Chapter 5
Hydrogen and Methane Generation from Biowaste: Enhancement and Upgrading via Bioelectrochemical Systems

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Abstract Bioelectrochemical systems (BESs) are emerging technologies that are based on catalyzing (bio-)anode and (bio-)cathode reactions from waste biomass by exoelectrogenic microorganisms. Microbial electrolysis cell (MEC), which is one of the BESs' technologies, is typically used to degrade organic wastes or wastewater for bioenergy recovery and biosynthesis. As one of the promising biotechnologies for resource recovery, value-added products have been obtained by MEC- or ME-integrated systems, such as hydrogen, methane, ethanol, etc. The fundamental reactions of (bio-)electron transport through anodic oxidation are well understood and allow us to increase reactor performance and efficiency. More attentions have

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been recently paid to cathode reactions on proton/electron transport and recovery, with or without microbial activities. Biogas upgrading systems have also been promoted in integrated systems, by combining bioelectrochemistry with various anaerobic processes. This chapter will focus on energy gas generation from waste organics involved in bioelectrochemical pathways and give an overview of bottlenecks and challenges related to this technology.

Keywords Bioelectrochemical systems · Microbial electrolysis cell · Hydrogen · Methane · Biowaste

5.1 Principle for Hydrogen and Methane Generation via Bioelectrochemical Systems

With the continuous stimulation of fossil fuel consumption and energy demand growth, the need to combine energy security with the development of a more sustainable energy sector is representing a severe global challenge. Some renewable resources are considered promising to harvest clean energy. Hydrogen is in principle an environmentally acceptable and clean energy vector that, however, is typically produced from non-renewable fossil fuels such as natural gas or water. In theory, bioelectrochemical systems (BESs) can be used to recover hydrogen from any biodegradable organics on the (bio-) cathode, by harnessing the biocatalytic electrolysis (bio-) anode, i.e., microbial electrolysis cell (MEC). Biocatalytic oxidation of organic matter to hydrogen can occur at both the anode and the cathode. In MEC, for instance, biodegradable organics can produce electrons through the biological oxidation at the anode. Electrons flow then through an external circuit (going from the anode to the cathode) and subsequently combine with protons to form hydrogen under a small voltage (0.2–1.2 V), necessary to overcome thermodynamic barriers (Cheng and Logan 2007). Additionally, H_2, being one of the most effective electron shuttles, can be exploited by microorganisms to produce small molecular compounds such as methane (Fig. 5.1). Compared with other H_2-/CH_4-producing technologies, MEC, as bioelectrochemical power-to-gas, which can convert renewable surplus electricity into hydrogen and methane, exhibits higher H_2-/CH_4-producing efficiency and wider diversity of substrate utilization, making it more advantageous, especially for the valorization of low concentration and/or complex organic matter (Logan et al. 2008).

5.1.1 Reactions Based on Extracellular Electron Transport

Based on whether there are microorganisms attached on the electrodes or not, MEC can be divided into four categories: (1) full-biological double-chambered (DC) bioanode/biocathode MEC; (2) full-biological single-chambered

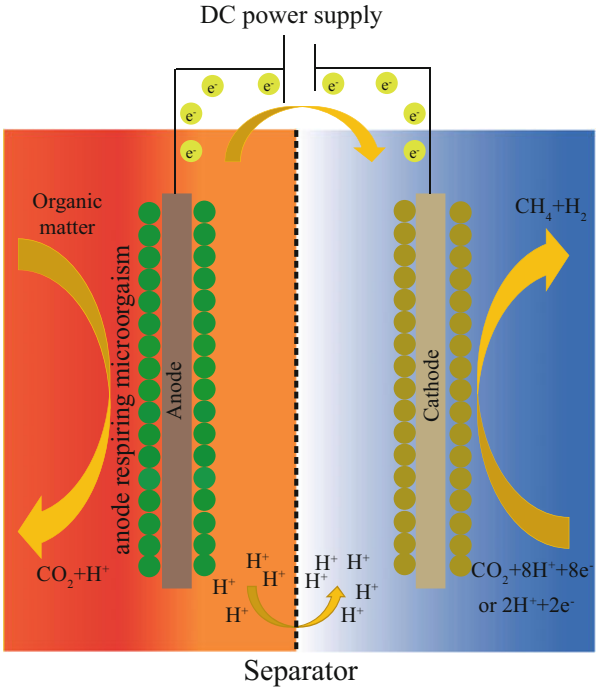

Fig. 5.1 Schematic of H_2 and CH_4 formation in the microbial electrolysis cell, MEC

(SC) bioanode/biocathode MEC; (3) half-biological double-chambered bioanode/cathode MEC; and (4) half-biological double-chambered anode/biocathode MEC.

In general, biodegradable organic matter is oxidized by anodic exoelectrogenic microorganisms and releases protons and carbon dioxide into the anolyte, while electrons transfer to the anode in a typical dual-chamber MEC (Hamelers et al. 2010).

[Anodic reaction]

$$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^-, E^0 = 0.187 \text{ and } E = -0.289 \text{ V vs.SHE} \quad (5.1)$$

Electrons flow typically through an external circuit, driven by an external voltage. Protons and carbon dioxide diffuse across the separator (such as ion exchange membranes, size-selective membranes, stacks of membranes, or the cloth) to the cathode, combining with electrons to be reduced to hydrogen or methane, depending on the cathodic potential, which drives the H_2/CH_4 production, affecting Gibbs' free energy (Jafary et al. 2015).

[Cathodic reaction]

Hydrogen formation:

$$8H^+ + 8e^- \rightarrow 4H_2, E^0 = 0 \text{ and } E = -0.412 \text{ V vs.SHE} \quad (5.2)$$

Methane formation:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O, E^0 = 0.227 \text{ V and } E = -0.248 \text{ V vs.SHE} \quad (5.3)$$

Microbial electrolysis for hydrogen production can be considered as an advantageous combination of conventional hydrogen production pathways. First, compared to dark fermentation, "hydrogen-producing microorganisms" of MEC cannot directly produce hydrogen but are capable of extracellular electron transfer, which play a pivotal role. Besides, MEC can also handle more types of organic substrates without the problem of fermentation end products, which provides a possible way to thoroughly and fully degrade organic matter. In addition, the efficiency of hydrogen production via dark fermentation (such as treated carbohydrate-rich wastewater) is limited (Angenent et al. 2004), due to thermodynamical limitations involving endothermic reactions (Hawkes et al. 2002; Kim et al. 2004; Oh et al. 2003). Second, compared with photosynthetic biological hydrogen production (photosynthesis, photo-fermentation), it is not restricted by light. Notably, photo-fermentation requires enormous reactor surface areas to improve electron transfer and overcome the diffuse nature of solar radiation and thermodynamical barriers, which are clearly not economically viable (Hallenbeck and Benemann 2002). Third, the cathode reaction has the same reaction as the electrolysis of hydrogen in the electrochemical method, except that the electron source of the anode is different. As a consequence, the electrons provided by the relevant microorganisms can save input energy, as demonstrated by the minimum applied potential. The theoretical minimum potential of hydrogen production reaction in MEC is 0.10 V (shown in the following, Eqs. (5.4) and (5.5)), whereas the theoretical minimum potential of hydrogen production in industrial electrolysis is 1.2 V.

For the hydrogen production in MEC, the reaction between proton and electron occurs only in the cathode; the semi-cell reaction of its electrochemical system is briefly described as:

$$\text{Anodic reaction} : NAD^+ + H^+ + 2e^- \rightarrow NADH, E^0 = -0.320 \text{ V vs.SHE} \quad (5.4)$$

$$\text{Cathodic reaction} : 2H^+ + 2e^- \rightarrow H_2, E^0 = -0.420 \text{ V vs.SHE} \quad (5.5)$$

To enable a nonspontaneous reaction, electrons are required to flow from the anode potential (−0.320 V, Eq. (5.4)) to the cathode potential (−0.420 V, Eq. (5.5)), namely, from the high point to the low point. Thereby, MEC needs extra energy to execute the reverse flow of electrons. Theoretically, this process needs to provide a potential of at least 0.1 V to overcome the energy barrier, whereas in practice, an additional voltage supply of minimum 0.13 V is needed to perform the cathode hydrogen production. The reason for the extra voltage required is that electrochemically active microorganisms or electroactive microorganisms (EAMs) consume

some of available energy to sustain their own growth, resulting in microbes to release electrons with a higher potential than the equilibrium potential. On the other hand, the voltage applied to the reactor will also be lost as the consequences of ohmic resistance of electrochemical bias and occurrence of overpotential (Rozendal et al. 2006). Nevertheless, hydrogen generated through water electrolysis actually requires at least a voltage of 1.6 V or more (Crow 1994; Rasten et al. 2003).

5.1.2 Functional Communities Involved in Bioelectrochemical Systems

The term EAMs refers to those microorganisms that can directly or indirectly donate electrons to an electrode (called exoelectrogens), or that accept electrons from the electrode (known as electrotrophs) (Table 5.1). Thus far, the exoelectrogens isolated from natural environment belong primarily to the phylum Proteobacteria and Firmicutes, mostly facultative anaerobic microorganisms, which are capable of gaining energy to sustain growth via anaerobic respiration and fermentation metabolism. Most exoelectrogens are Fe(III)-reducing bacteria (FRB), viz., the oxidized iron is the final electron acceptor of the respiratory chain (Lovley 2006). There are different strategies for transferring electrons to the anode, such as the mediated interspecies electron transfer (MIET) (Cai et al. 2020) and the direct interspecies electron transfer (DIET) (Logan et al. 2019; Lovley 2017). The latter requires direct contact of the outer membrane cytochromes and electron transport proteins associated with outer cell surfaces on electrically conductive materials. MIET includes: (1) self-generated mediators that facilitate the shuttling of electrons from the cells to the anode; (2) electrically conductive pili, capable of long-range electron transfer; and (3) diffusive exchange of electrons between species via soluble electron shuttles such as H_2 (electron-accepting microbes are methanogens) (Table 5.1).

5.2 Hydrogen as the Main Product Using Microbial Electrolysis Cells

Previously, the process of hydrogen generation via electrolyzing dissolved organic matter, using EAMs acting as catalyst, was named “biocatalyzed electrolysis” (Rozendal et al. 2006); subsequently, it was referred to “electrochemically assisted microbial production of hydrogen” (Liu et al. 2005). In earlier research on hydrogen production in MEC, the reactor basically had the similar configuration as the MFC reactor, composed of typical bipolar chamber structure made of glass, where two electrode chambers were isolated by proton exchange membrane (PEM). These initial studies primarily focused on hydrogen generation with acetate as the model compound in MEC. The experimental results showed that the coulomb efficiency

Table 5.1 Electroactive microorganisms (EAMs) in bioelectrochemical systems, BESs

Species	Taxonomy	Information	Reference
Proteus vulgaris	Proteobacteria	Chemically immobilized onto the surface of graphite felt electrodes, supporting continuous current production	Allen and Bennetto (1993)
Shewanella putrefaciens IR-1	α-Proteobacteria	The first observation of a direct electrochemical reaction via Fe(III)-reducing bacteria in BES	Kim et al. (1999)
Clostridium butyricum EG3	Firmicutes	The first reported gram-positive bacterium (Fe(III)-reducing bacterium) in microbial fuel cell (MFC) can ferment glucose to acetate, butyrate, CO_2, and H_2	Park et al. (2001)
Desulfuromonas acetoxidans	δ-Proteobacteria Geobacteraceae (family)	Anaerobic marine microorganism oxidizing acetate with concomitant reduction of elemental sulfur or Fe (III)	Bond et al. (2002)
Geobacter metallireducens	δ-Proteobacteria Geobacteraceae (family)	Oxidize a variety of aromatic contaminants (benzoate, toluene) with the reduction of Fe(III)	Bond et al. (2002)
Geobacter sulfurreducens	δ-Proteobacteria Geobacteraceae (family)	The first report of microbial electricity production solely by cells attached to an electrode without electron transfer mediator (potassium ferricyanide; thionine; neutral red; anthraquinone-2,6-disulfonate, AQDS); oxidize acetate or H_2	Bond and Lovley (2003)
Rhodoferax ferrireducens	β-Proteobacteria	Isolated from anoxic subsurface sediments; dissimilatory Fe(III)-reducing bacterium; electricity generation by direct oxidation of glucose in electron-shuttling mediatorless MFC	Chaudhuri and Lovley (2003)
Aeromonas hydrophila	δ-Proteobacteria	A facultative anaerobic bacterium, Fe (III)-reducing bacterium, can reduce nitrate and sulfate	Pham et al. (2003)
Pseudomonas aeruginosa	γ-Proteobacteria	Excrete redox mediators (pyocyanin)	Rabaey et al. (2004)
Desulfobulbus propionicus	δ-Proteobacteria Desulfobulbaceae (family)	The first example of sulfate-reducing bacteria that can preserve energy to support their growth by electron transfer to insoluble electron acceptors, such as Fe(III) oxide and electrodes, without the addition of exogenous electron-shuttling compounds	Holmes et al. (2004a)
Geopsychrobacter electrodiphilus	δ-Proteobacteria Geobacteraceae (family)	The first organism retrieved from an anode, able to effectively oxidize organic compounds at an electrode,	Holmes et al. (2004b)

(continued)

Table 5.1 (continued)

Species	Taxonomy	Information	Reference
		gram-negative bacterium with abundant c-type cytochromes	
Geothrix fermentans	Acidobacteria	The first report of a Fe(III)-reducing bacterium from outside the *Proteobacteria* family capable of complete oxidation of organic compounds linked to electrode reduction and synthesizing a soluble compound to enhance electrode reduction	Bond and Lovley (2005)
Escherichia coli	γ-Proteobacteria	The first reported that *E. coli*-catalyzed MFC with a carbon-based anode exhibited a higher power density without electron mediators	Zhang et al. (2006)
Enterobacter dissolvens	Proteobacteria	Gram-negative bacillus capable of utilizing phenanthrene and degrading xenobiotic compounds	She et al. (2006)
Hansenula anomala	Ascomycota	Yeast cells with redox enzymes present in their outer membrane (ferricyanide reductase, lactate dehydrogenase) could communicate directly with electrode surface and contribute to current generation in mediator-less MFC	Prasad et al. (2007)
Shewanella oneidensis DSP10	γ-Proteobacteria	Live in anaerobic and aerobic environments; can reduce metals with/without oxygen	Ringeisen et al. (2007)
Shewanella oneidensis MR-1	γ-Proteobacteria	Gram-negative facultative anaerobic bacterium able to exploit a broad range of electron acceptors	Bretschger et al. (2007)
		The reduction of the highly toxic hexavalent chromium Cr(VI) via biocathodes	Xafenias et al. (2013)
Rhodopseudomonas palustris DX-1	α-Proteobacteria	The first reported power production of 2.72 ± 60 W m^2 by a newly isolated strain of a photo(hetero)trophic purple non-sulfur bacterium	Xing et al. (2008)
Ochrobactrum anthropic YZ-1	α-Proteobacteria	The first reported an *Ochrobactrum* species can produce electricity, isolated via using a special U-Tube MFC	Zuo et al. (2008)
Desulfovibrio desulfuricans	δ-Proteobacteria	A sulfate-reducing bacterium was used to simultaneously remove sulfate and generate electricity in MFC	Zhao et al. (2008)
Acidiphilium cryptum	α-Proteobacteria	The first reported used an acidophile as the anode biocatalyst in MFC	Borole et al. (2008)
Klebsiella pneumoniae L17	γ-Proteobacteria	Utilize directly starch and glucose to generate electricity (DIET)	Zhang et al. (2008)

(continued)

Table 5.1 (continued)

Species	Taxonomy	Information	Reference
Thermincola sp. strain JR	Firmicutes	The first gram-positive bacterium isolated from a thermophilic MFC	Wrighton et al. (2008)
Geobacter lovleyi	Proteobacteria	Reductive dechlorination of tetrachlorethene	Strycharz et al. (2008)
Comamonas denitrificans	β-Proteobacteria	An exoelectrogenic denitrifying bacterium isolated by dilution to extinction	Xing et al. (2010)
Acetobacterium (genus)	Firmicutes	Electroacetogenesis; autotrophic microbiome	Marshall et al. (2013)

(CE, total recovery of electrons from acetate) of MEC could reach up to 60%, which was much higher than that of MFC, reported in the same period. More than 90% of electrons and protons generated via bacterial acetate oxidation were converted to hydrogen. When further considering the maximum conversion rate of hydrogen, assuming 78% of CE and 92% of electron recovery efficiency, it can be easily calculated that 1 mol of acetate can produce approximately 3 mol of hydrogen. Moreover, comparing the hydrogen production capacity of different organic acids (acetate and butyrate), the results showed that acetate was more favorable to the metabolism of microbes in MEC, with hydrogen yield from acetate being higher than butyrate. Preliminary results showed that CE of acetate and butyrate could reach 50–65%, whereas that of glucose only reached 14–21%, which implied the feasibility of exploiting MEC to produce hydrogen and simultaneously degrade end products of dark fermentation (Liu et al. 2005).

$$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2 \text{ (thermodynamically unfavorable)} \quad (5.6)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CO_2 + 2C_2H_2O_2 \quad (5.7)$$

$$C_6H_{12}O_6 \rightarrow 2H_2 + 2CO_2 + C_4H_8O_2 \quad (5.8)$$

The stoichiometric yield of hydrogen production from glucose as the substrate would be 12 mol H_2/mol glucose, but this process (Eq. (5.6)) would require a large amount of energy and is unlikely to occur ($\Delta G^{0'}$ =+3.2 kJ mol). This would be translated into extremely low hydrogen yields when hydrogen is produced from glucose, with acetate and butyrate as the only fermentation by-products. Theoretically, 4 mol H_2/mol glucose can be obtained if only acetate is produced, while only 2 mol H_2/mol glucose when butyrate is the exclusive end product. Not surprisingly, usually only 2–3 mol H_2/mol glucose can be produced in actual fermentation process (albeit some thermophilic strains, e.g., *Thermothoga* can also reach yields around 3.5 mol H_2/mol glucose) (Logan 2004). In a combined process with glucose fermentation to (2 moles of) acetate and subsequent conversion of acetate in an

MEC, the overall hydrogen production yield reached 8–9 mol H_2/mol glucose, while the supplied energy requirement (by external voltage) was equivalent to 1.2 mol H_2/mol glucose (Liu et al. 2005).

In principle, MFC and MEC have similar functional microbes, including bacteria capable of extracellular electron transfer and other collaborative bacteria. Consequently, in the early stage of MEC research, the start-up mode was basically to first adapt the inoculum to obtain the corresponding functional flora by virtue of MFC electricity production, and then shifting into the MEC reactor operation (Cheng and Logan 2007; Call and Logan 2008; Hu et al. 2008; Call et al. 2009; Guo et al. 2010). Thus, in order to obtain an anodic syntrophic consortium between fermentative and anode respiring bacteria (ARB), Montpart et al., for example, first utilized the effluent from an already working MFC, composed of ARB (Montpart et al. 2015). The inoculum was fed with acetate and propionate, and subsequently with sludge from culture flasks, containing fermentative bacteria, in order to develop the syntrophic consortium. Once the syntrophic consortium had colonized well in MFC, the biologically enriched anode was transferred into a single-chamber MEC, treating synthetic wastewater (comprising different complex carbon substrates, i.e., glycerol, milk, and starch) to evaluate hydrogen production (Montpart et al. 2015). In the study by Liu et al., the authors unraveled the effects of different MEC start-up modes on hydrogen production and microbial communities (Liu et al. 2010). Interestingly, the results indicated that the start-up conditions with applied voltages (MEC mode) had a strong influence on the performances of MEC reactors, from the perspective of both CE and COD removal efficiency, and presented larger effect on gas composition, especially on the production of hydrogen. The hydrogen production of the reactor, directly started as MEC, was generally higher than that of the one initially operated in the MFC mode. Microbial community analysis results further demonstrated that microbial communities developed in MECs were well separated from those present under start-up conditions, implying that reactor operation affected microbial community composition (Liu et al. 2010). Subsequently, Lee et al. collected the effluent from an acetate fed-batch MEC operated for over 9 months as an inoculation to the upflow single-chamber MEC, reaching a production rate of 4.3 ± 0.06 m^3 m^{-3} d^{-1} of H_2 with 27–49 kg m^{-3} d^{-1} removal rate of COD (Lee and Rittmann 2010).

Various pure substrates have been well investigated in two-chamber MECs for hydrogen production (Cheng and Logan 2007), including glucose, cellulose, and various fermentative products (acetate, butyrate, etc.). Near-stoichiometric yields have been obtained by those MEC tests. However, mixed substrates or complex organic matters are still leading to low conversion yields. In summary, simple or pure substrates, like acetate as model substrate, are employed in MECs for mechanism analysis of electron transfer or electron flow calculation, while mixed or complex substrates are commonly studied for scaling up reactors or practical treatment.

5.2.1 Simple Carbon Sources for Hydrogen Production

Acetate, a by-product of dark fermentation of glucose, was typically used as a model substrate to ferment hydrogen in MEC research.

Rozendal et al. first reported biocatalyzed acetate for electrohydrolysis via EAMs, inoculated from the effluent of an electrochemical cell, which was previously acclimatized with sludge from a full-scale upflow anaerobic sludge blanket (UASB) reactor, treating sulfate-rich papermill wastewater for five months (Rozendal et al. 2006). A relatively large double-chamber MEC reactor with a volume of up to 3.3 L, separated by a cation-selective membrane, was operated under an applied voltage of 0.5 V, achieving a hydrogen production rate of 0.02 $m^3\ m^{-3}\ d^{-1}$. The CE and cathodic electron recovery efficiency reached 92% and 57%, respectively. It is worth mentioned that the previous research pointed out the existence of methanogens at the anode, resulting in hydrogen loss, and speculated about the possible impacts, viz., the loss of partial CE and the decreasing numbers of electrons delivered to the anode by competing for consumption of acetate. Unfortunately, this study did not focus on the phenomenon of the electromethanogenesis and did not further analyze microbial communities. In contrast, the authors assumed that the abovementioned consumption was insignificant compared to the H_2 recovery loss at the cathode, because the H_2 generated at the cathode would diffuse to the anodic chamber and be used as electron donor for biocatalysis.

Chae et al. employed a two-chambered MEC, fed with acetate, to elucidate the effects of applied voltages on the hydrogen production. They found that the hydrogen yields generally increased with applied voltages (from 0.1 to 1.0 V), obtaining a maximum H_2 yield of 2.1 mol/mol acetate. Moreover, the higher voltage implied a higher electron loss at the anode, compared to that of the cathode (Chae et al. 2008). Jeremiasse and colleagues obtained the maximum H_2 production rate using acetate and applying 1.0 V (Jeremiasse et al. 2010). In fact, the applied voltage is crucial for hydrogen formation and also significantly affects the H_2 conversion efficiency. Although hydrogen can theoretically be produced at the cathode by applying a circuit voltage greater than 0.14 V (Rozendal et al. 2006; Liu et al. 2005), in reality, higher voltages are required due to the overpotential. In practice, cathodic hydrogen generation can be considered negligible when applying below 0.30 V (Hu et al. 2008; Chae et al. 2008).

Table 5.2 presents a comprehensive overview of MEC performances obtained in different studies, using simple carbon sources.

5.2.2 Complex Carbon Sources for Hydrogen Production

Hydrogen can be generated via biocatalytic electrolysis (MEC) with the potential to efficiently convert a variety of dissolved organic matter and refractory wastes from wastes or wastewaters. Even substrates that were previously considered to be

Table 5.2 Simple substrates used for hydrogen production in the microbial electrolysis cell, MEC

Simple carbon sources[a]	Reactor configuration[b]	Reactor size (L)[c]	Operation mode	Electrode materials		Applied voltage (V)	Current density ($A\ m^{-3}$)[d]	Hydrogen production rate ($m^3\ m^{-3}\ day^{-1}$)	Cathodic H_2 recovery (%)	Coulomb efficiency (%)	Reference
				Anode	Cathode						
Acetate	DC CEM[e]	3.3	Batch	Disk-shaped graphite felt	Disk-shaped titanium mesh	0.5	$0.47 \pm 0.07\ A\ m^{-2}$	0.02	57 ± 0.1	92 ± 6.3	Rozendal et al. (2006)
Acetate	DC AEM[f]	0.014	Batch	High-temperature NH_3-treated graphite granules	Carbon cloth coated with 0.5 mg cm^{-2} Pt	0.6	99	1.10	NA[g]	NA[g]	Cheng and Logan (2007)
Acetate	DC PEM[h]	0.018	Batch	Carbon felt	Perforated titanium plate	0.1–1.0	$0.04–2.91\ A\ m^{-2}$	0.052 (0.8 V)	~80 (0.8 V)	68.4 (0.8 V)	(Chae et al. (2008)
Acetate	DC AEM[f]	0.2	Continuous	Graphite felt	Ni foam	0.50, 0.53, 0.62, 0.70, 0.80, 0.85, 0.90, 0.95, 1.00	5704 ± 32 (1.00 V)	50 (1.00 V)	NA[g]	NA[g]	Jeremiasse et al. (2010)
Acetate	DC CEM[e]	0.12	Batch	Carbon brush	Carbon cloth (CC) modified with ~5 mg cm^2 CNTs, Pt/C, Fe/Fe_3C@C	0.8	1.36 ± 0.10 (CC) 1.30 ± 0.09 3.50 ± 0.12 2.60 ± 0.07	0.087 ± 0.007 (CC) 0.075 ± 0.006 0.230 ± 0.031 0.181 ± 0.011	67.7 ± 10.6 (CC) 66.6 ± 6.6 81.6 ± 5.0 79.8 ± 8.4	26.9 ± 1.1 (CC), 24.1 ± 3.0 39.8 ± 2.6 43.6 ± 0.8	Xiao et al. (2012)
Acetate	SC CEM[e] AEM[f]	3.3	Batch	Graphite felt	Membrane electrode assembly Supported by Pt coated with 50 g m^{-2} titanium mesh	1.0	2.25 ± 0.05 $2.37 \pm 0.04\ A\ m^{-2}$	0.33 ± 0 0.31 ± 0.01	101.4 ± 0.7 101.3 ± 0.6	22.8 ± 0.2 23.2 ± 1.5	Rozendal et al. (2007)
Acetate	SC	0.028	Batch	NH_3-treated graphite brush	Carbon cloth coated with 0.5 mg cm^{-2} Pt	0.2–0.8 with an interval of 0.1 V	292 (0.8 V)	3.12 (0.8 V)	96 ± 1 (0.8 V)	98 ± 0 (0.8 V)	Call and Logan (2008)
Acetate	SC	0.5	Batch pH=7, pH=5.8	Carbon cloth without wetproofing	Carbon cloth with 30% wetproofing coated with 0.5 mg cm^{-2} Pt	0.3, 0.4, 0.6	9.3 14 $A{\cdot}m^{-2}$	0.53 0.69	82 87	75 73	Hu et al. (2008)
Acetate	SC	0.018	Batch	Carbon cloth	Carbon cloth without wetproofing	0.3, 0.35, 0.4, 0.6	270 200 300 (0.6 V)	2.0 1.5 2.3 (0.6 V)	86 75 85 (0.6 V)	75 73 56 (0.6 V)	Hu et al. (2009)

(continued)

Table 5.2 (continued)

Simple carbon sources[a]	Reactor configuration[b]	Reactor size (L)[c]	Operation mode	Electrode materials		Applied voltage (V)	Current density (A m^{-3})[d]	Hydrogen production rate (m^3 m^{-3} day^{-1})	Cathodic H_2 recovery (%)	Coulomb efficiency (%)	Reference
				Anode	Cathode						
				without wetproofing	coated with NiMo, NiW, or Pt						
Acetate	SC	0.028	Batch	NH_3-treated graphite fiber brush	Stainless steel brush	0.6	188 ± 10	1.7 ± 0.1	84	NA[g]	Call et al. (2009)
Acetate	SC	0.14	Batch	Graphite spheres	Carbon felt	1.06 ± 0.08	51.4 ± 1.6	0.057 ± 0.02	98 ± 2	60 ± 2	Lee et al. (2009)
Acetate	SC without PEM[h] with PEM[h]	0.05	Continuous	Carbon felt	Gas diffusion electrode coated with 0.5 mg cm^{-2} Pt	1.0	4.7 1.8	6.32 1.22	NA[g]	~90 ~44	Tartakovsky et al. (2009)
Acetate	SC	0.032	Batch	NH_3-treated graphite brushes	SS 304 SS 316 SS 420 SS A286 Ni 201 Ni 400 Ni 625 Ni HX Pt	0.6, 0.9	100 ± 4 116 ± 1 122 ± 10 222 ± 4 127 ± 8 116 ± 9 160 ± 22 124 ± 14 129 ± 7 (0.9 V)	0.59 ± 0.01 0.35 ± 0.008 0.58 ± 0.007 1.50 ± 0.04 0.38 ± 0.004 0.41 ± 0.10 0.79 ± 0.27 0.55 ± 0.11 0.6 ± 0.06 (0.9 V)	53 ± 1 2 ± 6 43 ± 2 61 ± 3 27 ± 4 31 ± 5 43 ± 9 40 ± 8 47 ± 2 (0.9 V)	92 93 88 102 96 100 95 95 98 (0.9 V)	Selembo et al. (2009a)
Acetate	SC	0.15	Batch	Graphite granules	Mipor titanium tube coated with platinum	0.2–1.0 with an interval of 0.1 V	170	1.58 (1.0 V)	88	95	Guo et al. (2010)
Acetate	SC	0.125	Continuous HRT 1.6 h HRT 3.1 h HRT 6.5 h	Graphite fiber	Graphite fiber	1.43 ± 0.04 1.47 ± 0.05 1.49 ± 0.03	1630 ± 50 1590 ± 70 1470 ± 60	4.32 ± 0.46 3.70 ± 0.03 2.64 ± 0.10	24 ± 2 21 16	190 ± 7 230 310 ± 3	Lee and Rittmann (2010)
Acetate	SC	0.064	Batch	Graphite felt	Carbon cloth coated with 0.5 mg cm^{-2} Pt	0.5–1.0 with an interval of 0.1 V	621.3 ± 20.6 (0.8 V)	5.56 (0.8 V)	~90 (0.8 V)	NA[g]	Liang et al. (2011)
Acetate	SC	0.5	Continuous	316 L SS fiber felt	Pt-coated titanium mesh tube	Anode potential at −0.2 V vs. Ag/AgCl	10.6 A m^{-2}	6.65	>80	64–88	Feng et al. (2018)

Acetate	SC	0.03	Batch	Heat-treated graphite fiber brush	Carbon cloth with 30 wt.% wetproofed coated with 0.5 mg cm^{-2} Pt	Anode potential of −0.2 V vs. Ag/AgCl	24.6 A m^{-2}	NA[g]	80 ± 1	NA[g]	Cho et al. (2019)
Glucose	SC	0.028	Batch	Graphite brush	Wetproofed carbon cloth coated with Pt	0.5 0.9	115 ± 4 182 ± 31	0.83 ± 0.18 1.87 ± 0.30	51 ± 4 88 ± 5	127 ± 23 105 ± 10	Selembo et al. (2009b)
Glucose	SC	0.026	Batch (4 °C)	Graphite brush	Carbon cloth coated with 0.5 mg cm^{-2} Pt	0.6 0.8	38 ± 5 50 ± 7	0.25 ± 0.03 0.37 ± 0.04	61 ± 4 68 ± 4	82 ± 13 74 ± 8	Lu et al. (2012a)
Glucose	SC	0.026	Batch (25 °C)	Graphite brush	Carbon cloth coated with 0.5 mg cm^{-2} Pt	0.6	113 ± 4	1.01 ± 0.05	82 ± 5	59 ± 6	Lu et al. (2012a)

[a]Sodium acetate is considered as acetate in this table
[b]SC refers to the single-chamber MEC; DC refers to the double-chamber MEC
[c]The reactor size of all double-chamber MEC reactor refers to the effective working volume of each corresponding anode chamber
[d]Current intensity is normalized by reactor chamber volume (A m^{-3}), or by anode electrode projected area (A m^{-2})
[e]CEM, cation exchange membrane
[f]AEM, anion exchange membrane
[g]*NA* not available
[h]PEM, Nafion 117 proton exchange membrane (PEM)

unfitting for producing hydrogen, according to the endothermic conversion reactions, can now be valorized by means of MECs.

There is a limited amount of carbohydrates from waste activated sludge (WAS) suitable for utilization by hydrogen-producing microorganisms; thereby, low H_2 yield is typically harvested from the WAS fermentation. Lu et al. obtained H_2 yields of 15.08 ± 1.41 mg-H_2/g-VSS from alkaline-pretreated WAS, which was 2.66-fold of that with raw WAS (5.67 ± 0.61 mg-H_2/g-VSS) in the two-chamber MEC (TMEC). However, more than 13 times higher H_2 production rate was achieved in the single-chamber MEC (SMEC) with alkaline-pretreated WAS, compared to TMEC (Lu et al. 2012b). Besides carbohydrates, there were other substrates (including proteins and their acidification products, such as volatile fatty acids), supporting hydrogen generation in MECs. In addition, it was further confirmed that electrohydrogenesis can react on both the exo-polymeric compounds and the intracellular ones.

Crop castoffs are considered to be a feasible feedstock for dark fermentation to generate hydrogen, thanks to the simple operation and low-energy requirements (Ghimire et al. 2015); however, this process is always associated to the formation of various by-products, mainly volatile fatty acids such as acetate and butyrate (Pan et al. 2010; Xing et al. 2011). Therefore, the integration of dark fermentation with MEC represents an effective way to convert biomass and main fermentation dead-end products into hydrogen (Marone et al. 2017). In order to further enhance hydrogen yield, Li et al. first investigated the effect of pre-adaptation and acclimatization strategies of the MFC anode biofilm grown on diverse substrates and subsequently transferred to the MEC. A maximum H_2 production rate of 4.52 ± 0.13 m^3 m^{-3} d^{-1} under the highest current density of 480 ± 11 A m^{-3} was achieved in a pre-acclimatized anode fed with butyrate (applying 0.8 V), while the one treated with acetate reached 3.56 ± 0.22 m^3 m^{-3} d^{-1} and 346 ± 11 A m^{-3} (Li et al. 2017). Notably, the H_2 yields and removal efficiency of butyrate were substantially higher than in the case of any other substrates (i.e., corn stalk fermentation, ethanol, propionate, or even acetate) (Li et al. 2017).

Table 5.3 presents a comprehensive overview of complex carbon sources that have been used in MEC studies.

5.2.3 *Hydrogen Loss Evaluation for Microbial Electrolysis Cells*

In practice, hydrogen production is boosted in MECs during the initial operation; however, the production of methane is an inevitable consequence for long-term operation of the mixed flora reactor, in most cases. Undesired H_2 sinks, especially by methanogens, have been a serious issue in MEC operations, although H_2 has a low solubility (i.e., 0.0016 g H_2 can be dissolved into 1 kg water at 293 K). In order to inhibit methanogens' growth, MEC reactors can be put in aerobic conditions for

10 min after each feed cycle, then replenished with fresh medium, and finally flushed with oxygen-free gas to reestablish anaerobic conditions (Selembo et al. 2009b). Except for bioelectrodes exposed to air intermittently (Call and Logan 2008; Call et al. 2009; Lu et al. 2010), there are other strategies adopted to avoid methanogenesis: (1) operation under lower pH (Hu et al. 2008) or lower temperature conditions (Lu et al. 2011), ultraviolet irradiation (Hou et al. 2014a); (2) washout of methanogens by lowering hydraulic retention time (HRT) (Wang et al. 2009); (3) reducing carbonate concentration (Rozendal et al. 2008); and (4) methanogen inhibitor addition (e.g., 2-bromoethanesulfonate) (Chae et al. 2010). Unfortunately, the abovementioned strategies only focus on repressing methanogenesis but overlook other routes of H_2 consumption, including H_2 oxidized by exoelectrogens, or homoacetogenic microorganisms utilizing H_2 and CO_2 to synthesize acetate ($2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$) (Parameswaran et al. 2009). Both paths are commonly defined as hydrogen recycling between the anode and the cathode (Lee et al. 2009), which does not lead to dramatic H_2 loss, but improves the overpotential loss and prolongs duty cycle, eventually resulting in low H_2 recovery (Parameswaran et al. 2011). It seems to be essential to minimize the diffusion of H_2 toward the anode, to rapidly separate H_2 from the MEC reactor (Lee and Rittmann 2010). Instead of conducting top-down inhibition of methanogenesis, Lu et al. employed a novel approach to actively harvest H_2 by extracting it from the reactor, using a gas-permeable hydrophobic membrane and vacuum, leading to 3.32- to 4.29-folds higher H_2 yield than that of the conventional spontaneous release, without CH_4 detection (Lu et al. 2016). But the decreased biofilm growth, accumulation of foulants, and exorbitant cost related to the membrane will be a big challenge in the future.

5.3 Methane as the Main Product in Integrated Anaerobic Systems

Microbial electrolysis system can improve methane production by electrochemical enhancement process (Villano et al. 2011). Traditionally, the planktonic anaerobic bacteria (PAB) and electrochemically active bacteria (EAB) coexist in MEC and disperse in liquid and electrode surface, respectively (Cheng et al. 2009). Methane production partly depends on electron transfer function of PAB and EAB, which are responsible for the carbon dioxide reduction process. In addition, this process can also make use of electrons supplied by current. Hydrogenotrophic methanogens are generally regarded to exploit H_2 as the sole electron donor to reduce CO_2 for methanogenesis. In reality, recent research has clarified that the electron donor source of hydrogenotrophic methanogens is very extensive. There are mainly two ways through which methanogens directly acquire electrons: (1) supply of electrons through electrodes and (2) microorganisms with extracellular electron transport capability (Rotaru et al. 2014a; Fu et al. 2015).

Table 5.3 Complex substrates used for hydrogen production in the microbial electrolysis cell, MEC

Complex carbon sources	Reactor configuration[a]	Reactor size (L)[b]	Operation mode	Electrode material		Applied voltage (V)	Current density (A m^{-3})[c]	Hydrogen production rate (m^3 m^{-3} day^{-1})	Cathodic H_2 recovery (%)	Coulomb efficiency (%)	Reference
				Anode	Cathode						
Domestic wastewater	DC CEM[d]	0.192	Batch	Non-wetproofed carbon paper filled with graphite granules	Carbon paper coated with 0.5 mg cm^{-2} Pt	0.23 0.32 0.41 0.50 0.59	~0.18, ~0.2, ~0.26, ~0.38, ~0.40	0.0125 g-H_2/g-COD (0.50 V)	4.2 3.5 19.0 42.7 37.5	10.4 19.4 25.8 23.2 26.4	Ditzig et al. (2007)
Cellulose	SC	0.028	Batch FEI-Syn, SSI-Syn, SSI-Pr[e]	NH_3-treated graphite fiber brush	Flat carbon cloth coated with Pt (10% Pt/C)	0.5	NA[f]	0.59 ± 0.21 1.11 ± 0.13 1.02	49 ± 16 86 ± 7 89	110 ± 20 73 ± 3 90	Lalaurette et al. (2009)
P-glycerol	SC	0.028	Batch	Graphite brush	Wetproofed carbon cloth coated with Pt	0.5 0.9	116 ± 5, 221 ± 12	0.80 ± 0.008, 2.01 ± 0.41	64 ± 15 79 ± 18	99 ± 10, 104 ± 7	Selembo et al. (2009b)
B-glycerol	SC	0.028	Batch	Graphite brush	Wetproofed carbon cloth coated with Pt	0.3 0.5 0.6 0.8 0.9	15 ± 3 35 ± 8 59 ± 10 87 ± 11 63 ± 14	0 0.14 ± 0.06 0.30 ± 0.01 0.55 ± 0.28 0.41 ± 0.13	1 ± 0 45 ± 15 72 ± 19 52 ± 15 65 ± 14	37 ± 5 84 ± 11 65 ± 8 103 ± 11 91 ± 10	Selembo et al. (2009b)
Effluent of an ethanol-fed dark-fermentation CSTR[g]	SC	0.026	Batch	Carbon fiber brush	Wetproofed carbon cloth (30%) coated with Pt 0.5 mg cm^{-2} Pt (Pt/C: 20 wt.%)	0.2–0.8 with an interval of 0.1 V	135 (0.6 V)	1.41 ± 0.08 (0.6 V)	94 ± 4 (0.6 V)	87 ± 2 (0.6 V)	Lu et al. (2009)
Swine wastewater	SC	0.028	Batch D-S ND-S D-L ND-L[h]	Graphite-fiber brush	Carbon cloth coated with 0.5 mg cm^{-2} Pt	0.5	93 ± 22, 106 ± 6, 92 ± 13, 112 ± 25	0.8 ± 0.2 0.9 ± 0.2 1 ± 0.1 1 ± 0.1	53 ± 6 61 ± 12 58 ± 1 29 ± 2	43 ± 2 29 ± 17 48 ± 9 70 ± 2	Wagner et al. (2009)

Protein	SC	0.014	Batch	Graphite fiber	Carbon cloth coated with 0.5 mg cm^{-2} Pt	0.6, 0.8	132 ± 2 (0.6 V)	0.42 ± 7 (0.6 V)	29 ± 5 (0.6 V)	114 ± 9 (0.6 V)	Lu et al. (2010)
Potato-processing wastewater	SC	0.028	Batch	NH_3-treated graphite fiber brush	Carbon cloth with 30 wt.% wetproof coated with 0.5 mg cm^{-2} Pt	0.9	6.4 A m^{-2}	0.74	NA[f]	80	Cusick et al. (2011)
Winery wastewater	SC	1000	Continuous	Heat-treated graphite fiber brush	Stainless steel 304	0.9	7.4	0.19 ± 0.04	NA[f]	NA[f]	Cusick et al. (2011)
Methanol-rich industrial wastewater	SC	0.028	Batch	Heat-treated graphite fiber brush	Stainless steel 3004, carbon cloth coated with MoS_2, Pt	0.7	1.5 1.2 2.1 A m^{-2}	0.12 ± 0.02 0.08 ± 0.01 0.17 ± 0.03	NA[f]	12 ± 2 7 ± 0.5 10 ± 0.5	Tenca et al. (2013)
Food-processing wastewater	SC	0.028	Batch	Heat-treated graphite fiber brush	Stainless steel 3004, carbon cloth coated with MoS_2, Pt	0.7	1.0 1.0 2.4 A m^{-2}	0.12 ± 0.02 0.5 ± 0.06 0.41 ± 0.02	NA[f]	29 ± 2 26 ± 4 35 ± 2	Tenca et al. (2013)
Glycerol	SC	0.04	Batch	Graphite fiber brush	Graphite fiber cloth coated with 5 mg cm^{-2} Pt	0.8	NA[f]	NA[f]	NA[f]	NA[f]	Montpart et al. (2015)
Starch	SC	0.04	Batch	Graphite fiber brush	Graphite fiber cloth coated with 5 mg cm^{-2} Pt	0.8	NA[f]	NA[f]	NA[f]	NA[f]	Montpart et al. (2015)
Milk	SC	0.04	Batch	Graphite fiber brush	Graphite fiber cloth coated with 5 mg cm^{-2} Pt	0.8	NA[f]	NA[f]	NA[f]	NA[f]	Montpart et al. (2015)

(continued)

Table 5.3 (continued)

Complex carbon sources	Reactor configuration[a]	Reactor size (L)[b]	Operation mode	Electrode material		Applied voltage (V)	Current density (A m^{-3})[c]	Hydrogen production rate (m^3 m^{-3} day^{-1})	Cathodic H_2 recovery (%)	Coulomb efficiency (%)	Reference
				Anode	Cathode						
Corn stalk fermentation effluent	SC	0.064	Batch	Graphite felt	Carbon cloth coated with 0.5 mg cm^{-2} Pt (Pt/C: 20 wt%)	0.5 0.8	480 ± 11 (0.8 V)	2.41 ± 0.12 4.52 ± 0.13	NA[f]	72 ± 3 76 ± 2	Li et al. (2017)

[a]SC refers to the single-chamber MEC; DC refers to the double-chamber MEC

[b]The reactor size of all double-chamber MEC reactor refers to the effective working volume of each corresponding single anode/cathode chamber, not the total effective working volume, which is twice of that

[c]Current intensity is normalized by reactor chamber volume (A m^{-3}), or by anode electrode projected area (A m^{-2})

[d]*CEM* cation exchange membrane

[e]*FEI*, fermentation effluent inoculum, that is the MECs with brushes acclimatized to individual substrates or a synthetic effluent; *SSI*, the mixtures of solutions (in equal volumes) obtained from the MFCs acclimatized to individual substrates (that is the MECs with brushes acclimatized to a synthetic effluent); and a single-substrate inoculum, *SSI-Pr*, the predicted results for the synthetic effluent, which were calculated on the basis of the solution composition and the performance in MECs with individual substrates (Lalaurette et al. 2009)

[f]*NA* not available

[g]*CSTR* continuous stirred-tank reactor

[h]*D-S*, diluted wastewater with short cycle; *ND-S*, non-diluted wastewater with short cycle; *D-L*, diluted wastewater with long cycle; *ND-L*, non-diluted wastewater with long cycle. Short cycle, the batch ended immediately after max gas production; long cycle, batch ended when gas production finished

According to the type of substrate utilization, the potential mechanisms of methane formation in a bioelectrochemical system can be divided into two categories: one is through electron transfer, i.e., by means of DIET from the (bio-)cathode, and the other one is through interspecies hydrogen transfer among hydrogen-producing and hydrogen-consuming methanogens. Furthermore, in some cases, acetate and formic acid are formed by means of DIET from electrodes or via acetate- and formate-producing microorganisms (along with H_2 production); hence, acetate and formic acid can be further decarboxylated by acetotrophic methanogens to produce CH_4. Possible paths of methane formation on the (bio-)cathode are shown in Fig. 5.2.

5.3.1 *Microbial Extracellular Electron Transfer Driving Methane Production*

Carbon dioxide, methyl compounds, or acetate can be converted into methane in the microbial methanogenesis process. The fundamental pathways are shown in Fig. 5.3. There are two principal ways of obtaining electron donors for acetoclastic or hydrogenotrophic methanogens to generate methane: one is to directly harvest electrons through electrodes and the other is to utilize microorganisms capable of extracellular electron transport to capture electron donors.

Methanosaeta is a typical acetoclastic methanogen that exclusively uses acetate for methanogenesis. Morita et al. found that *Geobacter* was the dominant bacteria in microbial aggregates in cultured anaerobic digestion (AD) reactors, whereas *Methanosaeta* is the most abundant methanogen, indicating for the first time a possible DIET process with methanogenic wastewater aggregates. Microbial aggregates possess metallic-like conductance similar to the conductive pili of *Geobacter sulfurreducens* (Morita et al. 2011). Among microbial aggregates formed by the combination of *Geobacter metallireducens* and *Methanosaeta harundinacea*, *the former* can provide electrons to *the latter*. DIET between *Geobacter* and *Methanosaeta* can be used for methane formation (Rotaru et al. 2014b), which changed the viewpoint that the archaea *Methanosaeta* exclusively uses acetate to produce methane. In addition, metatranscriptomic analysis further revealed that *Methanosaeta* also has the capacity to reduce carbon dioxide for methane production in AD reactors. The relationship between *Geobacter* and *Methanosaeta* is similar to that between fermentation bacteria and syntrophic methanogens, which is based on electron transfer. Kaur et al. found that *Geobacter* exhibited a clear overwhelming competition for acetate utilization, compared to *Methanosaeta* in the open circuit (Kaur et al. 2014). Further, Jung et al. demonstrated positive correlation between external resistance and methanogenesis, also showing that the substrate competition among exoelectrogens and methanogens might be influenced by the same external resistance, thus suggesting that the anode potential can regulate the competition

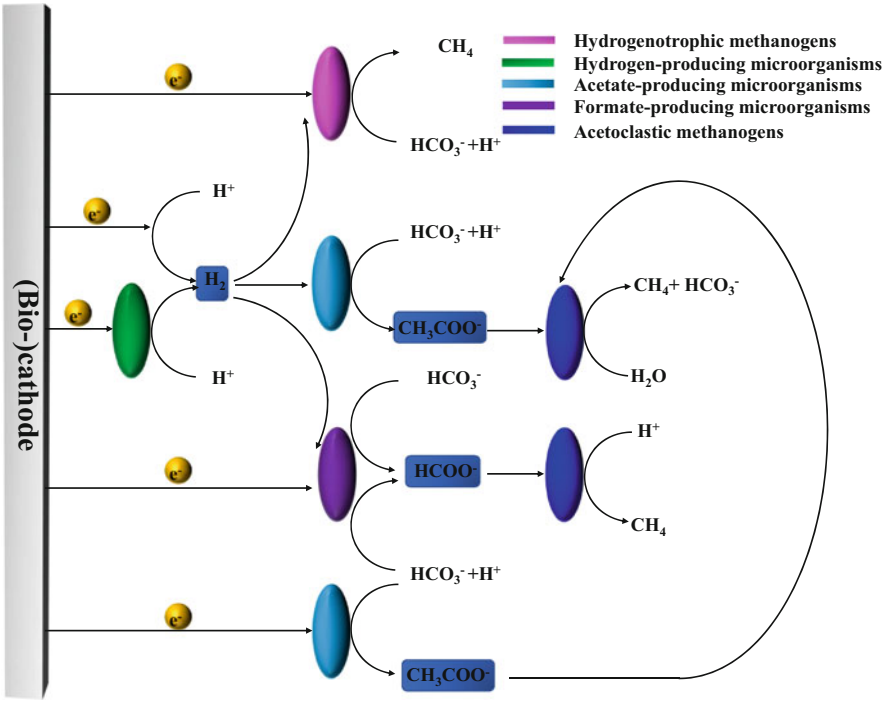

Fig. 5.2 Possible pathways for methane generation in the microbial electrolysis cell, MEC

between extracellular electron transfer bacteria and methanogens (Jung and Regan 2011).

Methanosarcina belongs to the facultative acetoclastic methanogen, which can utilize a wide variety of substrates, including acetate, methanol, methylamine, and hydrogen. Rotaru et al. found the evidence that *Methanosarcina barkeri* participated in DIET with *Geobacter metallireducens*. The study showed that the exploitation of activated carbon particles can replace pili for long-range electron transport, which implied that conductive materials can act as a substitute carrier for pili to perform electron transfer between species (Rotaru et al. 2014a). The close contact is necessary for the implementation of DIET, which may be attributed to the conductivity of pili, whose conductivity is ~5 mS cm^{-1} (Malvankar et al. 2011). The applied voltage, driving electrons through the external circuit and stimulating methane formation, is comparable to the transmission method through the pili. Nevertheless, on the contrary, the external circuit is not limited by the protein structure and the transmission scale, which can attain long-range electron transfer and display excellent conductivity.

Hydrogen and formic acid at the cathode can also become electron transfer mediators. This is different from DIET achieved by the conductive mediator, and the (bio-)cathode methanation process is in a way more controllable and expandable. On the other hand, the microbial electrosynthesis process based on carbon dioxide

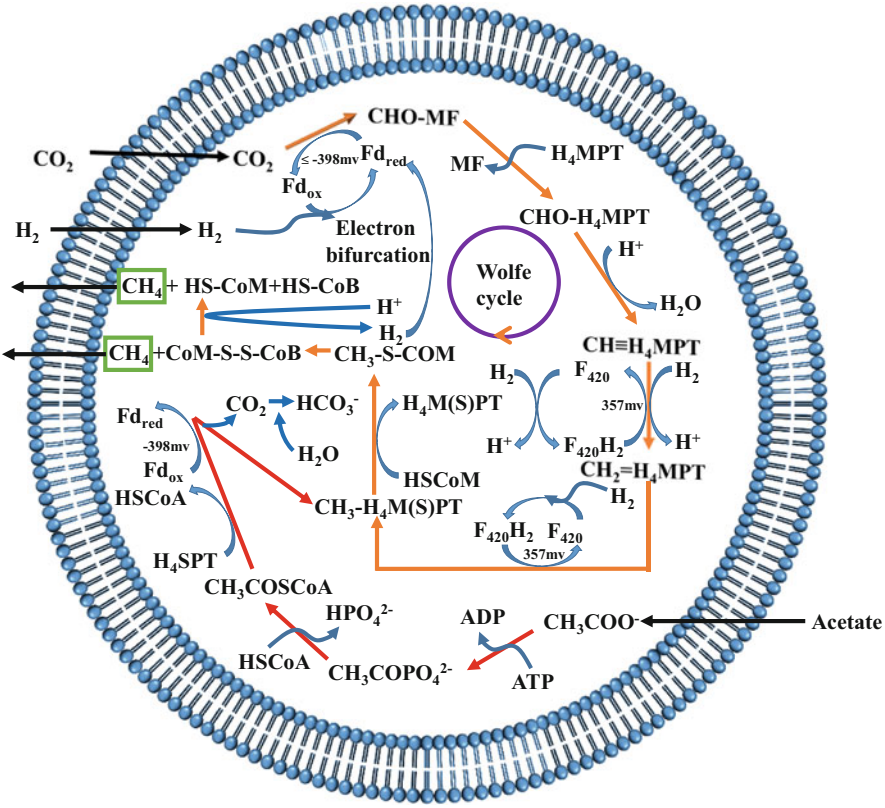

Fig. 5.3 Metabolic pathways of methanogens

reduction at the cathode can also promote the synthesis of chemical substances such as acetate and formic acid (Lee et al. 2017). Altogether, the (bio-)cathode methanogenesis process through DIET prevailingly comprises: (1) hydrogen produced by electrochemical processes that can diffuse into microorganisms to maintain microbial metabolism; (2) hydrogen produced by redox proteins (such as hydrogenase) then used as electron transport mediators; (3) since electrons can pass through the redox transmembrane protein, such as cytochrome c, they can be transferred from the electrodes into the microbes (Kumar et al. 2017). From a macro perspective, we can conclude that: (1) hydrogen evolution reaction (HER) occurs directly at the cathode (or from microorganisms on the electrode surface, $E^0 = -0.41$ V) and then hydrogen is absorbed by hydrogenotrophic methanogens and combined with carbonate to form methane (Wang et al. 2009); (2) certain methanogens receive electrons directly from the cathode and combine it with carbonate to generate methane ($E^0 = -0.24$ V) (Cheng et al. 2009; Van Eerten-Jansen et al. 2012); (3) homo-acetogens attached to the cathode surface receive electrons from the

electrode and synthesize acetate utilizing carbonate (Nevin et al. 2011), and acetotrophic methanogens utilize acetate to generate methane ($E^0 = -0.28$ V).

5.3.2 *Electricity-Stimulated Anaerobic Methanogenesis Process Using Different Substrates*

Conventional methanogenesis primarily depends on the hydrogen or formic acid for interspecies electron transfer in methanogenic environments (Stams and Plugge 2009). It is energetically difficult to complete proton reduction due to negative redox potential of NADH/$FADH_2$ (NAD^+/NADH, $E^0 = -0.32$ V, FADH/$FADH_2$, $E^0 = -0.22$ V), when hydrogen is used as the electron transport carrier. Therefore, anaerobic microorganisms usually use ferredoxin Fd (Fd_{ox}/Fd_{red}, $E^0 = -0.398$ V or lower), as a common redox mediator for catalyzing hydrogen evolution reaction (Stams and Plugge 2009). However, some methanogens do not possess cytochromes; thus, Fd and coenzyme F_{420} ($F_{420}/F_{420}H_2$, $E^0 = 0.357$ V) can help these methanogens, as the most vital hydrogen scavengers, without cytochrome to oxidize hydrogen at extremely low concentrations. Hydrogen is the common product at the cathode of BESs and is also the electron donor for hydrogenotrophic methanogens, as a bridge for converting biohydrogen to biomethane in MEC. Furthermore, hydrogenotrophic methanogens can be highly enriched at the cathode (Lovley 2017; Siegert et al. 2015), and there is also DIET that does not require hydrogen for catalysis (Cheng et al. 2009). Obviously, *Methanococcus maripaludis* with knocked out hydrogenases was able to directly obtain electrons from the cathode to reduce carbon dioxide into methane (Lohner et al. 2014).

In addition to the diffusion of hydrogen, formic acid can also be used as an electron intermediary to achieve interspecies electron transfer. The discovery of formic acid transfer pathway was due to the fact that the sole hydrogen transfer rate could not match the methane production rate from butyrate degradation in the bioreactor (Thiele and Zeikus 1988). However, only part of the methanogens can utilize formic acid, even though the transfer diffusion rate of formic acid is 100-fold that of hydrogen. Hence, formic acid also becomes an electron loss during the methanogenesis process. Moreover, it is extremely difficult to evaluate the contribution of hydrogen and formic acid to methanogenesis; on the other hand, the electron transfer process in traditional AD relies on two pathways, resulting in restricted possibilities for methane yield enhancement.

MECs, as emerging technologies for anaerobic wastewaters/wastes treatment and energy recovery, can be regarded as a practical integrative step to address some obstacles of AD, such as poor operational stability, low biogas yields, and qualities (Wang et al. 2020a). Importantly, the integrated electricity-stimulated anaerobic system can treat multiple wastes, regulate the establishment of microbial community structures and electron transfer paths, and dramatically improve energy efficiency and overall systems stability. Bo et al., for instance, employed waste activated sludge

as substrate in a MEC reactor (at an applied voltage of 1.0 V) for methane generation and obtained 2.3 times higher rates than the conventional AD (Bo et al. 2014). Furthermore, various kinds of biowastes can be employed in such microbial electrolysis integrated anaerobic systems for methane production, including black wastewater (Zamalloa et al. 2013), digested pig slurry (Cerrillo et al. 2016), distillery wastewater (Feng et al. 2017), food waste leachate (Lee et al. 2017), beer wastewater (Sangeetha et al. 2017), food waste (Zhi et al. 2019), etc. Table 5.4 presents a comprehensive overview of different substrates that have been tested so far.

5.4 The Energy Efficiency Calculation Formulas Involved in Microbial Electrolysis Cells

The performances of MEC reactors are typically evaluated using various parameters. In this section, we will present the main calculations, which are primarily based on the products associated to hydrogen and methane metabolism.

5.4.1 Essential Parameters Calculation

H_2 or CH_4 production rate (Y_{H_2}, Y_{CH_4}):

The daily volumetric H_2 or CH_4 production rates are obtained by dividing the produced volumetric H_2 or CH_4 yield by each cycle and normalize it with the effective working liquid volume of the reactor per day.

$$Y_{H_2} = \frac{A_{V_{H2}}}{t \cdot V_{\text{liquid}}} \tag{5.9}$$

$$Y_{CH_4} = \frac{A_{V_{CH4}}}{t \cdot V_{\text{liquid}}} \tag{5.10}$$

where Y_{H_2} and Y_{CH_4} are the H_2 or CH_4 production rate ($m^3\ m^{-3}$ reactor d^{-1}); $A_{V_{H_2}}$ and $A_{V_{CH_4}}$ are the average H_2 or CH_4 production for each batch (mL/batch); t represents the residence time of each batch (d, day); and V_{liquid} is the effective working volume of the reactor.

Coulomb Efficiency (CE)

Coulomb efficiency is used to measure the electron recovery efficiency of the microbial anode (Wang et al. 2020b). CE can be used as the metrics for evaluating the performance of the electrodes in BESs, combined with overpotential, which shows the energy loss at the electrodes (Hamelers et al. 2010). Coulombic efficiency can be an indicator to differentiate the involvement of anodic oxidation and acetoclastic methanogenesis in the removal of acetate. Calculations for the

Coulombic efficiency (CE) and COD removal efficiency (CRE) can be found in previous studies (Yang et al. 2019).

$$\mathrm{CE} = \frac{Q}{Q_T} \times 100\% \tag{5.11}$$

$$Q = It = \int i\mathrm{d}t \tag{5.12}$$

$$Q_T = \frac{b \cdot F \cdot m}{M} = \frac{b \cdot F \cdot (\mathrm{COD_{in}} - \mathrm{COD_{out}}) \cdot V_{\mathrm{liquid}}}{M_{\mathrm{O_2}}} \tag{5.13}$$

where Q is the actual amount of organic matter via anode microbial degradation; Q_T is the theoretical calculated amount of substrate oxidation; I is the current (A or C s^{-1}), which is calculated by Ohm's law ($I = V_{\mathrm{voltage}}/R_{\mathrm{ex}}$) from the voltage ($V_{\mathrm{voltage}}$) drop measured across the resistor (R_{ex}); t is the time (s); b is the total number of electrons transferred by oxidation (O_2) of 1 mol substrate, $b = 4$ (acetate); $F = 96{,}485$ C mol^{-1} is the Faraday constant; $\mathrm{COD_{inf}}$ is the initial chemical oxygen demand of substrate, before the reaction (mg L^{-1}); $\mathrm{COD_{eff}}$ is the final chemical oxygen demand of substrate after the reaction (mg L^{-1}); M is the relative molecular mass of the substrate (g mol^{-1}); $M_{\mathrm{O_2}} = 32\ \mathrm{g \cdot mol^{-1}}$ is the relative molecular mass of O_2.

Current Density (CD)

There are two approaches to express current density: the first is based on the projected electrode (anode or cathode) area (I_A, A m^{-2}) and the other is based on the total reactor effective working volume (I_V, A m^{-3}) (Wang et al. 2020c).

Electrode overpotential (EO), taking hydrogen generation as an example:
Anode and cathode overpotential are calculated as ($E_{\mathrm{eq,\ anode}}$, $E_{\mathrm{eq,\ cathode}}$):

$$\mathrm{EO} = E_{\mathrm{meas}} - E_{\mathrm{eq}} \tag{5.14}$$

where E_{meas} represents the measured anode/cathode potential (V vs. NHE), while E_{eq} is the equilibrium/theoretical anode/cathode potential (V vs. NHE), using the Nernst equation (based on the acetate).

$$\mathrm{CH_3COO^-} + 4\mathrm{H_2O} \rightarrow 2\mathrm{HCO_3^-} + 9\mathrm{H^+} + 8\mathrm{e^-}$$

$$E_{\mathrm{eq,anode}} = E^{0'}_{\mathrm{anode}} - \frac{\mathrm{RT}}{n_{\mathrm{substrate}}F} \ln\left(\frac{[\mathrm{CH_3COO^-}]}{[\mathrm{HCO_3^-}]^2[\mathrm{H^+}]^9}\right) \tag{5.15}$$

$$2\mathrm{H^+} + 2\mathrm{e^-} \rightarrow \mathrm{H_2}$$

Table 5.4 Different substrates used for methane production in the microbial electrolysis integrated anaerobic systems

Substrates	Reactor configuration[a]	Reactor size (L)	Operation mode	Retention time (day)	Electrode material		Applied voltage (V)	CH_4 production rate increase[b]	COD removal efficiency increase[b]	Reference
					Anode	Cathode				
Simulated black wastewater	DC without membrane	AD: 20 MEC: 24.2	Batch	AD: 20, 40 d; MEC: 3.5 d	Stainless steel mesh		2.0 ± 0.1	5	0	Zamalloa et al. (2013)
Anode: NaCl cathode: CO_2	DC CEM[c]	0.4	Batch	0.25	Carbon stick		Cathode potential from −0.6 to −1 V vs. Ag/AgCl	2.30 ± 0.34 mL (−0.9 V)	NA[e]	Zhen et al. (2015)
AD: pig slurry; anode: filtered digested pig slurry from AD; cathode: NaCl	DC CEM[c]	AD: 4, MEC: 0.5	Continuous	10 8 6.7 5 (AD)	Carbon felt	Stainless steel mesh 304	Anode potential at 0 V vs. SHE	1.35	1.65	Cerrillo et al. (2016)
Distillery wastewater	DC (UASB with SEA[d])	5.5	Continuous influent pH: 3.6 5.6 7.0 7.5	NA[e]	Fabricated with graphite fiber fabric, nickel chloride, multiwall carbon nanotube, and exfoliated graphite		0.5	1.719 1.604 1.783 1.7	1.062 1.123 1.115 1.099	Feng et al. (2017)
AD: pig slurry; anode:	DC CEM[c]	AD: 4, MEC: 0.5	Continuous	AD: 10 day, anode:	Carbon felt	Granular graphite	Cathode potential at −0.8 V vs. SHE	1.21	1.95	Cerrillo et al. (2018)

(continued)

Table 5.4 (continued)

Substrates	Reactor configuration[a]	Reactor size (L)	Operation mode	Retention time (day)	Electrode material		Applied voltage (V)	CH_4 production rate increase[b]	COD removal efficiency increase[b]	Reference
					Anode	Cathode				
filtered digested pig slurry from AD; cathode: $NaHCO_3$				32.4 h, cathode: 14.1 h						
Anaerobic activated sludge	SC	0.15	Batch	NA[e]	Ti/Ru alloy mesh plates		1.4 1.8	11.41 13.58	NA[e]	Guo et al. (2013)
Synthetic wastewater (sucrose)	SC (UASB)	2	Continuous	1	Carbon felt		0.8	3.9	1.31	Zhang et al. (2013)
Sodium acetate	SC (UASB)	10	Batch	0.25	Graphite rod		−0.4 −0.35 −0.3 −0.25 V (anode potential vs. Ag/AgCl)	1.39 1.57 1.67 1.93	1.35 1.57 1.72 1.97	Zhao et al. (2014)
Waste activated sludge	SC	0.18	Batch	0.5	Carbon felt	Stainless steel	0.4 1.0	2.3 (1.0 V)	3 (1.0 V)	Bo et al. (2014)
Waste sludge	SC	2	Batch	22 (digestion)	Fe tube	Graphite pillar	0.3 0.6	1.22 (0.3 V)	1.11 (0.3 V)	Feng et al. (2015)
Waste activated sludge	SC	0.5	Batch	2	Graphite brush	Carbon cloth coated with	0.8	3	NA[e]	Liu et al. (2016a)

						0.5 mg/cm^2 Pt				
Sodium acetate	SC	0.02	Batch	14	Granular activated carbon		0.55	5.3-6.6	5.6	Liu et al. (2016b)
Glucose	SC	0.27	Batch	6	Carbon fiber brush		0.5 0.7 1.0 1.5	1.15 1.24 1.27 1.16	1.19 1.21 1.25 1.40	Choi et al. (2017)
Food waste leachate	SC	15	Batch	20	Graphite mesh coated with Ni		0.3	1.7	NA[e]	Lee et al. (2017)
Artificial beer wastewater	SC	NA[e]	Continuous	0.5 0.75 1 1.5	Carbon brush	Nickel mesh	0.80 ± 0.01	1.54 2.10 3.04 based on the lowest MEC-AD	1.05, 1.16, 1.21 based on the lowest MEC-AD	Sangeetha et al. (2017)
Glucose and waste sludge	SC	0.8	Batch	24 h	Reticulated vitreous carbon		0.3 0.6	Glucose: 1.09, 1.10 Sludge: 1.131, 1.134	Glucose: 1.10 (0.3 and 0.6 V)	Gajaraj et al. (2017)
Food waste	SC	20	Batch (SBR[f])	20 d	Graphite carbon mesh coated with Ni		0.3	1.03	1.02	Park et al. (2018)
Raw sludge from the aeration tank of WWTP	SC	0.15	Batch	NA[e]	Ti/Ru alloy mesh		0.6, 0.8, 1.3, 1.8, 2.3	1.20–1.79 (0.6–1.8 V)	1.03–1.28 (0.6–1.8 V)	Xiao et al. (2018)
Food waste and sewage sludge	SC	0.18	Continuous	15 (SRT[g])	Carbon brush	Ti/RuO_2 graphite felt	0.2, 0.4, 0.8, 1.2	2.8 (0.4 V)	NA[e]	Zhi et al. (2019)

(continued)

Table 5.4 (continued)

Substrates	Reactor configuration[a]	Reactor size (L)	Operation mode	Retention time (day)	Electrode material		Applied voltage (V)	CH_4 production rate increase[b]	COD removal efficiency increase[b]	Reference
					Anode	Cathode				

[a]SC refers to single-chamber MEC; DC refers to double-chamber MEC
[b]The increased folds of CH_4 yield or production rate and COD removal efficiency (%), compared to the control
[c]*CEM*, cation exchange membrane
[d]*SEA*, the separator and electrode assembly that was stacked with the anode
[e]*NA*, not available
[f]*SBR*, sequencing batch reactors
[g]*SRT*, solids retention time

$$E_{\text{eq, cathode}} = E^{0'}_{\text{cathode}} - \frac{\text{RT}}{n_{\text{H}_2}F} \ln\left(\frac{P_{\text{H2}}}{[\text{H}^+]^2}\right) \tag{5.16}$$

where $E^{0'}_{\text{anode}} = 0.187$ V is the equilibrium anode potential at standard conditions; $T = 298$ K is the absolute temperature (10^5 Pa) (Logan et al. 2006); $n_{\text{substrate}} = 8$ is the number of electrons needed to generate H_2 by oxidizing one mol acetate (Table 5.5); $R = 8.314$ J K^{-1} mol^{-1} is the ideal gas law constant, under the standard biological condition, $E_{\text{eq, anode}} = -0.279$ V (Logan et al. 2008). $E^{0'}_{\text{cathode}}$ is the equilibrium cathode potential at standard conditions, assuming $[\text{H}^+] = 1$ mol L^{-1}, then $E^{0'}_{\text{cathode}} = 0$ V; $n_{\text{H}_2} = 2$ is the amount of electrons needed to generate 1 mol H_2 for hydrogen evolution reaction, under the standard biological condition, $E_{\text{eq, cathode}} = -0.414$ V (Logan et al. 2008). When at unit partial H_2 pressure and 30 °C (303 K), the cathode overpotential reduces to (Jeremiasse et al. 2010):

$$E_{\text{eq, cathode}} = -0.060\ \text{pH} \tag{5.17}$$

5.4.2 The Contribution for Production of Hydrogen and Methane in MECs

The source of hydrogen and methane is evaluated by comparing the volume generated by the current or detected by gas chromatograph, that is, the different contributions of H_2 or CH_4 production coming from the current or the substrate (acetate).

The theoretical production of hydrogen generated based on the measured current and substrate consumption ($T_{V_{\text{H}_2-\text{current}}}$, $T_{V_{\text{H}_2-\text{acetate}}}$) is given by:

$$T_{V_{\text{H}_2-\text{current}}} = \frac{\int It}{n_{\text{H}_2}\text{F}} V_m \tag{5.18}$$

$$T_{V_{\text{H}_2-\text{acetate}}} = \frac{b_{\text{O}_2}\Delta\text{COD}V_{\text{liquid}}}{2\text{M}_{\text{O}_2}} V_m \text{ (based on the mearsured COD conceration)} \tag{5.19}$$

$$T_{V_{\text{H}_2-\text{acetate}}} = \frac{b_{\text{H}_2}V_{\text{liquid}}\Delta C_{\text{acetate}}}{M_{\text{acetate}}} V_m \text{ (based on the mearsured acetate conceration)} \tag{5.20}$$

The theoretical production of methane generated based on the current and substrate consumption ($T_{V_{\text{CH}_4-\text{current}}}$, $T_{V_{\text{CH}_4-\text{acetate}}}$) is given by:

Table 5.5 The number of moles of electrons per mole of common substrate (n), based on the half-cell reactions

Substrate	$M_{substrate}$[a]	Half-cell reaction	$n_{substrate}$[b]
Acetate	60.05	$C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^-$	8
Formate	46.03	$CH_2O_2 \rightarrow CO_2 + 2H^+ + 2e^-$	2
Propionate	74	$C_3H_6O_2 + 4H_2O \rightarrow 3CO_2 + 14H^+ + 14e^-$	14
Lactate	90.08	$C_3H_6O_3 + 3H_2O \rightarrow 3CO_2 + 12H^+ + 12e^-$	12
1,3-Propanediol	76.09	$C_3H_8O_2 + 4H_2O \rightarrow 3CO_2 + 16H^+ + 16e^-$	16
Glycerol	92.09	$C_3H_8O_3 + 3H_2O \rightarrow 3CO_2 + 14H^+ + 14e^-$	14
Glucose	180.16	$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$	24

[a]$M_{substrate}$, the relative molecular mass of the substrate
[b]$n_{substrate}$, the number of moles of electrons per mole of common substrate consumed

$$T_{V_{CH_4-current}} = \frac{\int I\mathrm{d}t}{n_{CH_4}\mathrm{F}} V_m \tag{5.21}$$

$$T_{V_{CH_4-acetate}} = \frac{\Delta C_{acetate} V_{liquid}}{M_{acetate}} V_m \text{ (based on the mearsured acetate concentration)} \tag{5.22}$$

where $T_{V_{H_2-current}}$ and $T_{V_{CH_4-current}}$ represent the theoretical production rate of H_2 or CH_4 generated by the current for every batch, after being normalized to the effective working liquid volume of the reactor, per day ($m^3\ m^{-3}$ reactor d^{-1}); I is the current (A or C s^{-1}), calculated by Ohm's law ($I = V_{voltage}/R_{ex}$) from the voltage ($V_{voltage}$) drop, measured across the resistor (R_{ex}); t is the time (s); $\int I\mathrm{d}t$ is the coulombs produced by the current, (C); $n_{H_2} = 2$ for H_2 and $n_{CH_4} = 8$ for CH_4 are the number of electrons needed to generate 1 mole H_2 ($2H^+ + 2e^- \rightarrow H_2$) or CH_4 ($CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$); F = 96,485 C mol^{-1} is the Faraday constant; b_{O_2} is a conversion factor based on the stoichiometric relation between electrons in COD and H_2 gas, equaling to 1 mol H_2 per 16 g O_2; $b_{H_2} = 4$ is a conversion factor based on the stoichiometric conversion of the amount of one mol acetate consumed to generate the amount of mol equaling to 4 mol H_2 per 1 mol acetate ($CH_3COOH + 4H_2O \rightarrow 2CO_2 + 4H_2$); $V_m = 22.4$ L mol^{-1} is the gas constant. $T_{V_{H_2-acetate}}$ and $T_{V_{CH_4-acetate}}$ represent the theoretical volume of H_2 or CH_4 generated by the substrate (acetate) for every fed-batch, based on the acetate converted to methane ($CH_3COOH \rightarrow CH_4 + CO_2$) ($m^3\ m^{-3}$ reactor d^{-1}); ΔCOD and $\Delta C_{acetate}$ are the changes in substrate concentration with every fed-batch (mg L^{-1}); V_{liquid} is the effective working volume of the reactor, mL; $M_{acetate} = 60.05$ g mol^{-1} is the relative molecular mass of the substrate (acetate).

Thus,

$$\mathrm{CE} = \frac{T_{V_{\mathrm{H_2-current}}}}{T_{V_{\mathrm{H_2-acetate}}}} \text{ (based on the mearsured acetate conceration)} \tag{5.23}$$

5.4.3 The Energy Calculation Involved in MECs

The overall hydrogen recovery (R_{H2}) is given by:

$$R_{\mathrm{H2}} = \frac{A_{V_{\mathrm{H2}}}}{T_{V_{\mathrm{H_2-acetate}}}} = \frac{n_{\mathrm{H_2}}}{\frac{\int It}{nF}}$$
$$= \frac{2Fn_{\mathrm{H_2}}}{\int It} \text{ (based on the mearsured acetate conceration)} \tag{5.24}$$

Cathodic hydrogen recovery ($R_{\mathrm{H_2,cat}}$) is given by:

$$R_{\mathrm{H_2,cat}} = \frac{A_{V_{\mathrm{H_2}}}}{T_{V_{\mathrm{H_2-current}}}} \tag{5.25}$$

The overall hydrogen recovery is used to evaluate the ratio of recovered H_2 compared to the maximum potential H_2 recovery, based on the substrate utilization (Wagner et al. 2009). Cathodic hydrogen recovery is used to evaluate the fraction of electrons that form H_2 from the overall amount of electrons reaching the cathode, namely generating current.

Electron reduction efficiency (E_e) is given by:

$$E_e = \frac{Q_{\mathrm{CH_4}}}{Q} \times 100\% \tag{5.26}$$

$$Q_{\mathrm{CH_4}} = 8 \cdot n_{\mathrm{CH_4}} \cdot F \tag{5.27}$$

Ideal gas law:

$$n_{\mathrm{CH_4}} = \frac{PV}{RT} \tag{5.28}$$

where this equation is used under experimental condition (25 °C, 1 atm, i.e., 1011.325 kPa).

Electron reduction efficiency (E_e) is used to measure the capacity of electron reduction by catalysis at the cathode surface.

The total input energy (W_{input}) is given by:

$$W_{\text{input}} = W_{\text{electricity}} + W_{\text{substrate}} \tag{5.29}$$

where W_{input} is the total amount of energy added to the entire system, kJ; $W_{\text{electricity}}$ (W_E) is the amount of energy added to the circuit by the power source, kJ, adjusted for losses across the resistor; $W_{\text{substrate}}$ (W_S) is the amount of energy added by the substrate (kJ).

The input electricity energy (W_E) is given by:

$$W_E = \frac{\sum_1^n \left(IE_{ap}\Delta t - I^2 R_{\text{ex}}\Delta t\right)}{1000} \tag{5.30}$$

where E_{ap} is the voltage applied, using the power source (V); Δt is the time increment for n data points measured during a batch cycle (s); and R_{ex} is the external resistor (Ω).

The input substrate energy (W_S) is given by:

$$W_S = n_S \Delta H_S \tag{5.31}$$

where n_S represents the substrate consumed (in terms of number of moles) per batch cycle; ΔH_S is the heat of combustion of the substrate, $\Delta H_{\text{acetate}} = 870.28$ kJ mol^{-1}, $\Delta H_{\text{glycerol}} = 1655.4$ kJ mol^{-1}, $\Delta H_{\text{glucose}} = 2802.7$ kJ mol^{-1} (Selembo et al. 2009b).

The total gained energy (W_{gained}) is given by:

$$W_{\text{gained}} = W_{\text{H}_2} + W_{\text{CH}_4} = n_{\text{H}_2}\Delta \text{H}_{\text{H}_2} + n_{\text{CH}_4}\Delta \text{H}_{\text{CH}_4} \tag{5.32}$$

where W_{H_2} and W_{CH_4} are the energy content generated from H_2 or CH_4 (kJ); n_{H_2} and n_{CH_4} are the number of moles of H_2 or CH_4 produced during a batch cycle; $\Delta \text{H}_{\text{H}_2} = 285.83$ kJ mol^{-1} and $\Delta \text{H}_{\text{CH}_4} = 890$ kJ mol^{-1} are the calorific values of H_2 and CH_4, based on the heat of combustion (upper heating value).

The methane revenue (R_{CH_4}) is given by:

$$R_{\text{CH}_4} = P_e \frac{Y_{\text{CH}_4}\Delta \text{H}_{\text{CH}_4}}{V_m}\eta \tag{5.33}$$

where $P_e = 0.10$ £ kW^{-1} h^{-1} is the standard price of electricity (referenced from business rates in the UK) (Aiken et al. 2019); R_{CH_4} is the revenue from methane (£·m^{-3} reactor day^{-1}); $\eta = 35\%$ is the electrical efficiency with a combustion engine as converter.

5.4.4 The Energy Recovery Efficiency

The total energy recovery efficiency (η_{total}) is given by:

$$\eta_{\text{total}} = \eta_E + \eta_S \tag{5.34}$$

where η_{total} is the ratio of energy output evaluated by produced H_2 or CH_4 to the total energy input composed of electricity input and substrate (acetate) consumption in the entire system; η_E is the ratio of the energy content of H_2 or CH_4 produced to the input electrical energy required; and η_S is the ratio of output energy evaluated by produced H_2 or CH_4 to the input energy from the consumed acetate.

The electrical energy recovery efficiency (η_E) is given by:

$$\eta_E = \frac{W_{H_2}}{W_E} \text{ (based on } H_2 \text{ as the main biogas)} \tag{5.35}$$

$$\eta_E = \frac{W_{CH_4}}{W_E} \text{ (based on } CH_4 \text{ as the main biogas)} \tag{5.36}$$

The substrate energy recovery efficiency (η_S) is given by:

$$\eta_S = \frac{W_{H_2}}{W_S} \text{ (based on } H_2 \text{ as the main biogas)} \tag{5.37}$$

$$\eta_S = \frac{W_{CH_4}}{W_S} \text{ (based on } CH_4 \text{ as the main biogas)} \tag{5.38}$$

The conversion efficiency of substrate ($\eta_{\text{substrate}}$) is given by:

$$\eta_{\text{substrate}} = \frac{n_{H_2}\frac{V_2}{V_m}}{n_{\text{substrate}}\frac{C_{\text{substrate}}V_{\text{liquid}}}{M}} \times 100\% \text{(based on } H_2 \text{ as the main biogas)} \tag{5.39}$$

$$\eta_{\text{substrate}} = \frac{n_{CH_4}\frac{V_4}{V_m}}{n_{\text{substrate}}\frac{C_{\text{substrate}}V_{\text{liquid}}}{M}} \times 100\% \text{(based on } CH_4 \text{ as the main biogas)} \tag{5.40}$$

where $\eta_{\text{substrate}}$ is the substrate conversion efficiency; $n_{\text{substrate}}$ is the electron per single mole of substrate; n_{H_2} and n_{CH_4} are the electrons yielded by H_2 or CH_4; and $C_{\text{substrate}}$ is the substrate concentration (mg L^{-1}).

5.5 Efficiency Improvement for Electron Transport

The electron transfer process is critical for the methanogenesis on the microbe-electrode interface. The electron transfer process of the cathode is similar to that of the anode, except for the direct electron transfer by direct contact and an indirect electron transfer process using hydrogen as a mediator (Miriam et al. 2011). Previous studies have illustrated that hydrogen is an important electron intermediate in the formation of cathodic methane, as well as an important electron donor for basophilic hydrogenotrophic methanogens. Compared with other pathways (Fig. 5.3),

hydrogen as an electron donor has the advantage of facilitating the enrichment of a wider range of hydrogenotrophic methanogens by diffusion, which is beneficial to improve the methane production rate. Therefore, it is more advantageous to enhance the hydrogen-to-methane pathway by strengthening the hydrogen evolution reaction for the enrichment of hydrogenotrophic methanogens, viz., promoting the hydrogen-mediated electron transfer process.

The formation of hydrogen at the cathode primarily depends on the electrochemical reaction process. Different electrode materials can affect the electron transfer rate and thus restrict the rate of HER. Furthermore, the characteristics of the biofilm also trigger differences in electron recovery efficiency, which further affect hydrogen yield. On the reaction interface between electrodes and microorganisms, the final electron acceptor is influenced by microorganism types and material properties. Under the conditions of non-pure cultures and non-specific materials, the electrons transmitted to energy metabolism and anabolic processes are different (since diverse microorganisms have different electronic respiratory chains), consequentially resulting in a variety of products. As a consequence, many undesirable by-products are ultimately produced, which affects the electron recovery efficiency.

The microbial community structure plays a decisive role in the distribution of reactive products, and the properties of the electrode materials can cooperate with the microorganisms to capture certain specific electron acceptors, subsequently resulting in high electron transfer recovery. In order to further strengthen bioenergy recovery, the electron transfer process is primarily facilitated in terms of electrode material modification and microbial community regulation. On the one hand, it can promote the rate of electron transfer on the interface of the bioelectrodes, improving the ability of catalyzing HER on the cathode. Furthermore, it can increase the recovery efficiency of electron transfer to the target end products.

5.5.1 Cathode Materials Upgrading

To date, one of the main drawbacks of BESs large-scale application, particularly with MEC, is the demand for costly materials, e.g., platinum in cathode. These materials are often favored due to the dramatic electrocatalytic activity for H_2 evolution, although the performance is negatively influenced by a number of different components that can be found in waste streams. Therefore, more sustainable and low-cost cathodes for bioenergy production via BESs are becoming urgent (Villano et al. 2010). Recently, microbial biocathodes have exhibited more widespread applications, e.g., bioremediation systems for biological reduction of oxidized contaminants (Aulenta et al. 2008, b), biological reduction of nitrates to nitrogen (Clauwaert et al. 2007), or electrochemical reduction of CO_2 to CH_4 (Villano et al. 2010).

In general, upgrading electrode materials primarily focus on reducing the mass transfer resistance of materials and the catalytic resistance. Based on the low hydrogen evolution potential of nickel foam (NF), the high catalysis efficiency of

earth-abundant transition metal phosphides, and low cost, Cai et al. studied a one-step phosphorization of NF; the authors used phosphorous vapor to fabricate a 3D biphasic Ni_5P_4-NiP_2 nanosheet matrix, acting as an electron transfer cathodic tunnel for H_2, coupled with a bioanode (Cai et al. 2018). A productivity of 9.78 ± 0.38 mL H_2 d^{-1} cm^{-2} was obtained, which was 1.5-fold higher than NF alone, and even higher than that described for commercially available Pt/C of 5.28 mL d^{-1} cm^{-2} (Cai et al. 2016a) and 4.94 mL d^{-1} cm^{-2} (Hou et al. 2014b). In addition, in order to replace the precious metal Pt, many transition metals, e.g., molybdenum, stainless steel, nickel foam, and other materials, are used as the matrixes, which can be further modified to improve the electrocatalytic activity of the electrode. Selembo et al. compared the effects of different stainless steel alloys (SS 304, 316, 420, A286) and nickel alloys (Ni 201, 400, 625, HX) using sheet metal cathodes in MEC, on hydrogen production, and found SS A286 displayed the best performance of 1.5 m^3 H_2 m^{-3} d^{-1} at 0.9 V (Selembo et al. 2009a). Call et al. confirmed that the stainless steel brush cathode with specific surface area can achieve the maximum productivity of 1.7 ± 0.1 m^3 H_2 m^{-3} d^{-1} at 0.6 V, compared to graphite brush cathode and flat stainless steel cathode (Call et al. 2009). Similarly, Su et al. also found that 3D macroporous stainless steel fiber felt cathode with high electrochemical active surface area has superior catalytic properties for H_2 evolution, achieving 3.66 ± 0.43 m^3 H_2 m^{-3} d^{-1} (current density of 17.29 ± 1.68A m^{-2}) at 0.9 V (Su et al. 2016). Hrapovic et al. successfully electrodeposited Ni on porous carbon paper, as cathode, obtaining the maximum H_2 production rate of 5.4 m^3 m^{-3} d^{-1}, when Ni loaded between 0.2 and 0.4 mg cm^{-2}, on the contrary, no any increase of hydrogen production under the coelectrodeposition of Pt and Ni (Hrapovic et al. 2010).

Figure 5.4 presents a comprehensive overview of cathode materials that have been used in MEC studies to enhance hydrogen and methane production.

5.5.2 *Functional Microbial Community Regulation*

In the traditional methanogenesis process, various volatile acids (such as propionate, butyrate, valerate, etc.) need to be converted into acetate and hydrogen by acetogenesis, before being used by acetoclastic and hydrogenotrophic methanogens, respectively. It is generally believed that 70% of the source of methane is derived from acetate and 30% from the contribution of hydrogen (Angenent et al. 2004). However, the growth rate of methanogens is slower than that of fermentative microorganisms, which limits the increase in the rate of methanogenesis. In view of this limitation, a methanogenic process based on extracellular electron transfer is developed inside a conventional anaerobic bioreactor (Liu et al. 2016a). This pathway allows direct electron transfer at the anode, using coenzyme cytochrome c with both oxidized and reduced states, extending the substrate types for methanogenesis. During extracellular electron transport of methanogenesis, the EAB can utilize various types of substrates, such as acetate and propionate, while

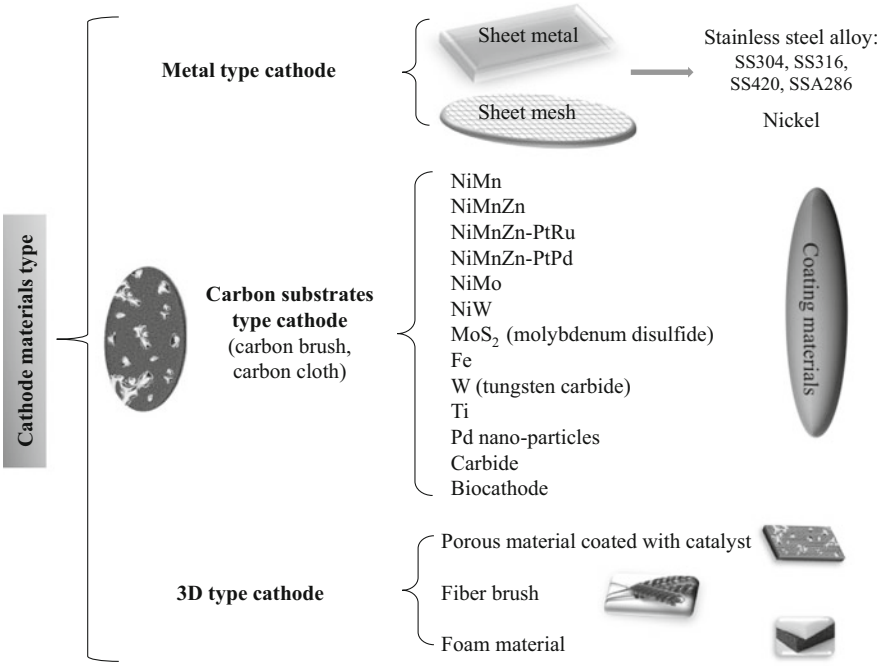

Fig. 5.4 Different cathode types of materials used in the microbial electrolysis cell, MEC

the electrons generated by oxidizing substrates can pass through an external circuit to drive the reduction of carbon dioxide at the cathode to form methane. Meanwhile, hydrogen also generates from electrons and protons, which can promote the growth of hydrogenotrophic methanogens, counteracting the limitations of traditional acetoclastic methanogens.

Cai et al. coupled the AD with the MEC to treat waste activated sludge and enhance methane generation (Cai et al. 2016b). Based on the results of Illumina MiSeq sequencing, methanogens were enriched in the cathode biofilm (particularly hydrogenotrophic *Methanobacterium* and acetoclastic *Methanosaeta)*, with two primary methanogenic pathways taking place at the cathode. This implied the possibility of increasing methane production, while reducing WAS digestion time, by controlling the bioelectrochemisty of the process.

Quorum sensing is an essential strategy for microbial community regulation and cell-to-cell communication in biofilms. The principle is that microbes secrete signaling molecules to affect the physiological activities of surrounding microorganisms, such as mobility, sporulation, biofilm formation, virulence, but also symbiosis, competition, toxicity, antagonism, antibiotics production, etc. (Miller and Bassler 2001). Acylated homoserine lactones (AHLs) are a representative signaling molecule that can also be used to regulate the interspecies communication process. AHLs

can be synthesized by bacteria like *Pseudomonas* sp. and degraded via chemical or biological pathways. In recent studies, it has been shown to enhance the respiratory activity associated with electron transfer, facilitating the capacity of electron transfer between cells and electrodes (Toyofuku et al. 2007). This increase in respiratory activity is mainly attributed to two strategies: one is by changing the physiological characteristics of microorganisms, including the cell membrane transmittance (Yong et al. 2013) and gene expression (Hu et al. 2015) (acting directly on the microorganism itself), and the other is by regulating processes related to electron shuttles for the biosynthesis, such as phenazines (Rabaey et al. 2005) (acting on the extracellular synthesis). Both strategies have been demonstrated to effectively increase the electrochemical activity of microorganisms. Cai et al. employed short-chain AHLs (3OC6), as intraspecific signaling molecules to modulate the biofilm community of bioelectrodes in single-chamber MECs. Surprisingly, the overall performance parameters of MECs with AHLs addition were significantly enhanced, including hydrogen yields, CE, electron recovery efficiency, and energy efficiency (Cai et al. 2016c). The lower internal resistance of reactors was verified via electrochemical impedance spectra (EIS). Noticeably, more EAB and fewer hydrogen scavengers, especially homo-acetogens *Acetoanaerobium* and *Acetobacterium*, and methanogens, especially hydrogenotrophic methanogen *Methanobrevibacter*, were detected in cathodic microbial aggregation (Fig. 5.5), which further confirmed the potential of regulating microbial communities by AHLs for strengthening electron transfer and hydrogen production in MEC, and impeding methanogenesis without any chemical inhibitors added (Cai et al. 2016c).

5.6 Bottlenecks and Challenges

MECs provide a promising potential to boost renewable hydrogen and methane generation from biowastes, possibly providing a new horizon to address imminent challenges in the energy sector, related to the rapidly growing population and fast developing industries. Importantly, MEC as an environmental-friendly technology not only displays a sustainable role in bioenergy recovery, but also simultaneously disposes and valorizes wastes. Undoubtedly, there are big challenges, such as fluctuating performances of MECs and costs of large-scale units, significantly constraining the transfer of bioelectrochemical technology from the laboratory to full scale. For example, system architecture, operating parameters, biological parameters determination, and techno-economic evaluation (such as products revenue, reproducibility, durability, scalability, etc.) need to be implemented and optimized, in order to bring this technology closer to the market.

Noticeably, there are four significant issues that need to be taken into consideration: (1) seeking alternative renewable energy, like solar, wind, waste heat, geothermal and marine energy, to improve sustainability or input energy saving; (2) increasing purities of biogas (H_2 or CH_4); (3) optimizing the electrode space layout to maximize efficiency in the limited reactor space; and (4) catalyzing the

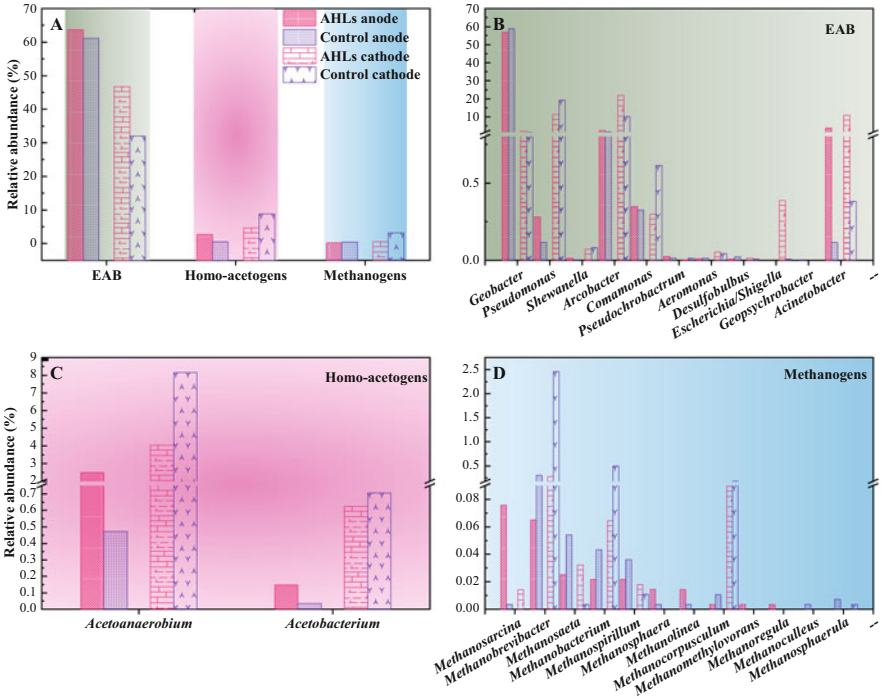

Fig. 5.5 The comparison of specific functional microbial community compositions on genus level between the anode and the cathode in the presence or absence of acylated homoserine lactones, AHLs. (**a**) is based on three functional microbial categories; (**b–d**) are subdivided into the specific categories of functional genera classification, including electrochemically active bacteria, EAB, homo-acetogens, and methanogens (reproduced from Cai et al. 2016c)

surface characteristics of the electrode and evaluating the electrochemical parameters of the composites. In the future, it will be strategic to integrate MECs with other waste treatment technologies to expand their scopes and applications. According to reactor configurations, alloy metal materials (like stainless steel mesh) will allow to form different configurations with large surface area, low overpotential, and low internal resistance of the system, at the same time promoting functional microorganisms adhesion to the electrodes. For the development of the electrode module system, it is necessary to pay more attention not only to the microstructure and the material properties of the electrodes, but also to three-dimensional electrode structure configuration with engineering application potential. The engineering application of MEC technology is promoted by modifying the conductive polymer on a single substrate, applying nanomaterials to improve electrode conductivity and specific surface area, and enhancing electron transfer performance and catalytic activity of bioelectrodes.

In conclusion, new assembly strategies will be explored, based on micro-nano interface of micro-electrode. Also, high-throughput sequencing, stable isotope

labeling, and other scientific methods are comprehensively employed to further reveal the function and structure of electrode microbial flora in depth, and shed light on microbial interactions, as well as extracellular electron transfer mechanism, based on electron mediator, nanowire, and cytochrome. Overall, these approaches are expected to provide technical and theoretical understanding for the development of viable and sustainable applications of MEC technology.

References

Aiken, D. C., Curtis, T. P., & Heidrich, E. S. (2019). Avenues to the financial viability of microbial electrolysis cells [MEC] for domestic wastewater treatment and hydrogen production. *International Journal of Hydrogen Energy, 44*(5), 2426–2434. https://doi.org/10.1016/j.ijhydene.2018.12.029

Allen, R. M., & Bennetto, H. P. (1993). Microbial fuel-cells - Electricity production from carbohydrates. *Applied Biochemistry and Biotechnology, 39*, 27–40. https://doi.org/10.1007/Bf02918975

Angenent, L. T., Karim, K., Al-Dahhan, M. H., Wrenn, B. A., & Domiguez-Espinosa, R. (2004). Production of bioenergy and biochemicals from industrial and agricultural wastewater. *Trends in Biotechnology, 22*(9), 477–485. https://doi.org/10.1016/j.tibtech.2004.07.001

Aulenta, F., Canosa, A., De Roma, L., Reale, P., Panero, S., Rossetti, S., et al. (2009a). Influence of mediator immobilization on the electrochemically assisted microbial dechlorination of trichloroethene (TCE) and cis-dichloroethene (cis-DCE). *Journal of Chemical Technology and Biotechnology, 84*(6), 864–870. https://doi.org/10.1002/jctb.2162

Aulenta, F., Canosa, A., Majone, M., Panero, S., Reale, P., & Rossetti, S. (2008). Trichloroethene dechlorination and H_2 evolution are alternative biological pathways of electric charge utilization by a dechlorinating culture in a bioelectrochemical system. *Environmental Science & Technology, 42*(16), 6185–6190. https://doi.org/10.1021/es800265b

Aulenta, F., Canosa, A., Reale, P., Rossetti, S., Panero, S., & Majone, M. (2009b). Microbial reductive dechlorination of trichloroethene to ethene with electrodes serving as electron donors without the external addition of redox mediators. *Biotechnology and Bioengineering, 103*(1), 85–91. https://doi.org/10.1002/bit.22234

Bo, T., Zhu, X. Y., Zhang, L. X., Tao, Y., He, X. H., Li, D. P., et al. (2014). A new upgraded biogas production process: Coupling microbial electrolysis cell and anaerobic digestion in single-chamber, barrel-shape stainless steel reactor. *Electrochemistry Communications, 45*, 67–70. https://doi.org/10.1016/j.elecom.2014.05.026

Bond, D. R., Holmes, D. E., Tender, L. M., & Lovley, D. R. (2002). Electrode-reducing microorganisms that harvest energy from marine sediments. *Science, 295*(5554), 483–485. https://doi.org/10.1126/science.1066771

Bond, D. R., & Lovley, D. R. (2003). Electricity production by Geobacter sulfurreducens attached to electrodes. *Applied and Environmental Microbiology, 69*(3), 1548–1555. https://doi.org/10.1128/aem.69.3.1548-1555.2003

Bond, D. R., & Lovley, D. R. (2005). Evidence for involvement of an electron shuttle in electricity generation by Geothrix fermentans. *Applied and Environmental Microbiology, 71*(4), 2186–2189. https://doi.org/10.1128/AEM.71.4.2186-2189.2005

Borole, A. P., O'Neill, H., Tsouris, C., & Cesar, S. (2008). A microbial fuel cell operating at low pH using the acidophile Acidiphilium cryptum. *Biotechnology Letters, 30*(8), 1367–1372. https://doi.org/10.1007/s10529-008-9700-y

Bretschger, O., Obraztsova, A., Sturm, C. A., Chang, I. S., Gorby, Y. A., Reed, S. B., et al. (2007). Current production and metal oxide reduction by *Shewanella oneidensis* MR-1 wild type and

mutants. *Applied and Environmental Microbiology, 73*(21), 7003–7012. https://doi.org/10.1128/AEM.01087-07

Cai, W., Liu, W., Han, J., & Wang, A. (2016a). Enhanced hydrogen production in microbial electrolysis cell with 3D self-assembly nickel foam-graphene cathode. *Biosensors & Bioelectronics, 80*, 118–122. https://doi.org/10.1016/j.bios.2016.01.008

Cai, W. W., Liu, W. Z., Sun, H. S., Li, J. Q., Yang, L. M., Liu, M. J., et al. (2018). Ni_5P_4-NiP_2 nanosheet matrix enhances electron-transfer kinetics for hydrogen recovery in microbial electrolysis cells. *Applied Energy, 209*, 56–64. https://doi.org/10.1016/j.apenergy.2017.10.082

Cai, W. W., Liu, W. Z., Yang, C. X., Wang, L., Liang, B., Thangavel, S., et al. (2016b). Biocathodic methanogenic community in an integrated anaerobic digestion and microbial electrolysis system for enhancement of methane production from waste sludge. *ACS Sustainable Chemistry & Engineering, 4*(9), 4913–4921. https://doi.org/10.1021/acssuschemeng.6b01221

Cai, W. W., Zhang, Z. J., Ren, G., Shen, Q. X., Hou, Y. N., Ma, A. Z., et al. (2016c). Quorum sensing alters the microbial community of electrode-respiring bacteria and hydrogen scavengers toward improving hydrogen yield in microbial electrolysis cells. *Applied Energy, 183*, 1133–1141. https://doi.org/10.1016/j.apenergy.2016.09.074

Cai, W. W., Liu, W. Z., Wang, B., Yao, H., Guadie, A., & Wang, A. J. (2020). Semiquantitative detection of hydrogen-associated or hydrogen-free electron transfer within methanogenic biofilm of microbial electrosynthesis. *Applied and Environmental Microbiology, 86*(17), e01056–01020. https://doi.org/10.1128/AEM.01056-20

Call, D., & Logan, B. E. (2008). Hydrogen production in a single chamber microbial electrolysis cell lacking a membrane. *Environmental Science & Technology, 42*(9), 3401–3406. https://doi.org/10.1021/es8001822

Call, D. F., Merrill, M. D., & Logan, B. E. (2009). High surface area stainless steel brushes as cathodes in microbial electrolysis cells. *Environmental Science & Technology, 43*(6), 2179–2183. https://doi.org/10.1021/es803074x

Cerrillo, M., Vinas, M., & Bonmati, A. (2016). Overcoming organic and nitrogen overload in thermophilic anaerobic digestion of pig slurry by coupling a microbial electrolysis cell. *Bioresource Technology, 216*, 362–372. https://doi.org/10.1016/j.biortech.2016.05.085

Cerrillo, M., Vinas, M., & Bonmati, A. (2018). Anaerobic digestion and electromethanogenic microbial electrolysis cell integrated system: Increased stability and recovery of ammonia and methane. *Renewable Energy, 120*, 178–189. https://doi.org/10.1016/j.renene.2017.12.062

Chae, K. J., Choi, M. J., Kim, K. Y., Ajayi, F. F., Chang, I. S., & Kim, I. S. (2010). Selective inhibition of methanogens for the improvement of biohydrogen production in microbial electrolysis cells. *International Journal of Hydrogen Energy, 35*(24), 13379–13386. https://doi.org/10.1016/j.ijhydene.2009.11.114

Chae, K. J., Choi, M. J., Lee, J., Ajayi, F. F., & Kim, I. S. (2008). Biohydrogen production via biocatalyzed electrolysis in acetate-fed bioelectrochemical cells and microbial community analysis. *International Journal of Hydrogen Energy, 33*(19), 5184–5192. https://doi.org/10.1016/j.ijhydene.2008.05.013

Chaudhuri, S. K., & Lovley, D. R. (2003). Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. *Nature Biotechnology, 21*(10), 1229–1232. https://doi.org/10.1038/nbt867

Cheng, Q., & Call, D. F. (2016). Hardwiring microbes via direct interspecies electron transfer: Mechanisms and applications. *Environmental Science. Processes & Impacts, 18*(8), 968–980. https://doi.org/10.1039/c6em00219f

Cheng, S., & Logan, B. E. (2007). Sustainable and efficient biohydrogen production via electrohydrogenesis. *Proceedings of the National Academy of Sciences of the United States of America, 104*(47), 18871–18873. https://doi.org/10.1073/pnas.0706379104

Cheng, S., Xing, D., Call, D. F., & Logan, B. E. (2009). Direct biological conversion of electrical current into methane by electromethanogenesis. *Environmental Science & Technology, 43*(10), 3953–3958. https://doi.org/10.1021/es803531g

Cho, S. K., Lee, M. E., Lee, W., & Ahn, Y. (2019). Improved hydrogen recovery in microbial electrolysis cells using intermittent energy input. *International Journal of Hydrogen Energy, 44* (4), 2253–2257. https://doi.org/10.1016/j.ijhydene.2018.07.025

Choi, K. S., Kondaveeti, S., & Min, B. (2017). Bioelectrochemical methane (CH_4) production in anaerobic digestion at different supplemental voltages. *Bioresource Technology, 245*(Pt A), 826–832. https://doi.org/10.1016/j.biortech.2017.09.057

Clauwaert, P., Rabaey, K., Aelterman, P., de Schamphelaire, L., Pham, T. H., Boeckx, P., et al. (2007). Biological denitrification in microbial fuel cells. *Environmental Science & Technology, 41*(9), 3354–3360. https://doi.org/10.1021/es062580r

Crow, D. R. (1994). *Principles and applications of electrochemistry*. Boca Raton: CRC Press. https://doi.org/10.1016/j.ijhydene.2003.08.017

Cusick, R. D., Bryan, B., Parker, D. S., Merrill, M. D., Mehanna, M., Kiely, P. D., et al. (2011). Performance of a pilot-scale continuous flow microbial electrolysis cell fed winery wastewater. *Applied Microbiology and Biotechnology, 89*(6), 2053–2063. https://doi.org/10.1007/s00253-011-3130-9

Ditzig, J., Liu, H., & Logan, B. E. (2007). Production of hydrogen from domestic wastewater using a bioelectrochemically assisted microbial reactor (BEAMR). *International Journal of Hydrogen Energy, 32*(13), 2296–2304. https://doi.org/10.1016/j.ijhydene.2007.02.035

Feng, H. J., Huang, L. J., Wang, M. Z., Xu, Y. F., Shen, D. S., Li, N., et al. (2018). An effective method for hydrogen production in a single-chamber microbial electrolysis by negative pressure control. *International Journal of Hydrogen Energy, 43*(37), 17556–17561. https://doi.org/10.1016/j.ijhydene.2018.07.197

Feng, Q., Song, Y. C., Yoo, K., Kuppanan, N., Subudhi, S., & Lal, B. (2017). Influence of neutralization in acidic distillery wastewater on direct interspecies electron transfer for methane production in an upflow anaerobic bioelectrochemical reactor. *International Journal of Hydrogen Energy, 42*(45), 27774–27783. https://doi.org/10.1016/j.ijhydene.2017.05.228

Feng, Y., Zhang, Y., Chen, S., & Quan, X. (2015). Enhanced production of methane from waste activated sludge by the combination of high-solid anaerobic digestion and microbial electrolysis cell with iron–graphite electrode. *Chemical Engineering Journal, 259*, 787–794. https://doi.org/10.1016/j.cej.2014.08.048

Fu, Q., Kuramochi, Y., Fukushima, N., Maeda, H., Sato, K., & Kobayashi, H. (2015). Bioelectrochemical analyses of the development of a thermophilic biocathode catalyzing electromethanogenesis. *Environmental Science & Technology, 49*(2), 1225–1232. https://doi.org/10.1021/es5052233

Gajaraj, S., Huang, Y. X., Zheng, P., & Hu, Z. Q. (2017). Methane production improvement and associated methanogenic assemblages in bioelectrochemically assisted anaerobic digestion. *Biochemical Engineering Journal, 117*, 105–112. https://doi.org/10.1016/j.bej.2016.11.003

Geppert, F., Liu, D., van Eerten-Jansen, M., Weidner, E., Buisman, C., & Ter Heijne, A. (2016). Bioelectrochemical power-to-gas: State of the art and future perspectives. *Trends in Biotechnology, 34*(11), 879–894. https://doi.org/10.1016/j.tibtech.2016.08.010

Ghimire, A., Frunzo, L., Pirozzi, F., Trably, E., Escudie, R., Lens, P. N. L., et al. (2015). A review on dark fermentative biohydrogen production from organic biomass: Process parameters and use of by-products. *Applied Energy, 144*, 73–95. https://doi.org/10.1016/j.apenergy.2015.01.045

Guo, K., Tang, X. H., Du, Z. W., & Li, H. R. (2010). Hydrogen production from acetate in a cathode-on-top single-chamber microbial electrolysis cell with a mipor cathode. *Biochemical Engineering Journal, 51*(1-2), 48–52. https://doi.org/10.1016/j.bej.2010.05.001

Guo, X. S., Liu, J. X., & Xiao, B. Y. (2013). Bioelectrochemical enhancement of hydrogen and methane production from the anaerobic digestion of sewage sludge in single-chamber membrane-free microbial electrolysis cells. *International Journal of Hydrogen Energy, 38*(3), 1342–1347. https://doi.org/10.1016/j.ijhydene.2012.11.087

Hallenbeck, P. C., & Benemann, J. R. (2002). Biological hydrogen production; fundamentals and limiting processes. *International Journal of Hydrogen Energy, 27*(11-12), 1185–1193. https://doi.org/10.1016/S0360-3199(02)00131-3

Hamelers, H. V., Ter Heijne, A., Sleutels, T. H., Jeremiasse, A. W., Strik, D. P., & Buisman, C. J. (2010). New applications and performance of bioelectrochemical systems. *Applied Microbiology and Biotechnology, 85*(6), 1673–1685. https://doi.org/10.1007/s00253-009-2357-1

Hawkes, F. R., Dinsdale, R., Hawkes, D. L., & Hussy, I. (2002). Sustainable fermentative hydrogen production: Challenges for process optimisation. *International Journal of Hydrogen Energy, 27*(11-12), 1339–1347. https://doi.org/10.1016/S0360-3199(02)00090-3

Holmes, D. E., Bond, D. R., & Lovley, D. R. (2004a). Electron transfer by Desulfobulbus propionicus to Fe(III) and graphite electrodes. *Applied and Environmental Microbiology, 70*(2), 1234–1237. https://doi.org/10.1128/aem.70.2.1234-1237.2004

Holmes, D. E., Nicoll, J. S., Bond, D. R., & Lovley, D. R. (2004b). Potential role of a novel psychrotolerant member of the family Geobacteraceae, *Geopsychrobacter electrodiphilus* gen. nov., sp. nov., in electricity production by a marine sediment fuel cell. *Applied and Environmental Microbiology, 70*(10), 6023–6030. https://doi.org/10.1128/AEM.70.(10).6023-6030.2004

Hou, Y., Luo, H., Liu, G., Zhang, R., Li, J., & Fu, S. (2014a). Improved hydrogen production in the microbial electrolysis cell by inhibiting methanogenesis using ultraviolet irradiation. *Environmental Science & Technology, 48*(17), 10482–10488. https://doi.org/10.1021/es501202e

Hou, Y., Zhang, B., Wen, Z. H., Cui, S. M., Guo, X. R., He, Z., et al. (2014b). A 3D hybrid of layered MoS_2/nitrogen-doped graphene nanosheet aerogels: An effective catalyst for hydrogen evolution in microbial electrolysis cells. *Journal of Materials Chemistry A, 2*(34), 13795–13800. https://doi.org/10.1039/c4ta02254h

Hrapovic, S., Manuel, M. F., Luong, J. H. T., Guiot, S. R., & Tartakovsky, B. (2010). Electrodeposition of nickel particles on a gas diffusion cathode for hydrogen production in a microbial electrolysis cell. *International Journal of Hydrogen Energy, 35*(14), 7313–7320. https://doi.org/10.1016/j.ijhydene.2010.04.146

Hu, H., Fan, Y., & Liu, H. (2008). Hydrogen production using single-chamber membrane-free microbial electrolysis cells. *Water Research, 42*(15), 4172–4178. https://doi.org/10.1016/j.watres.2008.06.015

Hu, H. Q., Fan, Y. Z., & Liu, H. (2009). Hydrogen production in single-chamber tubular microbial electrolysis cells using non-precious-metal catalysts. *International Journal of Hydrogen Energy, 34*(20), 8535–8542. https://doi.org/10.1016/j.ijhydene.2009.08.011

Hu, Y., Yang, Y., Katz, E., & Song, H. (2015). Programming the quorum sensing-based AND gate in *Shewanella oneidensis* for logic gated-microbial fuel cells. *Chemical Communications, 51*(20), 4184–4187. https://doi.org/10.1039/c5cc00026b

Jafary, T., Daud, W. R. W., Ghasemi, M., Kim, B. H., Jahim, J. M., Ismail, M., et al. (2015). Biocathode in microbial electrolysis cell; present status and future prospects. *Renewable and Sustainable Energy Reviews, 47*, 23–33. https://doi.org/10.1016/j.rser.2015.03.003

Jeremiasse, A. W., Hamelers, H. V. M., Saakes, M., & Buisman, C. J. N. (2010). Ni foam cathode enables high volumetric H_2 production in a microbial electrolysis cell. *International Journal of Hydrogen Energy, 35*(23), 12716–12723. https://doi.org/10.1016/j.ijhydene.2010.08.131

Jung, S., & Regan, J. M. (2011). Influence of external resistance on electrogenesis, methanogenesis, and anode prokaryotic communities in microbial fuel cells. *Applied and Environmental Microbiology, 77*(2), 564–571. https://doi.org/10.1128/AEM.01392-10

Kaur, A., Boghani, H. C., Michie, I., Dinsdale, R. M., Guwy, A. J., & Premier, G. C. (2014). Inhibition of methane production in microbial fuel cells: Operating strategies which select electrogens over methanogens. *Bioresource Technology, 173*, 75–81. https://doi.org/10.1016/j.biortech.2014.09.091

Kim, B. H., Kim, H. J., Hyun, M. S., & Park, D. H. (1999). Direct electrode reaction of Fe(III)-reducing bacterium, *Shewanella putrefaciens*. *Journal of Microbiology and Biotechnology, 9*

(2), 127–131. https://doi.org/10.1002/(SICI)1099-1514(199905/06)20:3<127::AID-OCA650>3.0.CO;2-I

Kim, I. S., Hwang, M. H., Jang, N. J., Hyun, S. H., & Lee, S. T. (2004). Effect of low pH on the activity of hydrogen utilizing methanogen in bio-hydrogen process. *International Journal of Hydrogen Energy, 29*(11), 1133–1140. https://doi.org/10.1016/j.ijhydene.2003.08.017

Kumar, A., Hsu, L. H.-H., Kavanagh, P., Barrière, F., Lens, P. N. L., Lapinsonnière, L., et al. (2017). The ins and outs of microorganism–electrode electron transfer reactions. *Nature Reviews Chemistry, 1*(3). https://doi.org/10.1038/s41570-017-0024

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. *International Journal of Hydrogen Energy, 34*(15), 6201–6210. https://doi.org/10.1016/j.ijhydene.2009.05.112

Lee, B., Park, J. G., Shin, W. B., Tian, D. J., & Jun, H. B. (2017). Microbial communities change in an anaerobic digestion after application of microbial electrolysis cells. *Bioresource Technology, 234*, 273–280. https://doi.org/10.1016/j.biortech.2017.02.022

Lee, H. S., & Rittmann, B. E. (2010). Significance of biological hydrogen oxidation in a continuous single-chamber microbial electrolysis cell. *Environmental Science & Technology, 44*(3), 948–954. https://doi.org/10.1021/es9025358

Lee, H. S., Torres, C. I., Parameswaran, P., & Rittmann, B. E. (2009). Fate of H_2 in an upflow single-chamber microbial electrolysis cell using a metal-catalyst-free cathode. *Environmental Science & Technology, 43*(20), 7971–7976. https://doi.org/10.1021/es900204j

Li, X., Zhang, R., Qian, Y., Angelidaki, I., & Zhang, Y. (2017). The impact of anode acclimation strategy on microbial electrolysis cell treating hydrogen fermentation effluent. *Bioresource Technology, 236*, 37–43. https://doi.org/10.1016/j.biortech.2017.03.160

Liang, D. W., Peng, S. K., Lu, S. F., Liu, Y. Y., Lan, F., & Xiang, Y. (2011). Enhancement of hydrogen production in a single chamber microbial electrolysis cell through anode arrangement optimization. *Bioresource Technology, 102*(23), 10881–10885. https://doi.org/10.1016/j.3biortech.2011.09.028

Liu, D., Zhang, L., Chen, S., Buisman, C., & Ter Heijne, A. (2016b). Bioelectrochemical enhancement of methane production in low temperature anaerobic digestion at 10 °C. *Water Research, 99*, 281–287. https://doi.org/10.1016/j.watres.2016.04.020

Liu, H., Grot, S., & Logan, B. E. (2005). Electrochemically assisted microbial production of hydrogen from acetate. *Environmental Science & Technology, 39*(11), 4317–4320. https://doi.org/10.1021/es050244p

Liu, S. Y., Charles, W., Ho, G., Cord-Ruwisch, R., & Cheng, K. Y. (2017). Bioelectrochemical enhancement of anaerobic digestion: Comparing single- and two-chamber reactor configurations at thermophilic conditions. *Bioresource Technology, 245*(Pt A), 1168–1175. https://doi.org/10.1016/j.biortech.2017.08.095

Liu, W., Wang, A., Cheng, S., Logan, B. E., Yu, H., Deng, Y., et al. (2010). Geochip-based functional gene analysis of anodophilic communities in microbial electrolysis cells under different operational modes. *Environmental Science & Technology, 44*(19), 7729–7735. https://doi.org/10.1021/es100608a

Liu, W. Z., Cai, W. W., Guo, Z. C., Wang, L., Yang, C. X., Varrone, C., et al. (2016a). Microbial electrolysis contribution to anaerobic digestion of waste activated sludge, leading to accelerated methane production. *Renewable Energy, 91*, 334–339. https://doi.org/10.1016/j.renene.2016.01.082

Logan, B. E. (2004). Extracting hydrogen and electricity from renewable resources. *Environmental Science & Technology, 38*(9), 160A–167A. https://doi.org/10.1021/es040468s

Logan, B. E., Call, D., Cheng, S., Hamelers, H. V., Sleutels, T. H., Jeremiasse, A. W., et al. (2008). Microbial electrolysis cells for high yield hydrogen gas production from organic matter. *Environmental Science & Technology, 42*(23), 8630–8640. https://doi.org/10.1021/es801553z

Logan, B. E., Hamelers, B., Rozendal, R., Schroder, U., Keller, J., Freguia, S., et al. (2006). Microbial fuel cells: Methodology and technology. *Environmental Science & Technology, 40* (17), 5181–5192. https://doi.org/10.1021/es0605016

Logan, B. E., & Rabaey, K. (2012). Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies. *Science, 337*(6095), 686–690. https://doi.org/10.1126/science.1217412

Logan, B. E., Rossi, R., Ragab, A., & Saikaly, P. E. (2019). Electroactive microorganisms in bioelectrochemical systems. *Nature Reviews. Microbiology, 17*(5), 307–319. https://doi.org/10.1038/s41579-019-0173-x

Lohner, S. T., Deutzmann, J. S., Logan, B. E., Leigh, J., & Spormann, A. M. (2014). Hydrogenase-independent uptake and metabolism of electrons by the archaeon *Methanococcus maripaludis*. *The ISME Journal, 8*(8), 1673–1681. https://doi.org/10.1038/ismej.2014.82

Lovley, D. R. (2006). Microbial fuel cells: Novel microbial physiologies and engineering approaches. *Current Opinion in Biotechnology, 17*(3), 327–332. https://doi.org/10.1016/j.copbio.2006.04.006

Lovley, D. R. (2017). Syntrophy goes electric: Direct interspecies electron transfer. *Annual Review of Microbiology, 71*, 643–664. https://doi.org/10.1146/annurev-micro-030117-020420

Lu, L., Hou, D., Wang, X., Jassby, D., & Ren, Z. J. (2016). Active H_2 harvesting prevents methanogenesis in microbial electrolysis cells. *Environmental Science & Technology Letters, 3*(8), 286–290. https://doi.org/10.1021/acs.estlett.6b00209

Lu, L., Ren, N., Xing, D., & Logan, B. E. (2009). Hydrogen production with effluent from an ethanol-H_2-coproducing fermentation reactor using a single-chamber microbial electrolysis cell. *Biosensors & Bioelectronics, 24*(10), 3055–3060. https://doi.org/10.1016/j.bios.2009.03.024

Lu, L., Ren, N. Q., Zhao, X., Wang, H. A., Wu, D., & Xing, D. F. (2011). Hydrogen production, methanogen inhibition and microbial community structures in psychrophilic single-chamber microbial electrolysis cells. *Energy & Environmental Science, 4*(4), 1329–1336. https://doi.org/10.1039/c0ee00588f

Lu, L., Xing, D., Liu, B., & Ren, N. (2012b). Enhanced hydrogen production from waste activated sludge by cascade utilization of organic matter in microbial electrolysis cells. *Water Research, 46*(4), 1015–1026. https://doi.org/10.1016/j.watres.2011.11.073

Lu, L., Xing, D., Ren, N., & Logan, B. E. (2012a). Syntrophic interactions drive the hydrogen production from glucose at low temperature in microbial electrolysis cells. *Bioresource Technology, 124*(3), 68–76. https://doi.org/10.1016/j.biortech.2012.08.040

Lu, L., Xing, D., Xie, T., Ren, N., & Logan, B. E. (2010). Hydrogen production from proteins via electrohydrogenesis in microbial electrolysis cells. *Biosensors & Bioelectronics, 25*(12), 2690–2695. https://doi.org/10.1016/j.bios.2010.05.003

Malvankar, N. S., Vargas, M., Nevin, K. P., Franks, A. E., Leang, C., Kim, B. C., et al. (2011). Tunable metallic-like conductivity in microbial nanowire networks. *Nature Nanotechnology, 6* (9), 573–579. https://doi.org/10.1038/nnano.2011.119

Marone, A., Ayala-Campos, O. R., Trably, E., Carmona-Martinez, A. A., Moscoviz, R., Latrille, E., et al. (2017). Coupling dark fermentation and microbial electrolysis to enhance bio-hydrogen production from agro-industrial wastewaters and by-products in a bio-refinery framework. *International Journal of Hydrogen Energy, 42*(3), 1609–1621. https://doi.org/10.1016/j.ijhydene.2016.09.166

Marshall, C. W., Ross, D. E., Fichot, E. B., Norman, R. S., & May, H. D. (2013). Long-term operation of microbial electrosynthesis systems improves acetate production by autotrophic microbiomes. *Environmental Science & Technology, 47*(11), 6023–6029. https://doi.org/10.1021/es400341b

Miller, M. B., & Bassler, B. L. (2001). Quorum sensing in bacteria. *Annual Review of Microbiology, 55*(1), 165–199. https://doi.org/10.1146/annurev.micro.55.1.165

Miriam, R., Federico, A., Marianna, V., & Angenent, L. T. (2011). Cathodes as electron donors for microbial metabolism: Which extracellular electron transfer mechanisms are involved? *Bioresource Technology, 102*(1), 324–333. https://doi.org/10.1016/j.biortech.2010.07.008

Montpart, N., Rago, L., Baeza, J. A., & Guisasola, A. (2015). Hydrogen production in single chamber microbial electrolysis cells with different complex substrates. *Water Research, 68* (68C), 601–615. https://doi.org/10.1016/j.watres.2014.10.026

Morita, M., Malvankar, N. S., Franks, A. E., Summers, Z. M., Giloteaux, L., Rotaru, A. E., et al. (2011). Potential for direct interspecies electron transfer in methanogenic wastewater digester aggregates. *mBio, 2*(4), e00159–e00111. https://doi.org/10.1128/mBio.00159-11

Nevin, K. P., Hensley, S. A., Franks, A. E., Summers, Z. M., Ou, J., Woodard, T. L., et al. (2011). Electrosynthesis of organic compounds from carbon dioxide is catalyzed by a diversity of acetogenic microorganisms. *Applied and Environmental Microbiology, 77*(9), 2882–2886. https://doi.org/10.1128/AEM.02642-10

Oh, S. E., Van Ginkel, S., & Logan, B. E. (2003). The relative effectiveness of pH control and heat treatment for enhancing biohydrogen gas production. *Environmental Science & Technology, 37* (22), 5186–5190. https://doi.org/10.1021/es034291y

Pan, C. M., Zhang, S. F., Fan, Y. T., & Hou, H. W. (2010). Bioconversion of corncob to hydrogen using anaerobic mixed microflora. *International Journal of Hydrogen Energy, 35*(7), 2663–2669. https://doi.org/10.1016/j.ijhydene.2009.04.023

Parameswaran, P., Torres, C. I., Lee, H. S., Krajmalnik-Brown, R., & Rittmann, B. E. (2009). Syntrophic interactions among anode respiring bacteria (ARB) and Non-ARB in a biofilm anode: electron balances. *Biotechnology and Bioengineering, 103*(3), 513–523. https://doi.org/10.1002/bit.22267

Parameswaran, P., Torres, C. I., Lee, H. S., Rittmann, B. E., & Krajmalnik-Brown, R. (2011). Hydrogen consumption in microbial electrochemical systems (MXCs): the role of homo-acetogenic bacteria. *Bioresource Technology, 102*(1), 263–271. https://doi.org/10.1016/j.biortech.2010.03.133

Park, H. S., Kim, B. H., Kim, H. S., Kim, H. J., Kim, G. T., Kim, M., et al. (2001). A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to Clostridium butyricum isolated from a microbial fuel cell. *Anaerobe, 7*(6), 297–306. https://doi.org/10.1006/anae.2001.0399

Park, J., Lee, B., Tian, D., & Jun, H. (2018). Bioelectrochemical enhancement of methane production from highly concentrated food waste in a combined anaerobic digester and microbial electrolysis cell. *Bioresource Technology, 247*, 226–233. https://doi.org/10.1016/j.biortech.2017.09.021

Pham, C. A., Jung, S. J., Phung, N. T., Lee, J., Chang, I. S., Kim, B. H., et al. (2003). A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Aeromonas hydrophila*, isolated from a microbial fuel cell. *FEMS Microbiology Letters, 223*(1), 129–134. https://doi.org/10.1016/s0378-1097(03)00354-9

Prasad, D., Arun, S., Murugesan, M., Padmanaban, S., Satyanarayanan, R. S., Berchmans, S., et al. (2007). Direct electron transfer with yeast cells and construction of a mediatorless microbial fuel cell. *Biosensors & Bioelectronics, 22*(11), 2604–2610. https://doi.org/10.1016/j.bios.2006.10.028

Rabaey, K., Boon, N., Hofte, M., & Verstraete, W. (2005). Microbial phenazine production enhances electron transfer in biofuel cells. *Environmental Science & Technology, 39*(9), 3401–3408. https://doi.org/10.1021/es048563o

Rabaey, K., Boon, N., Siciliano, S. D., Verhaege, M., & Verstraete, W. (2004). Biofuel cells select for microbial consortia that self-mediate electron transfer. *Applied and Environmental Microbiology, 70*(9), 5373–5382. https://doi.org/10.1128/AEM.70.9.5373-5382.2004

Rasten, E., Hagen, G., & Tunold, R. (2003). Electrocatalysis in water electrolysis with solid polymer electrolyte. *Electrochimica Acta, 48*(25-26), 3945–3952. https://doi.org/10.1016/j.electacta.2003.04.001

Ringeisen, B. R., Ray, R., & Little, B. (2007). A miniature microbial fuel cell operating with an aerobic anode chamber. *Journal of Power Sources, 165*(2), 591–597. https://doi.org/10.1016/j.jpowsour.2006.10.026

Rotaru, A. E., Shrestha, P. M., Liu, F., Markovaite, B., Chen, S., Nevin, K. P., et al. (2014a). Direct interspecies electron transfer between *Geobacter metallireducens* and *Methanosarcina barkeri*. *Applied and Environmental Microbiology, 80*(15), 4599–4605. https://doi.org/10.1128/AEM.00895-14

Rotaru, A. E., Shrestha, P. M., Liu, F. H., Shrestha, M., Shrestha, D., Embree, M., et al. (2014b). A new model for electron flow during anaerobic digestion: Direct interspecies electron transfer to *Methanosaeta* for the reduction of carbon dioxide to methane. *Energy & Environmental Science, 7*(1), 408–415. https://doi.org/10.1039/c3ee42189a

Rozendal, R. A., Hamelers, H. V. M., Euverink, G. J. W., Metz, S. J., & Buisman, C. J. N. (2006). Principle and perspectives of hydrogen production through biocatalyzed electrolysis. *International Journal of Hydrogen Energy, 31*(12), 1632–1640. https://doi.org/10.1016/j.ijhydene.2005.12.006

Rozendal, R. A., Hamelers, H. V., Molenkamp, R. J., & Buisman, C. J. (2007). Performance of single chamber biocatalyzed electrolysis with different types of ion exchange membranes. *Water Research, 41*(9), 1984–1994. https://doi.org/10.1016/j.watres.2007.01.019

Rozendal, R. A., Jeremiasse, A. W., Hamelers, H. V., & Buisman, C. J. (2008). Hydrogen production with a microbial biocathode. *Environmental Science & Technology, 42*(2), 629–634. https://doi.org/10.1021/es071720+

Sangeetha, T., Guo, Z. C., Liu, W. Z., Gao, L., Wang, L., Cui, M. H., et al. (2017). Energy recovery evaluation in an up flow microbial electrolysis coupled anaerobic digestion (ME-AD) reactor: Role of electrode positions and hydraulic retention times. *Applied Energy, 206*, 1214–1224. https://doi.org/10.1016/j.apenergy.2017.10.026

Selembo, P. A., Merrill, M. D., & Logan, B. E. (2009a). The use of stainless steel and nickel alloys as low-cost cathodes in microbial electrolysis cells. *Journal of Power Sources, 190*(2), 271–278. https://doi.org/10.1016/j.jpowsour.2008.12.144

Selembo, P. A., Perez, J. M., Lloyd, W. A., & Logan, B. E. (2009b). High hydrogen production from glycerol or glucose by electrohydrogenesis using microbial electrolysis cells. *International Journal of Hydrogen Energy, 34*(13), 5373–5381. https://doi.org/10.1016/j.ijhydene.2009.05.002

She, P., Bo, S., Xing, X. H., van Loosdrecht, M., & Liu, Z. (2006). Electrolytic stimulation of bacteria Enterobacter dissolvens by a direct current. *Biochemical Engineering Journal, 28*(1), 23–29. https://doi.org/10.1016/j.bej.2005.08.033

Siegert, M., Yates, M. D., Spormann, A. M., & Logan, B. E. (2015). *Methanobacterium* dominates biocathodic archaeal communities in methanogenic microbial electrolysis cells. *ACS Sustainable Chemistry & Engineering, 3*(7), 1668–1676. https://doi.org/10.1021/acssuschemeng.5b00367

Stams, A. J., & Plugge, C. M. (2009). Electron transfer in syntrophic communities of anaerobic bacteria and archaea. *Nature Reviews. Microbiology, 7*(8), 568–577. https://doi.org/10.1038/nrmicro2166

Strycharz, S. M., Woodard, T. L., Johnson, J. P., Nevin, K. P., Sanford, R. A., Loffler, F. E., et al. (2008). Graphite electrode as a sole electron donor for reductive dechlorination of tetrachlorethene by *Geobacter lovleyi*. *Applied and Environmental Microbiology, 74*(19), 5943–5947. https://doi.org/10.1128/AEM.00961-08

Su, M., Wei, L. L., Qiu, Z. Z., Wang, G., & Shen, J. Q. (2016). Hydrogen production in single chamber microbial electrolysis cells with stainless steel fiber felt cathodes. *Journal of Power Sources, 301*, 29–34. https://doi.org/10.1016/j.jpowsour.2015.09.108

Tartakovsky, B., Manuel, M. F., Wang, H., & Guiot, S. R. (2009). High rate membrane-less microbial electrolysis cell for continuous hydrogen production. *International Journal of Hydrogen Energy, 34*(2), 672–677. https://doi.org/10.1016/j.ijhydene.2008.11.003

Tenca, A., Cusick, R. D., Schieuano, A., Oberti, R., & Logan, B. E. (2013). Evaluation of low cost cathode materials for treatment of industrial and food processing wastewater using microbial electrolysis cells. *International Journal of Hydrogen Energy, 38*(4), 1859–1865. https://doi.org/10.1016/j.ijhydene.2012.11.103

Thiele, J. H., & Zeikus, J. G. (1988). Control of interspecies electron flow during anaerobic digestion: Significance of formate transfer versus hydrogen transfer during syntrophic methanogenesis in flocs. *Applied and Environmental Microbiology, 54*(1), 20–29.

Toyofuku, M., Nomura, N., Fujii, T., Takaya, N., Maseda, H., Sawada, I., et al. (2007). Quorum sensing regulates denitrification in *Pseudomonas aeruginosa* PAO1. *Journal of Bacteriology, 189*(13), 4969–4972. https://doi.org/10.1128/JB.00289-07

Van Eerten-Jansen, M. C. A. A., Heijne, A. T., Buisman, C. J. N., & Hamelers, H. V. M. (2012). Microbial electrolysis cells for production of methane from CO_2: Long-term performance and perspectives. *International Journal of Energy Research, 36*(6), 809–819. https://doi.org/10.1002/er.1954

Villano, M., Aulenta, F., Ciucci, C., Ferri, T., Giuliano, A., & Majone, M. (2010). Bioelectrochemical reduction of CO_2 to CH_4 via direct and indirect extracellular electron transfer by a hydrogenophilic methanogenic culture. *Bioresource Technology, 101*(9), 3085–3090. https://doi.org/10.1016/j.biortech.2009.12.077

Villano, M., Monaco, G., Aulenta, F., & Majone, M. (2011). Electrochemically assisted methane production in a biofilm reactor. *Journal of Power Sources, 196*(22), 9467–9472. https://doi.org/10.1016/j.jpowsour.2011.07.016

Wagner, R. C., Regan, J. M., Oh, S. E., Zuo, Y., & Logan, B. E. (2009). Hydrogen and methane production from swine wastewater using microbial electrolysis cells. *Water Research, 43*(5), 1480–1488. https://doi.org/10.1016/j.watres.2008.12.037

Wang, A. J., Liu, W. Z., Cheng, S. A., Xing, D. F., Zhou, J. H., & Logan, B. E. (2009). Source of methane and methods to control its formation in single chamber microbial electrolysis cells. *International Journal of Hydrogen Energy, 34*(9), 3653–3658. https://doi.org/10.1016/j.ijhydene.2009.03.005

Wang, B., Liu, W. Z., Zhang, Y. F., & Wang, A. J. (2020a). Intermittent electro field regulated mutualistic interspecies electron transfer away from the electrodes for bioenergy recovery from wastewater. *Water Research, 185*, 116238. https://doi.org/10.1016/j.watres.2020.116238

Wang, B., Liu, W., Cai, W., Li, J., Yang, L., Li, X., et al. (2020b). Reinjection oilfield wastewater treatment using bioelectrochemical system and consequent corrosive community evolution on pipe material. *Journal of Bioscience and Bioengineering, 129*(2), 199–205. https://doi.org/10.1016/j.jbiosc.2019.09.001

Wang, B., Liu, W. Z., Zhang, Y. F., & Wang, A. J. (2020c). Bioenergy recovery from wastewater accelerated by solar power: Intermittent electro-driving regulation and capacitive storage in biomass. *Water Research, 175*, 115696. https://doi.org/10.1016/j.watres.2020.115696

Wrighton, K. C., Agbo, P., Warnecke, F., Weber, K. A., Brodie, E. L., DeSantis, T. Z., et al. (2008). A novel ecological role of the Firmicutes identified in thermophilic microbial fuel cells. *The ISME Journal, 2*(11), 1146–1156. https://doi.org/10.1038/ismej.2008.48

Xafenias, N., Zhang, Y., & Banks, C. J. (2013). Enhanced performance of hexavalent chromium reducing cathodes in the presence of *Shewanella oneidensis* MR-1 and lactate. *Environmental Science & Technology, 47*(9), 4512–4520. https://doi.org/10.1021/es304606u

Xiao, B. Y., Chen, X., Han, Y. P., Liu, J. X., & Guo, X. S. (2018). Bioelectrochemical enhancement of the anaerobic digestion of thermal-alkaline pretreated sludge in microbial electrolysis cells. *Renewable Energy, 115*, 1177–1183. https://doi.org/10.1016/j.renene.2017.06.043

Xiao, L., Wen, Z. H., Ci, S. Q., Chen, J. H., & He, Z. (2012). Carbon/iron-based nanorod catalysts for hydrogen production in microbial electrolysis cells. *Nano Energy, 1*(5), 751–756. https://doi.org/10.1016/j.nanoen.2012.06.002

Xing, D., Cheng, S., Logan, B. E., & Regan, J. M. (2010). Isolation of the exoelectrogenic denitrifying bacterium *Comamonas* denitrificans based on dilution to extinction. *Applied Microbiology and Biotechnology, 85*(5), 1575–1587. https://doi.org/10.1007/s00253-009-2240-0

Xing, D., Zuo, Y., Cheng, S., Regan, J. M., & Logan, B. E. (2008). Electricity generation by *Rhodopseudomonas palustris* DX-1. *Environmental Science & Technology, 42*(11), 4146–4151. https://doi.org/10.1021/es800312v

Xing, Y., Fan, S. Q., Zhang, J. N., Fan, Y. T., & Hou, H. W. (2011). Enhanced bio-hydrogen production from corn stalk by anaerobic fermentation using response surface methodology. *International Journal of Hydrogen Energy, 36*(20), 12770–12779. https://doi.org/10.1016/j.ijhydene.2011.07.065

Yang, L. H., Cheng, H. Y., Ding, Y. C., Su, S. G., Wang, B., Zeng, R., et al. (2019). Enhanced treatment of coal gasification wastewater in a membraneless sleeve-type bioelectrochemical system. *Bioelectrochemistry, 129*, 154–161. https://doi.org/10.1016/j.bioelechem.2019.05.013

Yong, Y. C., Yu, Y. Y., Yang, Y., Liu, J., Wang, J. Y., & Song, H. (2013). Enhancement of extracellular electron transfer and bioelectricity output by synthetic porin. *Biotechnology and Bioengineering, 110*(2), 408–416. https://doi.org/10.1002/bit.24732

Zamalloa, C., Arends, J. B., Boon, N., & Verstraete, W. (2013). Performance of a lab-scale bio-electrochemical assisted septic tank for the anaerobic treatment of black water. *New Biotechnology, 30*(5), 573–580. https://doi.org/10.1016/j.nbt.2013.01.009

Zhang, J., Zhang, Y., Quan, X., & Chen, S. (2013). Effects of ferric iron on the anaerobic treatment and microbial biodiversity in a coupled microbial electrolysis cell (MEC)--anaerobic reactor. *Water Research, 47*(15), 5719–5728. https://doi.org/10.1016/j.watres.2013.06.056

Zhang, L. X., Zhou, S. G., Zhuang, L., Li, W. S., Zhang, J. T., Lu, N., et al. (2008). Microbial fuel cell based on *Klebsiella pneumoniae* biofilm. *Electrochemistry Communications, 10*(10), 1641–1643. https://doi.org/10.1016/j.elecom.2008.08.030

Zhang, T., Cui, C., Chen, S., Ai, X., Yang, H., Shen, P., et al. (2006). A novel mediatorless microbial fuel cell based on direct biocatalysis of *Escherichia coli*. *Chemical Communications, 21*, 2257–2259. https://doi.org/10.1039/b600876c

Zhao, F., Rahunen, N., Varcoe, J. R., Chandra, A., Avignone-Rossa, C., Thumser, A. E., et al. (2008). Activated carbon cloth as anode for sulfate removal in a microbial fuel cell. *Environmental Science & Technology, 42*(13), 4971–4976. https://doi.org/10.1021/es8003766

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. *Scientific Reports, 4*, 6658. https://doi.org/10.1038/srep06658

Zhen, G., Kobayashi, T., Lu, X., & Xu, K. (2015). Understanding methane bioelectrosynthesis from carbon dioxide in a two-chamber microbial electrolysis cells (MECs) containing a carbon biocathode. *Bioresource Technology, 186*, 141–148. https://doi.org/10.1016/j.biortech.2015.03.064

Zhi, Z., Pan, Y., Lu, X., Zhen, G., Zhao, Y., Zhu, X., et al. (2019). Electrically regulating co-fermentation of sewage sludge and food waste towards promoting biomethane production and mass reduction. *Bioresource Technology, 279*, 218–227. https://doi.org/10.1016/j.biortech.2019.01.142

Zuo, Y., Xing, D., Regan, J. M., & Logan, B. E. (2008). Isolation of the exoelectrogenic bacterium *Ochrobactrum anthropi* YZ-1 by using a U-tube microbial fuel cell. *Applied and Environmental Microbiology, 74*(10), 3130–3137. https://doi.org/10.1128/AEM.02732-07