Microbial Electrolysis Cells for Waste Biorefinery: A State of the Art Review

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Abstract

Microbial electrolysis cells (MECs) is an emerging technology for energy and resource recovery during waste treatment. MECs can theoretically convert any biodegradable waste into H₂, biofuels, and other value added products, but the system efficacy can vary significantly when using different substrates or are operated in different conditions. To understand the application niches of MECs in integrative waste biorefineries, this review provides a critical analysis of MEC system performance reported to date in terms of H₂ production rate, H₂ yield, and energy efficiency under a variety of substrates, applied voltages and other crucial factors. It further discusses the mutual benefits between MECs and dark fermentation and argues such integration can be a viable approach for efficient H₂ production from renewable biomass. Other marketable products and system integrations that can be applied to MECs are also summarized, and the challenges and prospects of the technology are highlighted.

Keywords: Microbial Electrolysis Cell, Wastewater, Biorefinery, Microbial Electrochemical Technology, Biohydrogen, Bioelectrochemical Systems, Microbial Electrochemical Cells

1. Introduction

The increasing awareness of food-water-energy nexus is compelling the transformation of traditional energy intensive, treatment focused wastewater treatment into water resource recovery facilities. Current treatment of municipal wastewater accounts for approximately 3% of global electricity consumption and 5% of non-carbon dioxide greenhouse gas emissions (Li et al., 2015; Lu et al., 2015). In the United States, the publicly owned treatment works consume approximately 30.2 billion kWh every year for treating 12 trillion gallons of municipal wastewater, and these wastewater plants are often the largest energy consumers in a city. However, considering the energy content embedded in wastewater is 2–4 times the energy used for treatment, future utilities can become energy positive when novel technologies are deployed for energy recovery (McCarty et al., 2011). Moreover, these facilities will also recover other value-added resources such as nutrients, metals, chemicals, and clean water and therefore become close loop waste biorefineries with maximized productivity and efficiency.

Microbial electrolysis cells (MECs) are an emerging technology that will become an important part of future waste biorefinery plants for waste to product conversion. Fig. 1 shows the schematic of an example MEC, which utilizes exoelectrogenic microbes at the anode to convert biodegradable substrates, such as organic matter in wastewater, into electrical current and release protons (H^+). The electrons are then transferred to the cathode to reduce the protons for H_2 production (Liu et al., 2005; Rozendal et al., 2006). Because the microbial electrolysis is endothermic (positive Gibbs free energy) so it won't occur

spontaneously unless hydrogen partial pressure is extremely low. Therefore, in order to produce H₂ on the cathode, current generation needs to be forced by applying a small voltage between the two electrodes. In general, the applied voltage (0.2–0.8 V) for overcoming the thermodynamic barrier is much lower than traditional water electrolysis (1.8–3.5 V) and can be supplied by a small solar panel, low-grade heat, or microbial fuel cells (MFCs) (Kadier et al., 2015). The gap of energy input between microbial and pure electrochemical electrolysis is provided by chemical energy stored in the organics. In addition to H₂, other inorganic and organic chemicals, such as CH₄, H₂O₂ and formic acid, have also been generated using a similar process (Wang & Ren, 2013; Zhang & Angelidaki, 2014). Earlier reviews addressed MEC principles and evaluation metrics, reactor configurations and materials, system performance, and economic considerations (Escapa et al., 2016; Kadier et al., 2015; Kundu et al., 2013; Liu et al., 2010; Zhang & Angelidaki, 2014). The intent of this present review is not to duplicate these previous reviews. Instead, this review focuses on understanding the niche of MECs for waste biorefineries in terms of organic to H₂ conversion, as well as new directions in MECs. Microbial catalyzed anode organic oxidation and cathodic H₂ production are the focus of the study.

Among the products that can be generated in microbial electrochemical systems (MESs), H₂ from MEC is considered a very promising route with near term commercialization potential. This is not only because MECs demonstrated superior H₂ production performance compared with other bio-H₂ technologies such as fermentation or photobiological processes, but also for the reason that MEC system efficiency is much higher than other MESs such as

electricity from MFCs (Lee et al., 2010). H₂ is easier to store and transport than decentralized electricity, and the demand of H₂ has been rapidly increasing with the growth of fuel cell market and other H₂-consuming industries (chemical, petrochemical, fertilizer, biorefinery, metallurgy, etc.). US Department of Energy Fuel Cell Technology Office (FCTO) identified MEC as a key technology to be developed in order to meet the cost goals of \$2-4/gge (gasoline gallon equivalent) H₂ from renewable biomass (Randolph & Studer, 2013).

Since the invention of the MEC technology in 2005, the number of journal articles has been steadily increasing. Fig. 2 shows the number of articles focusing on MEC H₂ production that were published between 2005-2015. More importantly, these articles reported that during the past decade, the H₂ production rate and yield have increased by orders of magnitudes, with rate increased from less than 0.1 m³ H₂/m³ reactor/day to the highest of 50 m³ H₂/m³ reactor/day, and H₂ yield increased from below 50% to nearly 100% (Fig. 2B, Table 1). However, simply reading these literature data cannot gain the specific understanding on in what condition the MEC provides the most benefits to waste refinery, and how to integrate MECs with other processes to maximize substrate conversion to H₂. These questions are important, because previous studies found that the MEC H₂ performance was highly dependent on substrate composition, microbial communities, and other factors. For example, more H₂ can be produced from acetate, alcohols or glucose, but poor performance was found from proteins or domestic wastewater (Kadier et al., 2014). This review aims to provide a state-of-the-art analysis on how did the key parameters such as substrate, reactor volume and applied voltage affect MEC H₂ performance (current, H₂ yield, H₂ rate, energy efficiency,

etc.), and what is the best strategy for MEC to maximize the H₂ generation efficiency from complex organic substrates. The review also identifies the key challenges faced by the MEC technology and discussed the pathways of solving these problems to achieve the H₂ cost goals in large scale systems.

2. The Benefits and Niche Applications of MEC for Waste Biorefinery

2.1. How much hydrogen energy was recovered in MECs from different substrates

MECs can theoretically convert any biodegradable waste into H₂ and other products, but the production efficacy can vary significantly when using different substrates. To understand the niche applications of MECs in waste biorefineries, it is critical to understand how substrates affect MEC performance. [Table 1](#) classifies 9 categories of substrates that have been used in MECs for H₂ production, and the specific system performance under different substrates is summarized in [Fig. 3](#). While many studies used defined substrates such as volatile fatty acids, alcohols, and proteins, the utilization of different mixed waste streams from municipal and industrial sources represent the real world potential of MECs. Though reactor configuration, microbial communities, and operation conditions also influence system performance, they are not focused in this review as these factors are built upon the specific needs of substrate conversion. Previous reviews provided excellent references on these topics ([Kadier et al., 2015](#); [Kadier et al., 2014](#); [Kundu et al., 2013](#)).

[Fig. 3](#) shows how 4 key performance metrics are influenced by the substrates used in MECs in different scaled reactor systems. Current density (I , A/m²), H₂ production rate (Q ,

m³/m³/d), overall H₂ recovery (R_{H_2} , %) and energy efficiency relative to electrical input (η_E , %) were chosen because they represent the different aspects of the metrics. Volumetric current density is obtained by normalizing current to the reactor liquid volume, and it has shown a strong positive correlation with H₂ production. This correlation is true for most studies in which proton is the only electron acceptor, but it may not be accurate when other electron acceptors are present as they also consume electrons and generate other products. Further discussions on this point will be discussed in section 2.6 and 3.1. H₂ production rate measures how fast the H₂ is evolved from per volume of reactor, which is a crucial economic measurement, because it directly relates to the dimension of the system and speed of production. Overall H₂ recovery is used to evaluate H₂ yield, which is a ratio of produced H₂ in experiments to the theoretical maximum. The use of percentage allows direct comparison among different substrates, because the maximum possible H₂ yield from various substrates are different. The energy efficiency is defined as the energy content of produced H₂ to the input electrical energy required. This efficiency can be used to evaluate whether a microbial electrolysis process is energy positive or negative. For waste biorefinery, substrates are generally considered “free”, so the embedded chemical energy is not included into a calculation for energy efficiency (Logan et al., 2008).

As shown in Fig. 3, the majority of the studies were conducted in lab scales with reactor volumes below 100 mL. Among the 55 articles that contain sufficient information for analysis, a general observation is that smaller reactors showed higher system performance. Gradual declines in current density, production rate and efficiency were observed along with

dimension increase. The highest performing MEC to date was reported by *Jeremiasse et al.* using a 40 mL two-chamber reactor, 1 V voltage, and new Ni-foam cathode. They obtained a maximum H₂ rate of 50 m³/m³/day with a current density 5704 A/m³ (*Jeremiasse et al., 2010*). In contrast, several pilot scale systems (100-1000L) reported lower H₂ production rates (< 0.02 m³/m³/day) and lower energy efficiencies (< 70%) (*Cusick et al., 2011; Heidrich et al., 2013*). While performance drop is expected during scale-ups, it is worth noting that all these pilot scale systems were treating real wastewater instead of pure chemicals, and the production rates were not considered poorly low compared with smaller reactors (< 1 L) using similar real waste (*Fig. 3*). The largest MEC reported to date was by Cusick et al. and installed in the Napa Wine Co. (NWC), in Oakville, CA, USA. The 1000 L, single chamber MEC was operated for 100 days and showed a maximum current density of 7.4 A/m³. It produced H₂ at the beginning at a rate of 0.07 m³/m³/day with a decent COD removal of 62%. However, methanogenesis led to H₂ consumption after 43 days, and methane became the dominant product (*Cusick et al., 2011*).

Among the substrates investigated, acetate showed the best conversion performance, with rates up to 6.3–50 m³/m³/day (*Cheng & Logan, 2011; Jeremiasse et al., 2010; Liang et al., 2011; Tartakovskya et al., 2009*). These rates are comparable to dark-fermentation using sugars. However, these results were obtained in the most favorable but not realistic conditions, with the reactor volumes ranged from 10–50 mL and applied voltage of 1–1.5V. As fermentation products, volatile fatty acids (VFAs) with more than two carbon atoms, such as propionic, butyric, valeric and lactic acids also showed relatively higher production rates of

0.072–1.04 m³/m³/day in MECs, though the rates were lower than acetate. In a similar context, dark fermentation effluents and lignocellulosic biorefinery byproducts contain high concentrations of organic acids, alcohol and monosaccharide or disaccharide, which are favorable substrates by exoelectrogenic microbes at the anode. These waste streams also yielded higher H₂ rates up to 3.4–4.3 m³/m³/day (Lewis et al., 2015; Li et al., 2014). In contrast, fermentable substrates including a variety of sugars, polysaccharides (cellulose and starch) and glycerol showed lower H₂ production, with glucose and pure glycerol obtaining the highest rates of 1.01–2.01 m³/m³/day (Lu et al., 2012c; Selembo et al., 2009), while recalcitrant and polymeric substrates showed very low rates < 0.2 m³/m³/day (Catal, 2015; Cheng & Logan, 2007). Pure proteins showed even lower performance (0.05–0.54 m³/m³/day) for H₂ production in MECs despite a high current is generated (Lu et al., 2010). Cellulose fermentation wastewaters containing high concentrations of proteins also showed the decline of H₂ production compared to synthetic wastewater with similar composition but without proteins (Nam et al., 2014). This may explain that why domestic wastewater and waste activated sludge containing high content of proteins produced low H₂ in MECs. Because almost all known exoelectrogens prefer simple substrates for extracellular electron transfer, they are not effective in directly oxidizing polymeric materials or complex wastes, rather they generally require cooperation with polymer-degrading bacteria, often fermenters, for the organics to energy conversion (Lu et al., 2012c; Ren et al., 2007). This suggests that the combination of fermentation and MEC can be a viable pathway for enhance H₂ production from biomass containing waste streams (details in Section 2.3).

Although H₂ production rates varied among substrates, high H₂ yields were obtained in most MECs, especially when compared with fermentation, which has an upper limit of 33% of theoretical H₂ yield (4 mole H₂ per mole hexose) due to incomplete organic degradation. For pure non-fermentable organics such as VFAs and alcohols, many studies reported higher than 80% H₂ recovery. Several acetate MECs even achieved higher than 90% recovery. Relatively lower recoveries observed in mixed wastewater including fermentation effluents, wastewater streams, and lignocellulosic biorefinery byproducts, but most studies still achieved higher than 30% of recovery. H₂ yield from proteins and waste sludge are among the lowest, ranged from 3–72%, presumably due to similar reasons of low degradability and loss of electrons to competing mechanisms.

2.2. How much hydrogen energy was recovered in MECs under different external voltages

[Fig. 3D](#) shows that most studies obtained a higher than 100% of energy efficiency, indicating the recovered H₂ contained more energy than the energy used by the external small voltage. MEC H₂ evolution does not occur spontaneously in experimental conditions, so the small voltage is used to boost current generation for proton reduction. For example, the potential of acetate oxidation at the anode is –0.285 V under standard biological condition (pH = 7, T = 298.15 K, P = 1 atm), while the potential of proton reduction at the cathode is –0.414 V. Therefore, theoretically an applied voltage of $(-0.414) - (-0.285) = -0.129$ V will be needed to make the reaction exothermic ([Logan et al., 2008](#)). In practice, a small voltage of 0.2–1.0 V is applied due to presence of overpotential lost in the system. [Fig. 4](#) shows the effects of applied voltage on the 4 evaluation parameters in MECs. It can be seen that for current

density, H₂ production rate and yield, higher external voltages led to higher performance within each substrate used, because increased voltage facilitates proton transfer and reduction (Fig. 4A-C). However, higher voltage also costs more external energy, which led to reduced energy efficiency. (Fig. 4D). Fig. 4 shows that a voltage of 0.6–0.8 V can maintain an optimum balance the H₂ production and energy efficiency. While most studies used programmable power sources for demonstration purpose, the external voltage has been supplied by renewable power sources, such as solar panels, waste heats, salinity gradient, and microbial fuel cells (Zhang & Angelidaki, 2014). Though not reported to date, larger scale MECs may be powered by other renewable sources, such as wind, geothermal, or hydropower. Most renewable power sources are facing major challenges in electricity storage and transportation, so the conversion of underutilized electricity to H₂ in MECs offers a desirable gaseous product with higher value that can be stored locally and transported over long distance.

2.3. The integration of MEC with fermentation can achieve high H₂ rate and yield

Because MECs showed slow carbohydrate and protein degradation and low H₂ production, it may not be an efficient standalone process for waste biorefinery treating complex wastewater. However, when an MEC is coupled with fermentation as a pretreatment, complete and efficient conversion can be achieved. Many anaerobic bacteria can produce H₂ during dark fermentation of fermentable substrates, and the highest production rate was reported as high as 189 m³/m³/day from sucrose (Lo et al., 2009). However, dark fermentation has an intrinsic upper limit of 4 mol H₂/mol hexose due to incomplete oxidation

(Eq. 1), which means it can only achieve up to 33% of the theoretical H₂ yield (12 mol H₂/mol hexose) (Eq. 2). Most experimental results only showed around 17% efficiency (~2 mol/mol), with the majority of the electrons wasted in the fermentation products (Lee et al., 2010). Moreover, because of this incomplete conversion, fermentation cannot be an effective waste treatment process, because the effluent still contains high concentration of organics that require further treatment.

However, fermentation can be a great pretreatment for MECs, as it breaks down recalcitrant polymers into short chain metabolites, so an MEC can further oxidize these organics to more H₂ and improve effluent quality (Eq. 3) (Lalaurette et al., 2009; Lu et al., 2009). When combined, the two H₂ producing streams can be merged to achieve high overall yield and rate, and the produced CO₂ maybe recirculated through electrolytes for pH buffering. MECs can be configured to produce high purity H₂ in two-chamber systems, and they also showed viability to as low as 4 °C after mature anode biofilm was established. (Lu et al., 2011; Lu et al., 2012c). These features also complement fermentation in waste refineries. Table 2 summarizes the individual performance of the two processes as well as their mutual benefits when combined for converting actual biomass waste to high-rate, high-yield, and high-quality H₂.

Several studies have reported the synergy between the two processes. Lu *et al.* found that

by combining ethanol type of fermentation with an MEC, H_2 yield from molasses wastewater was enhanced by 538% from 1.6 mol- H_2 /mol-hexose in fermentation alone to the 10.2 mol- H_2 /mol-hexose in an integrated system (Fig. 5), achieving an 85% H_2 recovery. H_2 production rate was also enhanced by 201%, from 0.7 to 2.11 m³ H_2 /m³/d (Lu et al., 2009). The ethanol type fermentation is an ideal pathway because ethanol and acetic acids are the major fermentation products that can be easily used in MECs (Lu et al., 2009). Fig. 5 also shows other integrated systems for enhanced H_2 production from cellobiose, corn stover lignocellulose, sugar beet juice, corn stalk, cellulose and glycerol (Chookaew et al., 2014; Dhar et al., 2015; Lalaurette et al., 2009; Li et al., 2014; Wang et al., 2011). All systems showed performance increase when fermentation was combined with an MEC. The overall H_2 yield was enhanced by 42% to 538%, with an average enhancement of 225%. The overall production rate increased by 2% to 400%, with an average enhancement of 148%.

2.4. Integration of MEC with AD for enhanced CH_4 production

Anaerobic digestion is a popular waste treatment technology to recover biogas during sludge and other high strength organic waste degradation. The produced biogas has been used for heat or electricity recovery. In general AD biogas contains approximately 60% of CH_4 and 40% CO_2 , so in order to increase efficiency many efforts are being made to increase the CH_4 content. Several recent studies showed that the integration of AD and MEC may accomplish this goal. When a pair of MEC electrodes inserted in an AD reactor, the produced H_2 from the MEC cathode could react with the CO_2 to produce CH_4 *in situ* by hydrogenotrophic methanogens (Bo et al., 2014). Compared with traditional AD systems, the integrated reactor

obtained very high content of CH₄ up to 98%. It was also reported that CH₄ yield, COD removal and carbon recovery was increased by 24–230%, 130–300% and 55–56% compared with traditional AD reactor, respectively. In another study, an iron anode and a graphite cathode were inserted into AD for waste activated sludge treatment. With an applied voltage of 0.3 V, the anode served as a source of electrons by self-oxidation and release of Fe²⁺, which decreased oxidative-reductive potential (ORP) and enhanced the activities of enzymes associated with hydrolysis-acidification. This approach boosted the growth of methanogens and enhanced CH₄ production by 22.4% (Feng et al., 2015).

2.5. Integration of MEC in biorefineries for production of H₂ and commodity chemicals

Biorefineries that utilize lignocellulosic biomass as feedstock to produce renewable biofuels and bioproducts are an integral part of the renewable energy portfolio. Current biorefineries produce large amount of wastewater that contains biodegradable organics, which can be used in MECs for additional energy production. Lignocellulosic biomass feedstock is generally pretreated using thermal, chemical, or biological methods to produce hydrolysate, which then is fermented to biofuels such as ethanol. During the pretreatment process, large amount of inhibitors, such as furanic and phenolic compounds, furfural and hydroxymethylfurfural, are generated and become a major impediment of downstream fermentation. MECs can be placed after pretreatment to utilize these inhibitors as substrates and produce H₂ and other bioproducts, and the removal of these chemicals will increase fermentation efficacy (Zeng et al., 2015). In addition, the fermentation effluent contains a variety of biodegradable organics,

such as residual sugar, ethanol and organic acids, which are ideal substrates for MECs as
aforementioned (Borole & Mielenz, 2011; Lewis et al., 2015). Borole and Mielenz estimated
that around 750-8900 m³/hr and 1260-7200m³/hr of H₂ could be produced by MECs from
existing starch-based and lignocellulosic-based biorefineries in the USA, respectively. MEC
can also be used to upgrade the straw hydrolysate to produce purified phenolic compounds
and H₂ by selective removal of carbohydrates and fatty acids (Thygesen et al., 2011). It was
estimated that 10-20% of the biorefinery cost is associated with water and wastewater, so the
utilization of pretreatment and fermentation effluents by MECs not only will facilitate water
reuse but also produces H₂ and other value-added chemicals for onsite and offsite uses
(Borole & Mielenz, 2011). For example, for biomass pyrolysis for bio-oil production, the
produced H₂ can be used *in situ* for downstream hydrogenation process, which will eliminate
the polar and oxygen-rich compounds and make biofuels more stable (Lewis et al., 2015;
Zeng et al., 2015).

2.6. MEC for organic chemical production via microbial electrosynthesis

While traditionally MEC has been referred as a H₂ producing process, different research
groups expanded the MEC definition to include microbial electrosynthesis (MES). Instead of
reducing H₂O and producing H₂, the electrons or H₂ derived from the cathode are used to
reduce CO₂ and other chemicals into organic compounds that can be precursors for
transportation fuels and value-added bioproducts. The MES process greatly expands the
benefits of microbial electrochemical systems by converting CO₂ into easily storable and
distributable products such as organic acids and alcohols, and therefore many excellent

reviews have been published to illustrate its potentials (Rabaey & Rozendal, 2010; Wang & Ren, 2013).

Most MES studies were conducted by using a potentiostat to poise a fixed biocathode potential and utilize water electrolysis or chemical oxidation at the abiotic counter electrode (anode) to provide electrons for electrosynthesis. These studies are not discussed in this review as they are not directly utilizing waste biomass, but the findings can be found in previous reviews on the topic. The earliest MES studies using wastewater as a feedstock was by Cheng, et al. (2009), who reported that CH₄ could be produced in an MEC reactor (Cheng et al., 2009). Follow-up studies hypothesized that methanogens grown on the cathode can accept electrons directly from electrode or indirectly use intermediate electron donor, such as cathodic H₂, for the reduction of CO₂ to CH₄ (Villano et al., 2010). It is still not clear which route(s) dominate CO₂ reduction and chemical production, partially because all studies used poised cathode potentials that are lower than H₂-evolution potential of -0.414 V (versus standard hydrogen electrode, SHE) under standard biological conditions ($T = 25\text{ }^{\circ}\text{C}$, $P = 1\text{ bar}$, $\text{pH} = 7$). H₂ produced at the cathode may be consumed rapidly *in situ* inside biofilm and served as an electron donor to indirectly reduce CO₂ even if no H₂ could be detected. Different microbial community analyses showed similar results that hydrogenotrophic methanogens dominated methanogenesis in MECs in spite of the presence or absence of acetate (Lu et al., 2012b), which makes it difficult to draw a conclusion on the mechanisms of direct or indirect electron transfer between the cathode and biofilm. The experimental setup of using a cathode potential above H₂-evolution potential as well as non-hydrogenotrophic

pure culture or mutant would help identify the potential pathway of direct electron transfer. MECs have also been reported to produce H₂O₂, formic acid, and other chemicals on the abiotic cathode using electrons from organic oxidation in the anode chamber, and details can be found in recent MES reviews ([Wang et al., 2015](#)).

3. Challenges of the MEC Technology

The MEC platform carries good potentials for waste refineries. With optimal system integration and operation, MECs are capable of converting organic waste into renewable energy and products with high yields and high rates. For example, the integrated fermentation-MEC has been able to achieve >85% H₂ yield and >10 m³/m³/day production rate from organic wastes, and some calculations even predicted the H₂ production rate can reach to 100 m³/m³/day after system optimization ([Liu et al., 2010](#)). However, there are still many challenges remain, and they need to be overcome in order for MECs to be applied in large scale systems ([Randolph & Studer, 2013](#)).

3.1. Undesired electron sinks reduce MEC H₂ yield

When H₂ is the target product in MECs, its yield can be significantly reduced over time due to several undesired electron sinks of other metabolisms. Methanogenesis commonly occurs in MECs especially in single-chamber systems without separators. This leads to CH₄ instead of H₂ production after a period of time, which was reported by many studies including the first pilot MEC using winery wastewater ([Cusick et al., 2011](#)). Most studies show that hydrogenotrophic methanogens were responsible for the H₂ re-consumption and CH₄

generation, as acetoclastic methanogens were generally outcompeted by acetate oxidizing exoelectrogens in low acetate environment such as MECs (Lu et al., 2012b). The hydrogenotrophic methanogenesis can be a serious problem because the production of 1 mole of CH₄ consumes 4 moles of H₂ ($4\text{H}_2 + \text{CO}_2 = \text{CH}_4 + 2\text{H}_2\text{O}$), and these microbes are able to scavenge H₂ at nanomolar concentrations. Separators help block H₂ diffusion and methanogen migration, but they just slow down methanogenesis without solving the problem (Lu et al., 2010). Several strategies have been employed to inhibit methanogenesis in MECs including physical, chemical, and biological methods (Table 3). The continued addition of chemical inhibitors may be effective, but it also increases operational costs and results in new potential contamination. The exposure to O₂ has limited effects on methanogen growth, because they are protected by other anaerobic or facultative microorganisms in the biofilm, and high DO may cause damage on anaerobic exoelectrogens. The reduction of hydraulic retention time by increasing flow rate or applied voltage cannot eliminate methanogens from the reactor either. Although the use of low temperature and radiation achieved good performance in limiting CH₄ production at the beginning, they face problems of high operational cost and complex operation. In addition, the activities of methanogens would recover once these operations are stopped (Lu et al., 2012b). Finding out an effective and sustainable way to suppress methanogenesis is a major task ahead for MECs.

Current density is commonly used to represent H₂ production potential, as shown in Fig. 3. However, such correlation is not always accurate due to the presence of competing electron acceptors. If proton is the only electron acceptor in the MECs, the theoretical moles of H₂

produced can be calculated from current using an equation $n = \frac{\int_{t=0}^t Idt}{2F}$ (Logan et al., 2008).

Based on this assumption, the higher current produced the higher H₂ production rate will be observed. However, many studies reported a disconnection between these two parameters, that is, higher current didn't result in higher H₂ production. For example, proteins such as bovine serum albumin or peptone were reported to have very low H₂ yield despite high current, and further investigations revealed that certain intermediate products such as amino acid cysteine, could serve as an electron shuttle and transfer electrons from *Geobacter sulfurreducens* to poorly crystalline Fe(III) oxides and thus generate current (Doong & Schink, 2002; Lu et al., 2010). Other undesired H₂-scavengers include H₂-utilizing exoelectrogens and homoacetogens. They either directly produce current from H₂ consumption or transform H₂ to acetate, which in turn is converted back to current, resulting in an internal electron loop that artificially boosted current but not H₂ yield. To limit these reactions, rapid H₂ harvesting can be effective to minimize H₂ residence time in the liquid. More efficient materials and reactor configurations, as well as new H₂ harvesting methods are needed for this task.

3.2. Materials, reactor configurations, and substrate characteristics for MEC scale-ups

The advancement of MEC reactor configurations and materials for catalysts, electrodes, and separators has greatly increased system performance in the past decade. The current MEC H₂ production rate is in the range of 10 m³/m³/day, which is 2-3 orders of magnitude higher than that obtained in initial MEC prototypes (Fig. 2) (Liu et al., 2005; Rozendal et al., 2006). In the meantime, the cost has also been dramatically reduced via the development of new

catalysts and separators to replace platinum and ion exchange membranes (Jeremiasse et al., 2010). More details on these advancements can be found in recent reviews (Kadier et al., 2015; Kundu et al., 2013) and therefore are not replicated in this study. However, the capital cost of MEC H₂ production is still about ten times higher than advanced abiotic alkaline electrolyzers despite similar electrode materials were tested. This is mainly due to the reason that the H₂ production rates of MECs are still far below those achieved by alkaline water electrolysis (Escapa et al., 2016; Lee et al., 2010; Lu et al., 2015). In order to achieve the cost goals of \$2-4/gge identified by the US DOE, the cost of MEC H₂ production needs to be reduced by another order magnitude. Several key development efforts are needed for MEC to become a viable option for waste biorefinery. First, the over potential loss on the cathode and high cost are still major bottlenecks. The cost of cathode and its catalyst may account for around 47% of the total reactor cost (Kundu et al., 2013). Recent development of non-precious metal or non-metal catalysts showed great promises. These materials include Ni, MoS₂, stainless steel and other transition metal alloys, N-doped activated carbon, carbon nanotube, graphene, and biocathodes that use microorganisms as the catalysts. Many studies showed these new catalysts were able to achieve similar or even higher H₂ rate than Pt-based cathodes, but more studies are needed to investigate their sustained performance in scaled systems and long term operation using real waste streams. Carbon based materials have been proven viable and scalable for MEC applications as the anode, because they are relatively low cost, easy to manufacture, resistant to corrosion, and can have high surface area with scalability. Other anode materials with higher conductivity such as stainless steel and titanium were investigated, but their performance was poor. Different separator materials or

non-separator MECs were also investigated to reduce H₂-resumption and reduce electrode distance and therefore minimize internal resistance loss, and detailed summaries can be found in recent reviews ([Escapa et al., 2016](#)).

When real waste streams are used MECs, the system performance is significantly influenced by the wastewater characteristics. Low conductivity and alkalinity, and variation of organic loading are among major challenges for MEC waste biorefinery applications. Most lab studies use phosphate or other buffer solutions to boost conductivity and buffer capacity, but it is not a sustainable approach for scaled systems. Domestic wastewater has a conductivity around 1 mS/cm, about 10 times lower than buffered solutions, and that's why studies used raw wastewater feedstocks showed lower H₂ production ([Fig. 3](#)). The low buffer capacity in raw wastewater also limit mass transfer and proton availability for H₂ evolution, which lead to decreased performance and pH imbalance. Reactor optimization and operation can help alleviate such problems, such as using compact designs with membrane (separator)-electrode assemblies (MEA) and conduct cathode-anode recirculation. Detailed discussions on reactor system development and scale up can be found in previous reviews ([Kadier et al., 2015](#)). The aforementioned fermentation-MEC integration can also be beneficial for high strength waste treatment, as the solution conductivity is enhanced by the production of organic acids during fermentation, and the alkaline catholyte can be recirculated to buffer the acidity generated during fermentation ([Lu et al., 2009](#)). Recent studies also showed earth-abundant silicate minerals can be used in MECs to enhance solution conductivity and buffer capacity, which greatly facilitated H₂ production and CO₂

sequestration (Lu et al., 2015).

3. 3. Microbial syntrophic interactions and competitions

The utilization of complex organics present in waste streams require the understanding of microbial syntrophic interactions among different functional microorganisms. However, such understanding is currently limited. Because most known exoelectrogens can only extract electrons from simple organic substrates such as volatile fatty acids (VFAs) and alcohols to produce current, they require the cooperation from polymer-degrading bacteria (fermenters) to firstly break down the complex polymers such as cellulose and protein to simple and usable organics (Parameswaran et al., 2009; Ren et al., 2007). Characterization of microbial interactions will provide insights on the processes required to convert waste into H₂ or other biorefinery productions. Furthermore, such understanding may lead to tailored system design and operation for targeted product generation via controlled microbial communities and activities. Fig. 6 shows three main routes of syntrophic interactions in MECs, and they include (A) interactions between the aforementioned fermentative bacteria and exoelectrogens, with the first group converting complex substrates into simpler organics for the second group to generate current; (B) interactions among fermentative bacteria, homoacetogens, and exoelectrogens. H₂ derived from fermentation or electrolysis is used to reduce CO₂ by homoacetogens to acetate ($\text{HCO}_3^- + 2\text{H}_2 + 0.5\text{H}^+ \leftrightarrow 0.5\text{CH}_3\text{COO}^- + 2\text{H}_2\text{O}$), which then can be oxidized by exoelectrogens; and (C) interactions between fermentative bacteria and methanogens. Fermentation products acetate or H₂ can be used by methanogens for CH₄ production (Lu et al., 2012c). In addition, there are also competitions between

exoelectrogens and methanogens as mentioned before, which may occur on both the anode and the biocathode. Previous reviews provided excellent discussions on substrate utilization kinetics of exoelectrogens and microbial-electrode or interspecies electron transfers, but more studies are needed to understand these microbial syntrophic interactions for waste-to-electron conversion (Lee et al., 2010; Lovley & Nevin, 2011). The synergy between the groups for substrate utilization and alleviation on product inhibition also need further investigations. Current studies on microbial communities focus only on the general structures rather than the functions carried out by each microbial groups, yet such functions are especially crucial in MECs due to the complex role played by H_2 both as a product and an electron donor. It would be very valuable to not only reveal what communities are present in the system, but also to understand what roles each group is playing within the consortium using metagenomics library based approach.

3. 4. Effluent quality

MECs as a waste treatment process are able to significantly reduce organic concentration. However, recent studies discussed that microbial electrochemical technology as an anaerobic process has difficulties producing an effluent quality that meets discharge standard (<30 mg/L biochemical oxygen demand and suspended solids), because electrical current production can only be sustained at ~100 mg/L chemical oxygen demand. In this context, membranes can complement such shortcomings by controlling effluent quality and also providing an enriched stream for potential nutrient recovery. In addition to externally connecting an MEC with a membrane filter, Katuri et al. (2014) reported a novel anaerobic electrochemical membrane

bioreactor by using an electrically conductive hollow fiber microfiltration membrane as the MEC cathode (Katuri et al., 2014). Although most H_2 was converted to CH_4 due to methanogenesis (83% CH_4 and < 1% H_2), higher than 95% of the COD (acetate) was removed. The gas bubbles helped alleviate membrane fouling. The net energy consumption of the system was 0.27 kWh/m³, which was smaller than that of 1–2 kWh/m³ needed for traditional aerobic wastewater treatment. To meet the water reuse or discharge standard and make MEC a viable process for waste treatment, more studies are needed to improve effluent quality.

4. Conclusions

The MEC platform holds great potentials for future waste biorefineries. MECs convert biodegradable waste into value-added energy carriers and bioproducts, making the system potentially energy-positive and carbon-negative. By integrating MECs with fermentation, high H_2 yield and rate can be achieved from high strength biomass waste. By utilizing novel materials and reactor configurations, costs can be decreased, and system performance will be improved. Challenges lie in better understanding of the syntrophy and competition among different microbial groups, so methods for promoting syntrophic interactions or minimizing energy and product losses can be developed for system scale up and application.

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Figure captions

Fig.1. Schematic of a single chamber microbial electrolysis cell (MEC).

Fig.2. (A) Number of published journal articles on microbial electrolysis for hydrogen production and (B) reported high performance studies between 2005 and 2015 containing key words of “electrochemically assisted hydrogen production”, “biocatalyzed electrolysis”, “electrohydrogenesis”, “microbial electrolysis”, “**bioelectrochemical system**” and “**microbial electrochemical cell**”. (Source: Scopus on 1/2016; document type: Journal; Language: English; duplicates were removed from searching results)..

Fig.3. Performance of microbial electrolysis cells (MECs) with different working volume and feeding substrates: (A) current density, (B) H₂ production rate, (C) overall H₂ recovery, and (D) energy efficiency relative to the electrical input.

Fig.4. Performance of microbial electrolysis cells (MECs) with different applied voltages and feeding substrates: (A) current density, (B) H₂ production rate, (C) overall H₂ recovery, and (D) energy efficiency relative to the electrical input.

Fig.5. Enhancement of (A, C) H₂ yields and (B, D) production rates from different substrates by integrating fermentation with MEC.

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Table 1. The variety of substrates used in MECs for H₂ production.

Substrate type	Specific source	Representative Studies
Acetate		(Call & Logan, 2008; Cheng & Logan, 2011; Cheng & Logan, 2007; Hu et al., 2008; Jeremiasse et al., 2010; Liang et al., 2011; Lu et al., 2011; Rozendal et al., 2006; Tartakovskya et al., 2009; Yang et al., 2015)
Non-fermentable organics	Butyric acid, Lactic acid, Propionic acid, Valeric acid	(Cheng & Logan, 2007; Yang et al., 2015)
Fermentable organics	Glucose, Galactose, Mannose, Xylose, Arabinose, Maltose, Saccharose, Cellobiose, Starch, Cellulose, Pure glycerol Biodiesel byproduct glycerol	(Catal, 2015; Cheng & Logan, 2007; Lu et al., 2012c; Montpart et al., 2015; Selembo et al., 2009)
Protein	Bovine serum albumin, Peptone	(Lu et al., 2010)
Low-strength wastewater	Domestic wastewater	(Ditzig et al., 2007; Escapa et al., 2015; Gil-Carrera et al., 2013; Heidrich et al., 2013)
High-strength wastewater	Potato processing and dairy manure, Industrial and food processing wastewater, Swine wastewater, Winery wastewater	(Cusick et al., 2011; Kiely et al., 2011; Tenca et al., 2013; Wagner et al., 2009)
Lignocellulosic biorefinery byproducts	Furanic and phenolic wastewater, Aqueous stream of switchgrass pyrolysis, De-oiled refinery wastewater, Hydrolysate of wheat straw pyrohydrolysis	(Lewis et al., 2015; Ren et al., 2013; Thygesen et al., 2011; Zeng et al., 2015)
Fermentation effluent	Cellulose fermentation, Xylose fermentation, Corn cob hydrolysate fermentation, Spent wash fermentation, Corn stalk fermentation, Landfill leachate fermentation, Corn stover lignocellulose fermentation, Cellobiose fermentation, Sugar beet juice fermentation, Dark fermentation, Molasses wastewater fermentation, Crude glycerol fermentation	(Chookaew et al., 2014; Dhar et al., 2015; Lalaurette et al., 2009; Li et al., 2014; Lu et al., 2009; Mahmoud et al., 2014; Modestra et al., 2015; Nam et al., 2014; Wang et al., 2011)
Waste activated sludge (WAS)	Raw WAS, Fermentative treatment WAS	(Liu et al., 2012; Lu et al., 2012a)

Table 2. The metrics of current status and synergy between fermentation and MECs (Data from Fig. 5).

Properties	Fermentation	MEC	When combined
H ₂ yield (%)	14-27	23-71	>85
H ₂ rate (m ³ H ₂ /m ³ reactor/day)	0.2-10	0.1-3.4	>10
Complex biomass degradation	Yes but not complete	No	Yes and complete
Complete organic to H ₂ conversion	No	Yes	Yes
Produce H ₂ at high yield	No	Yes	Yes
Produce H ₂ at high rate	Yes	No	Yes
Produce H ₂ at low temperature	Difficult	Possible	Possible
Produce H ₂ with high purity	No	Possible	Possible
Treat wastewater for Reuse/discharge	No	Possible	Possible
Waste biorefinery application	Long-term	Long-term	Nearer-term

Table 3. Different strategies reported for methanogenesis inhibition in MECs.

Inhibition strategy	Effectivity	Challenges	Reference
Methanogen inhibitors	Effective	Unsustainable	(Chae et al., 2010)
Reactor air exposure	Moderate	Exoelectrogens damage	(Call & Logan, 2008; Hu et al., 2008)
Short hydraulic retention time	Limited	Poor organics degradation	(Clauwaert & Verstraete, 2009)
Low pH	Limited	Exoelectrogens damage	(Chae et al., 2010; Hu et al., 2008)
Temperature shock	Limited	Difficult operation	(Chae et al., 2010)
Carbonate limitation	Limited	None reported	(Clauwaert & Verstraete, 2009)
Low temperature	Effective	Territorial restrictions	(Lu et al., 2011; Lu et al., 2012b)
Ultraviolet irradiation	Effective	High cost	(Hou et al., 2014)
Electrolytic O ₂ production	Moderate	Aerobic biofilm growth and energy intensive	(Tice & Kim, 2014)