-W., Chen, Y.-Z., Wang, H.-L., Yu, H.-Q., 2009. Manipulating the hydrogen production from acetate in a microbial electrolysis cell–microbial fuel cell-coupled system. J. Power Sources 191(2), 338-343.

81. Sun, M., Sheng, G.P., Zhang, L., Xia, C.R., Mu, Z.X., Liu, X.W., Wang, H.-L., Yu, H.Q., Qi, R., Yu, T., 2008. An MEC-MFC-coupled system for biohydrogen production from acetate. Environ. Sci. Technol. 42(21), 8095-8100.

82. Varanasi, J.L., Veerubhotla, R., Pandit, S. and Das, D., 2019.

Biohydrogen production using microbial electrolysis cell: recent advances and future prospects, in: Mohan, S. V., Varjani, S. and Pandey, A. (Eds.), Microbial Electrochemical Technology, Elsevier, pp. 843-869.

83. Vincent, K.A., Parkin, A., Armstrong, F.A.J.C.r., 2007. Investigating and exploiting the electrocatalytic properties of hydrogenases. 107(10), 4366-4413.

84. Wang, A., Sun, D., Cao, G., Wang, H., Ren, N., Wu, W.-M., Logan, B.E., 2011. Integrated hydrogen production process from cellulose by combining dark fermentation, microbial fuel cells, and a microbial electrolysis cell. Bioresour. Technol. 102(5), 4137-4143.

85. Xing, D., Yang, Y., Li, Z., Cui, H., Ma, D., Cai, X., Gu, 2020. Hydrogen Production from Waste Stream with Microbial Electrolysis Cells, in: Wang, A., Liu, W., Zhang, B., Cai, W. (Eds.), Bioelectrosynthesis: Principles and Products, Technologies for Value-Added. Wiley-Vch Verlag Gmbh & Co. Kgaa, pp. 39-70.

86. Xu, Y., Jiang, Y., Chen, Y., Zhu, S., Shen, S., 2014. Hydrogen production and wastewater treatment in a microbial electrolysis cell with a biocathode. Water Environ. Res. 86(7), 649-653.

87. Yadav, T., Mungray, A.A., Mungray, A.K., 2016. Effect of multiwalled carbon nanotubes on UASB microbial consortium. Environ. Sci. Rec. 23(5), 4063-4072.

88. Yang, E., Mohamed, H.O., Park, S.-G., Obaid, M., Al-Qaradawi, S.Y.,

Castaño, P., Chon, K., Chae, K.-J., 2020. A review on self-sustainable microbial electrolysis cells for electro-biohydrogen production via coupling with carbon-neutral renewable energy technologies. Bioresour. Technol. 124363.

89. Yasri, N., Roberts, E.P., Gunasekaran, S., 2019. The electrochemical perspective of bioelectrocatalytic activities in microbial electrolysis and microbial fuel cells. Energy Rep. 5, 1116-1136.

90. Yoro, K.O., Daramola, M.O. 2020. CO_2 emission sources, greenhouse gases, and the global warming effect. in: Rahimpour, M. R., Farsi, M. and Makarem, M. A. (Eds.), Advances in carbon capture, Elsevier, pp. 3-28.

91. Zhao, N., Liang, D., Li, X., Meng, S., Liu, H., 2021. Hydrophilic porous materials provide efficient gas-liquid separation to advance hydrogen production in microbial electrolysis cells. Bioresour. Technol. 125352.

92. Zhao, N., Liang, D., Meng, S., Li, X.J.I.J.o.H.E., 2020. Bibliometric and content analysis on emerging technologies of hydrogen production using microbial electrolysis cells. Int. J. Hydrog. Energy. 45, 33310-33324.

93. Zhao, Z., Zhang, Y., Chen, S., Quan, X., Yu, Q., 2014. Bioelectrochemical enhancement of anaerobic methanogenesis for high organic load rate wastewater treatment in a up-flow anaerobic sludge blanket (UASB) reactor. Sci. Rep. 4(1), 1-8.

Figure and Table captions:

Fig. 1 Schematic diagram of basic two-chamber and single-chamber MEC

Fig. 2 Example of microbial electrolysis cell (MEC) process for biohydrogen production: (a) half and full biological start-up of biocathode MEC; (b) microbial fuel cell (MFC) powered MEC; (c) dye Sensitized Solar Cell (DSSC) powered MEC; (d) photocathode MEC

Table 1 Examples of promoting strategies for improving biohydrogen production in MECs

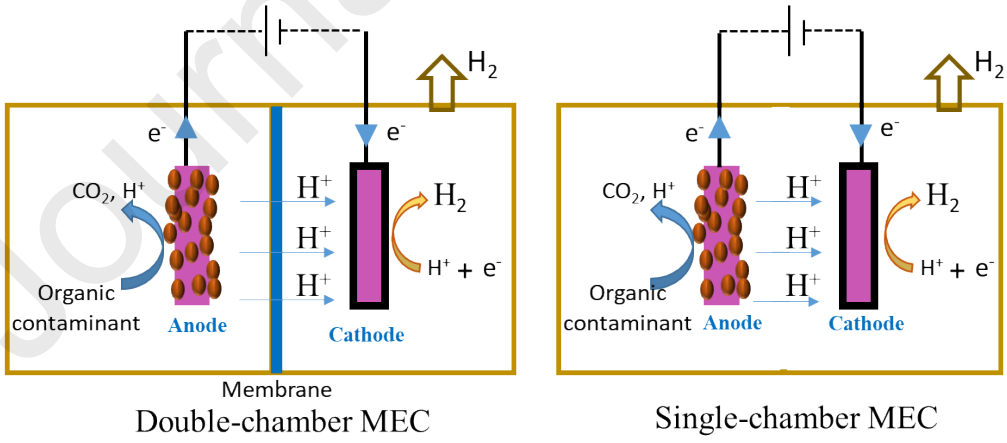

Fig. 1 Schematic diagram of basic two-chamber and single-chamber MEC

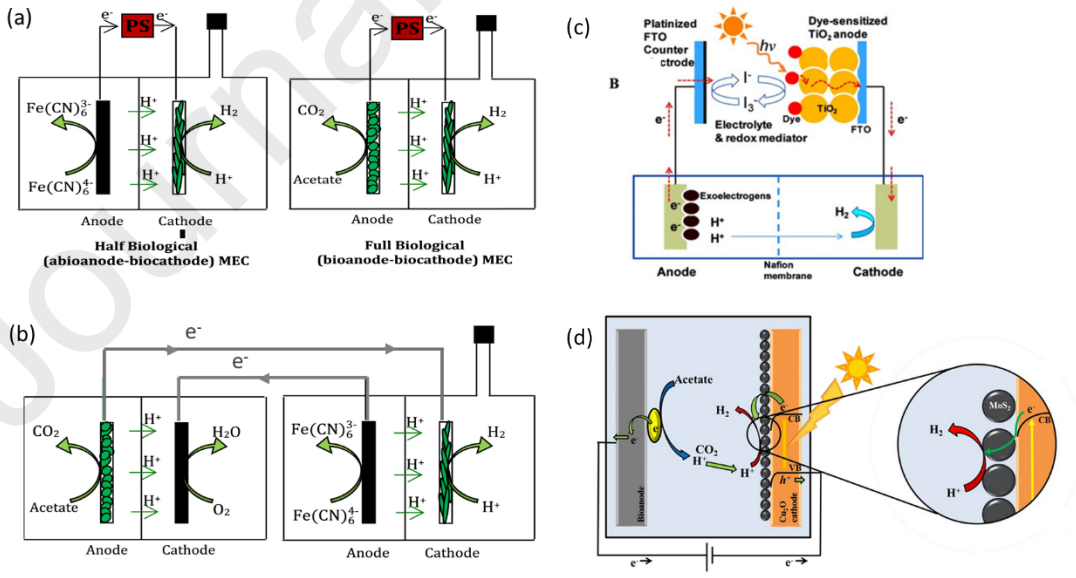

Fig. 2. Example of microbial electrolysis cell (MEC) process for biohydrogen production: (a) half and full biological start-up of biocathode MEC; (b) microbial fuel

cell (MFC) powered MEC; (c) dye Sensitized Solar Cell (DSSC) powered MEC; (d) photocathode MEC (Fischer & Reviews, 2018; Jafary et al., 2019; Jeon et al., 2018).

Table 1 Examples of promoting strategies for improving biohydrogen production in MECs

Impact factors	Promoting strategies	Advanced methods	Performance	References
Microorganisms	Inhibiting methanogens growth	Enriching denitrifying bacteria in MEC systems	H_2 production increased 100% compared to the control	(Hardhi et al., 2018)
		Adding *Pseudomonas stutzeri* as biological control of methanogenesis	76.28% reduction of methane, H_2 production was 128% higher than the control reactor	(Pratiwi et al., 2020)
		Supplement of carbon nanomaterials (2%) to the cathode chamber	Effectively suppresses H_2 consumption and methane production	(Fujinawa et al., 2019)
		High voltage application	H_2 production rates increased 33.3 times by increasing the voltage from 0.2 V to 1.0 V	(Guo et al., 2010)
		Rapid H_2 extraction using a gas-permeable hydrophobic membrane and vacuum	3.3 - 4.4 times higher of H_2 production than the control system	(Lu et al., 2016)
		Operating under negative pressure (40.52 kPa)	>4 times higher of H_2 production than the control system	(Feng et al., 2018)
		Using hydrophilic porous membranes to replay the conventional proton exchange membrane	1.14 times higher H_2 production than that using proton exchange membrane	(Zhao et al., 2021)
Anode materials	Advanced anode materials	Recycled carbon fibre anode	5.9 times higher of H_2 production rate and 93% less expensive than similar graphite anodes	(Carlotta et al., 2020)

		Composed anode of stainless steel and plasma-pretreated carbon cloth (CCP) (COMBP)	40% and 18% higher H_2 production rate than the COMB and CCP anodes,	(Rozenfeld et al., 2019)
Cathode and catalysts	Biocathode	Sulphate reducing bacteria enriched biocathode	6 times improvement of H_2 production compared to the non-inoculated graphite felt cathode	(Jafary et al., 2017)
		Sulphate reducing bacteria enriched biocathode	Lower HER onset potential of biocathode (500 mV) than the abiotic control system	(Jafary et al., 2019)
		"Direct-starting" biocathode	H_2 recovery (71.22 ± 8.98%) and production rate (0.428 ± 0.054 m^3 H_2/m^3/days) slightly higher than those obtained with the Pt/C cathode MEC	(Dai et al., 2019)

Highlights

- Novel strategies for inhibiting methanogens in MECs are reviewed.
- Biocathodes are promising alternatives to noble metal catalytic cathodes.
- Advanced MEC systems can enhance low-cost biohydrogen production.
- Challenges and perspectives concerning biohydrogen production were clearly stated.