# Chapter 11
# Biofuel Production from Bioelectrochemical Systems


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## 1 Introduction

Environmental pollution and the global energy crisis call for new renewable technologies to support a more sustainable society. Microbial electrolysis cells (MECs) and microbial electrosynthesis cells can degrade organic matter and pollutants in wastewater; when producing biofuels, they offer promising renewable energy technologies for carbon dioxide ($CO_2$) reduction and wastewater treatment. In the two bioelectrochemical systems, hydrogen and methane ($CH_4$) can be easily produced by applying a voltage of 0.2–0.6 V, and other value-added products, such as acetate, ethanol, hydrogen peroxide, and formic acid, also can be produced at low overpotentials [1–3]. Many challenges still face these bioelectrochemical systems, such as the low production rate of biofuels, hydrogen re-oxidation, and the difficult separation of liquid products. In this chapter, we review the recent progress in electrodes and reactor configurations, electrode materials, electron transfer mechanism, and applications of the two systems.

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## 2 Hydrogen Production from MECs

### 2.1 Working Principle of MECs

Similar to a typical dual-chamber microbial fuel cell (MFC), a typical MEC reactor consists of an anode and a cathode chamber, which are separated by an ion exchange membrane. On the anode, exoelectrogenic microorganisms (such as the *Geobacter* and *Shewanella* species) colonize on the surface and oxidize organic substrates (such as acetate and glucose in wastewater) to produce electrons and protons. The generated electrons transfer to the anode via direct or indirect electron transfer and pass through the circuit to the cathode. On the cathode, the electrons combine with the protons permeating from the anode chamber to produce hydrogen. During this operation, both the anode and cathode chambers are maintained at anaerobic conditions (Fig. 1).

Unlike the reactions in a MFC, the reaction in an MEC cannot spontaneously occur because of its irreversibility. According to the Nernst equation, under typical biological conditions (T = 25 °C, p = 1 bar, and pH 7.0), the standard potential of $H^+/H_2$ (the cathode reaction) can be calculated as shown in the following equations:

$$2H^+ + 2e^- \rightarrow H_2 \tag{1}$$

$$E_{cat} = -\frac{RT}{2F}\ln\left(\frac{p_{H_2}}{[H^+]^2}\right) \tag{2}$$

where $p_{H_2}$ is the partial pressure of hydrogen, $F$ = 96,485 (C/mol; the Faraday constant), $T$ is the temperature, and $R$ is the ideal gas constant. Under standard biological conditions, the cathode potential is equal to −0.414 V versus the standard hydrogen electrode (SHE). The potential of the bioanode in an MEC, which uses acetate as an electron donor, can be calculated using the following equations:

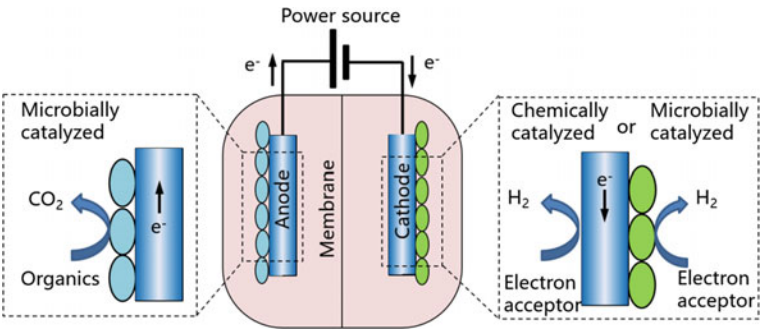


**Fig. 1** The working principle of microbial electrolysis cell

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad (3)$$

$$E_{an} = E_{an}^0 - \frac{RT}{8F} \ln\left(\frac{[CH_3COO^-]}{[HCO_3^-]^2[H^+]^9}\right) \quad (4)$$

Under standard biological conditions, the anode potential is equal to −0.279 V versus SHE. Therefore, the voltage required for the operation of an MEC is $E_{eq}$ = (−0.414 V) − (−0.279 V) = − 0.14 V.

In dark fermentation, various organic acids (such as acetate) are considered as end-products, which cannot be degraded by microorganisms [4, 5]. Notably, these end products can be degraded by the bioanodes of MECs. For a reaction that occurs spontaneously, the Gibbs free energy ($\Delta G_r$) must be negative, but the conversion of such end-products to hydrogen yields a positive $\Delta G_r$ in dark fermentation. In MECs, acetate and protons are commonly used as the electron donor and electron acceptor, respectively. Under biological conditions, the reaction equation and Gibbs free energy (ΔGr″) of acetate oxidation to hydrogen are as follows [4]:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + H^+ + 4H_2,\ \Delta Gr'' = +104.6\ \text{kJ/mol} \quad (5)$$

The positive Gibbs free energy means that acetate cannot be spontaneously fermented to hydrogen in the MECs, and thus additional energy has to be added to MECs to realize the reaction. According to thermodynamics, the applied voltage needs to be larger than ΔGr″/nF, where n is the amount of electrons involved in the reaction (for hydrogen production, n = 2), and F is the Faraday constant. The voltage calculated from thermodynamics is referred as the equilibrium voltage, $E_{eq}$. For MECs that use acetate as the electron donor under standard biological conditions, the voltage is

$$E_{eq} = -\Delta Gr''/nF = -104.6 \times 10^3/4 \times 2/96485 = -0.14\ \text{V} \quad (6)$$

where the negative sign indicates that the reaction is not spontaneous.

In practice, a voltage between 0.2 and 0.6 V is required for an efficient hydrogen production rate because of the overpotentials on the electrodes, ohmic losses, and concentration losses in the systems. Yet, the input voltage is still substantially lower than the voltage necessary for conventional water electrolysis (in practice, greater than 1.6 V) [6].

## 2.2 MEC Systems

### 2.2.1 Configuration

Various MEC configurations have been proposed for high-efficiency hydrogen production [4, 7]. Generally, MEC configurations can be classified into dual-chamber and single-chamber reactors. In the dual-chamber reactors, the anode and cathode chambers are divided by a separator (mainly ion exchange membranes), by which the anodic and cathodic reactions cannot be affected by each other. The dual-chamber can minimize hydrogen re-oxidation by microorganisms in the anode chamber [8] and can prevent the mixing of hydrogen generated in the cathode chamber and the $CO_2$ generated in the anode chamber. The H-type reactor is a typical dual-chamber reactor that has been widely used in MEC experiments [4, 9, 10]. This kind of reactor has a high internal resistance because of the large distance between the anode and cathode and the small size of the separating membrane [10], and these issues largely limit the performance of MECs. There are various approaches to enhancing the hydrogen production performance of H-type MECs, such as increasing the size of the membrane relative to the electrode-projected surface areas [5, 11], using a high surface area electrode [12, 13], and reducing the distance between the anode and cathode [14]. For example, Cheng et al. clamped an anion exchange membrane (AEM) between the anode (30 mm in diameter, 20 mm long; 14 ml) and cathode chambers (30 mm in diameter, 40 mm long; 28 ml), and obtained a hydrogen production rate of 1.1 $m^3$-$H_2$ $m^{-3}$ $d^{-1}$ at an applied voltage of 0.6 V [5]. Liu et al. used graphite granules as the anode and a graphite rod that was inserted into the granules as an electron conductor, significantly increasing the surface area of the anode [15]. Because higher anode surfaces are suitable for the attachment of microorganisms on the anode, a higher MEC performance was obtained by using this anode in contrast to that in a plain carbon cloth anode. Inspired by the membrane-electrode-assembly in proton exchange membrane fuel cells (PEMFCs), Rozendal et al. proposed an MEC reactor using the membrane-electrode-assembly module, in which the anode and cathode are pressed onto the two sides of the ion exchange membrane. Using the membrane-electrode-assembly module, the distance between the anode and cathode are significantly reduced, thereby resulting in a higher hydrogen production performance [11].

The cation exchange membrane (CEM) and AEM are commonly used separators between the anode and cathode chamber in MECs. Cations (such as $Na^+$, $K^+$, and $NH_4^+$) and anions (such as $OH^-$) can pass through the CEM and AEM, respectively. However, when the CEM (especially a Nafion membrane) is used in an MEC, cation species (such as $Na^+$, $K^+$, and $NH_4^+$) other than protons are responsible for the positive charge transport through the membrane because of their much higher concentration than the protons in the cathodic liquid (pH 7.0). As a result, the protons consumed at the cathode cannot be replenished by the protons generated at the anode, leading to a pH increase in the cathode chamber and a pH decrease in

the anode chamber, leading to a loss of voltage according to the Nernst equation. To deal with this problem, Tartakovsky et al. proposed using a J-cloth, a material without electrical conductivity, as the separator in the MECs [14]. Cations and anions can both pass through a J-cloth, resulting in equal pHs in the anode and cathode. A bipolar membrane, which consists of a cation-selective layer and an anion-selective layer, also has been used, because it can dissociate water to $H^+$ and $OH^-$ under a reverse bias direct current field, thereby controlling the pH of the anodic and cathodic liquid [16, 17].

No matter what kind of separators are used, the existence of separators between the anode and cathode chamber can increase the ohmic resistance. Therefore, a configuration without separators (i.e., single-chamber reactors) was proposed to reduce the ohmic resistance and increase the performance of MECs [11, 15, 18, 19]. Call et al. reported the first single-chamber reactor for hydrogen production in an MEC and achieved a hydrogen production rate of $3.12 \pm 0.02$ $m^3$-$H_2$ $m^{-3}$ $d^{-1}$ at an applied voltage of 0.8 V [18]. Although it has been reported that hydrogen can be re-oxidized by anode-respiring bacteria [8], Call and Logan [18] demonstrated that it was possible to achieve a high hydrogen recovery and production rate in single-chamber MECs, potentially reducing the costs of MECs and enabling the construction of simpler designs. This process, however, resulted in other negative impacts because it eliminated the ion exchange membranes, such as hydrogen consumption by methanogens [20, 21] and the gas mixing of the $CO_2$ that was produced by the bioanodes. For example, Cusick et al. constructed a pilot-scale MEC that was inoculated with winery wastewater and reported that $CH_4$ was the main gas product that resulted from the long operation cycles [21]. Therefore, the primary challenge of developing a single-chamber MEC is to avoid $CH_4$ production, especially when complex inoculum are used.

### 2.2.2 Electrodes Materials and Cathode Catalysts

Carbon materials are commonly used as electrode materials in MECs. On the anode, the catalytic reactions are essentially the same as those in the MFC anodes. Thus, the materials used as anodes in MFCs also can be used as anode materials in MECs. For example, carbon cloth, carbon paper, graphite felt, graphite granules, and carbon brushes are commonly used as anode materials in MECs [12].

Carbon materials are commonly used also as the cathode materials in MECs. The reaction rate is relatively slow on plain carbon electrodes because of the high overpotentials. To reduce the overpotentials, metal catalysts, such as platinum (Pt), nickel (Ni), and stainless steel, are often used at the cathodes. Among them, Pt is the most investigated catalyst because of its low overpotential (0.05 V at 15 A $m^{-2}$) for hydrogen evolution under optimized conditions (at a pH of 6.2 for the phosphate buffer) [12, 22]. Cheng and Logan [5] constructed the first MEC reactor that used Pt (0.5 mg $cm^{-2}$) as the catalyst on the cathode and obtained a hydrogen production rate of 1.1 $m^3$-$H_2$ $m^{-3}$ $d^{-1}$ at an applied voltage of 0.6 V. Call and Logan [18, 23] used a Pt catalyst on a carbon cloth in a single-chamber MEC and obtained

hydrogen production rates of 3.12 ± 0.02 $m^3$ $m^{-3}$ of reactor liquid volume per day at an applied voltage of 0.8 V with a hydrogen recovery of 96%.

The application of Pt in cathode catalysts is largely limited due to its high cost, however. In addition, Pt can be easily poisoned by sulfide, which is a common constituent of wastewater [4, 23]. Further studies found that nickel (Ni) alloys and stainless steel (SS) were promising catalysts because of their availability, low overpotentials, and stability in wastewater [23]. Selembo et al. [24] investigated the influence of different SS and Ni alloys on the hydrogen production rate in MECs. They showed that stainless steel A286 was superior to Pt sheet metal in terms of its cathodic hydrogen recovery (61 vs. 47%), overall energy recovery (46 vs. 35%), and maximum volumetric hydrogen production rate (1.5 vs. 0.68 $m^3$ $m^{-3}$ $d^{-1}$) at an applied voltage of 0.9 V. Although Ni 625 was better than other Ni alloys, it did not perform as well as stainless steel A625. They also reported that the performance of stainless steel and Ni cathodes could be further increased by electrodepositing a nickel oxide layer onto the sheet metal, although the performance of the nickel oxide cathodes decreased over time because of a reduction in the stability of the oxides. To further improve the hydrogen production rate, three-dimensional (3D) materials were also used as cathode materials because of their high specific surface area. Many nonprecious materials were used as 3D cathodes. Call et al. [25] showed that a stainless steel brush cathode, 2.5 cm long and 2.5 cm in diameter with a specific surface area of 810 $m^2$ $m^{-3}$, achieved a hydrogen production rate and efficiencies similar to those achieved with a Pt-catalyzed carbon cloth. The hydrogen production rate of the stainless steel brush was 1.7 ± 0.1 $m^3$-$H_2$ $m^{-3}$ at an applied voltage of 0.6 V. Zhang et al. [26] studied the effect of the stainless steel mesh size on the performance of MECs. They showed that a stainless steel mesh with a relatively thick wire size (0.02 cm), a medium pore size (0.02 cm), and a specific surface area of 66 $m^2$ $m^{-3}$ had the best performance with a hydrogen production rate of 2.1 ± 0.3 $m^3$-$H_2$ $m^{-3}$ and hydrogen recovery of 98 ± 4% at an applied voltage of 0.9 V.

Graphene and carbon nanotubes (CNTs), materials with a good conductivity and excellent performance for the modification of bioanodes in MFCs, also were used in the MEC cathodes. A 3D hybrid of layered $MoS_2$/nitrogen-doped graphene nanosheet aerogels were used as cathode catalysts in MECs [27]. A high current density of 0.36 mA $cm^{-2}$ and a hydrogen production rate of 0.19 $m^3$-$H_2$ $m^{-3}$ $d^{-1}$ was achieved at a 0.8 V bias. Hou et al. suggested that the outstanding performance of the hybrid cathode benefited from its 3D conductive networks, porous structure, and strong synergic effects between the $MoS_2$ nanosheets and N-gas. Cai et al. [28] constructed a cathode using 3D self-assembly Ni foam-graphene in MECs. In this study, improved electrochemical activity and effective mass diffusion were achieved after coating the Ni foam with graphene. The average hydrogen production rate was comparable to that of the Pt/C (1.32 ± 0.07 $m^3$-$H_2$ $m^{-3}$ $d^{-1}$) catalyst at an applied voltage of 0.8 V. Dai et al. synthesized a series of nano-$Mg(OH)_2$/graphene composites via the hydrothermal method [29]. The cathode with this composite exhibited good stability, and its current density was comparable to that of the Pt/C cathode. CNTs, widely used in super-capacitors and MFCs, also can be

used as a base material to synthesize nanoparticles as cathode catalysts in MECs. Wang et al. [30] used a CNT-based electrode as an alternative to Pt in a single chamber MEC and achieved a hydrogen production rate of 1.42 $m^3\ m^{-3}\ day^{-1}$ with a current density of 192 A $m^{-3}$ at an applied voltage of 0.9 V. Furthermore, conductive polymers, which have been used in various electrochemical devices [31, 32], also have attracted significant attention for their applications in MECs. For example, polyaniline was used to modify the cathode with multi-walled CNTs [33], and a hydrogen production rate of 1.04 $m^3\ m^{-3}\ day^{-1}$ and current density of 163 A $m^{-3}$ were achieved.

## 2.3 *Biocathode Catalyzing $H_2$ Evolution in MECs*

### 2.3.1 Development of Biocathodes

Inspired by the electricity generation using exoelectrogenic microorganisms on bioanodes, researchers proposed to use microorganisms as the catalysts on the cathode to produce hydrogen in MECs. Rozendal et al. reported on the first biocathode that was capable of catalyzing hydrogen evolution in MECs. The biocathode was achieved through a three-phase biocathode startup procedure, which turned an acetate- and hydrogen-oxidizing bioanode into a hydrogen-producing biocathode by reversing the electrode's polarity [34]. Compared to the plan graphite felt, the hydrogen production rate of the biocathode significantly increased. Jeremiasse et al. demonstrated the proof-of-concept of an MEC in which both the anode and cathode reaction were catalyzed by microorganisms. At an applied voltage of 0.5 V and a cathode potential of −0.7 V versus SHE, a maximum current density of 1.4 A $m^{-2}$ and 3.3 A $m^{-2}$ were achieved, respectively. In contrast, a control cathode (graphite felt without a biofilm) only showed a current density of 0.3 A $m^{-2}$ at a potential of −0.7 V versus SHE [35].

Biocathodes still have room for improvement when compared with the current density generated by the cathodes with metal catalysts. For example, current densities in the range of 4–10 A $m^{-2}$ are typically achieved when Pt is used on the cathode, whereas it was only around 1.2 A $m^{-2}$ with a biocathode at a cathode potential of −0.7 V versus SHE [34]. However, the biocathode possesses other attractive advantages, as it is inexpensive, not easily poisoned by wastewater, and capable of self-regeneration.

Various methods have been proposed to modify the cathode surface to further improve the hydrogen production rate of biocathodes. For example, CNTs, graphene, and polymers have been used to modify biocathodes [36, 37]. Polyaniline was reported to improve the electrode's bioaffinity and electron transfer [38]. Carbon nanotubes were found to reinforce the electrochemical activity of the electrode. Chen et al. [36] used polyaniline and CNTs to modify a biocathode and achieved a hydrogen production rate of 0.67 $m^3\ m^{-3}\ day^{-1}$ at an applied voltage of 0.9 V. They reported that some electrode characteristics, such as the number of

active positions [39], ability of electron transfer [38, 40], and specific surface area [41], could be improved by using polyaniline and multi-walled CNTs. Graphene [37] was also used to promote the performance of the hydrogen production of biocathodes. Su et al. constructed a biocathode modified by graphene and assessed the performance of this biocathode under different cathode potentials [37]. At a cathode potential of −0.9 V versus SHE, the hydrogen production rate of the modified biocathode achieved 2.49 ± 0.23 $m^3$ $m^{-3}$ $day^{-1}$, which was about three times higher than that of the unmodified biocathode. In their research, the hydrogen production performance of the modified biocathode was similar to that of the cathode that was catalyzed by Pt and superior to that of the stainless steel mesh cathode at −0.9 V versus SHE. In addition to the surface modification of the cathode, the employment of microorganisms with better catalytic properties also improved performance. Fu et al. [42] used thermophilic microorganisms to develop a hydrogen producing biocathode with the advantages of thermophilic microorganisms, such as a higher reaction activity and greater durability [43, 44]. At a potential of −0.8 V versus SHE, the thermophilic biocathode achieved a current density of 1.28 ± 0.15 A $m^{-2}$ and a hydrogen production rate of 376.5 ± 73.42 mmol $m^{-2}$ $day^{-1}$ at 55 °C, which were around 10 times higher than those same values achieved with noninoculated electrodes.

Notably, in MECs with biocathodes, hydrogen can be further converted to $CH_4$ by methanogens, which were commonly enriched with hydrogen and $CO_2$ [20, 21, 45]. To improve the hydrogen production rate in MECs, several approaches have been used to inhibit the growth of methanogens. For example, biocathodes have been exposed to air to inhibit the growth of methanogens [18]. A specific inhibitor for methanogens also has been applied [46].

### 2.3.2 Microbial Ecosystem of Biocathodes

Microorganisms adhering on cathode surfaces can catalyze hydrogen production, but it is still not well understood how those microorganisms catalyze the reaction. To understand the working principle of a biocathode, Croese et al. analyzed the microbial community of a mixed culture biocathode [47]. They reported that the bacterial population consisted of 46% *Proteobacteria*, 25% *Firmicutes*, 17% *Bacteroidetes*, and 12% other phyla. They also found that the *Desulfovibrio* species were the dominant microorganisms at the biocathode. Fu et al. analyzed the bacterial community of a thermophilic biocathode [42] and found that *Firmicutes* was the dominant phylum (77.4%), followed by *Coprothermobacter* (19.8%).

Few studies have examined the electron transfer manners of microorganisms adhering on cathodes. It has been hypothesized that the electron transfer between the electrode and the microorganisms may be possible reverse reactions of those in the bioanodes, as some similarities were found between anodic bacteria and cathodic bacteria. For example, the genomes of the *Desulfovibrio* species encode several c-type cytochromes and multicopper proteins, which show homologies to the proteins involved in the electron donation in the *Geobacter* species (a main

bacteria species in bioanodes). Similar to the pilin-like appendages that were reported to be electron transfer structures in the *Geobacter* sp., the *D. vulgaris* flagellar appendages are involved in a physical association during syntrophic growth with other microbes and also might be involved in the adherence to electrodes. These similarities suggest that the mechanism of extracellular electron transfer by the *Desulfovibrio* species could be, at least partly, similar to the electron transfer mechanisms at the bioanodes [47].

## 2.4 Development and Application of MECs

### 2.4.1 MFC-MEC Coupled Systems

In theory, an applied voltage of 0.14 V is required to drive the production of hydrogen in MECs [4]. In practice, a voltage of 0.6 V or more is required for high-efficiency hydrogen production because of the overpotentials [4, 18]. Notably, the open circuit voltage of a typical MFC can reach as high as 0.8 V [48]; thus, a high-efficiency hydrogen production may be achieved by using an MFC to power an MEC, creating an MFC-MEC coupled system. In this system, hydrogen can be harvested from substrates, and no external power supply is required. Min et al. reported the first demonstration of an MFC-MEC coupled system, which combined a single-chamber MFC with an air cathode and a dual-chamber MEC. Using acetate (0.1 g $L^{-1}$) as the electron donor in both the MFC and MEC, the hydrogen production rate of the system reached 2.2 ± 0.2 mL $L^{-1}$ $d^{-1}$. The cathodic hydrogen recovery and overall systemic Columbic efficiency were 88%–96% and 28%–33%, respectively. The overall systemic hydrogen peak production was 1.21 mol-$H_2$/mol-acetate [49]. Performance of the coupled system was further investigated under different configurations: When the resistor changed from 10 Ω to 10 kΩ, the results showed that the hydrogen production rate varied in the range of 2.9 ± 0.2–0.2 ± 0.0 mL $L^{-1}$ $d^{-1}$. The hydrogen production rate increased significantly when the MFCs were connected in a series, whereas it slightly decreased when the MFCs were connected in parallel [50].

### 2.4.2 Photo-Microbial Coupled System

As an environmentally friendly approach to generating hydrogen, the direct utilization of renewable energy (such as solar) is an obvious but still challenging choice. A dye-sensitized solar cell (DSSC)-driven MEC system was reported in the literature [51–54], where an external solar cell taking the place of the electrical bias was coupled with an MEC device to supply the required additional energy. Furthermore, a solar-powered MEC system integrating the microbial anode and semiconductor photocathode (such as $Cu_2O$ [55], $TiO_2$ [56]) has been shown to

generate hydrogen effectively. It can minimize the material preparation and device fabrication costs of a DSSC-driven MEC.

### 2.4.3 MEC-Fermentation Coupled Systems

Because of the thermodynamic limitations (refer to Sect. 2.1), many organic compounds produced by dark fermentation cannot be further degraded into hydrogen via fermentation [4, 5]. An MEC can be coupled with fermentation to further degrade these dead-end products. For example, Lu et al. fed a single-chamber MEC with the effluent that was produced in an ethanol-type fermentation reactor. The MEC achieved a hydrogen production rate of $1.41 \pm 0.08$ $m^3$ $L^{-3}$ $d^{-1}$ at an applied voltage of 0.6 V, much higher than that ($0.70$ $m^3$ $L^{-3}$ $d^{-1}$) of the fermentation reactor [57]. MECs also were used to degrade the fermentation effluent of recalcitrant substrates, such as lignocellulose and cellobiose. Lalaurette et al. achieved a hydrogen production rate of $0.96 \pm 0.16$ L $L^{-1}$ $d^{-1}$ (cellobiose) and $1.00 \pm 0.19$ L $L^{-1}$ $d^{-1}$ (lignocellulose), respectively, when the MECs were fed with the fermentation effluent of lignocellulose and cellobiose [58]. Yan et al. fed MFCs with the fermentation effluent of xylose and corncob hydrolysate. When a current was generated, the MFCs were used as MECs to produce hydrogen. The hydrogen production rates of 41.7 and 23.3 mmol per mol-acetate were achieved with the xylose and corncob hydrolysate effluent, respectively [59]. The fermentation effluents of cellulose [60] and glycerol [61] were also used as electron donors in MECs.

### 2.4.4 MECs for Wastewater Treatment

It has been reported that 7.6 kJ $L^{-1}$ energy was obtained from domestic wastewater [62], indicating that wastewater contains abundant energy. Both MFCs and MECs were used to recover energy from wastewater. MECs have some advantages over MFCs from both an economic and environmental perspective [63, 64]. Several MEC reactors were designed for wastewater treatment. Ditzig et al. [65] designed the first MEC that used domestic wastewater as the substrate. A double-chamber reactor was used to treat domestic wastewater at the anode chamber with applied voltages of 0.2–0.6 V. The MEC was operated in the fed-batch mode and removed COD almost completely (87–100%). The hydrogen yield (ca. 10% of the theoretical value) was low because of the low conversion of the substrate and hydrogen loss.

Laboratory results at the pilot scale must be used to assess the practical application of MECs and to estimate the durability of their critical components, such as the electrodes and membranes. Cusick et al. constructed the first pilot-scale MEC to treat actual wastewater from a winery plant [21]. The MEC was a 1,000 L-volume single-chamber reactor that used graphite fiber brushes as anodes and SS mesh as cathodes. The MEC achieved a hydrogen production rate of 0.2 L $L^{-1}$ $d^{-1}$ and an

average soluble COD removal of 62%. The produced gas, however, was mainly composed of $CH_4$ (86%) and $CO_2$, with trace amounts of hydrogen, because the produced hydrogen was further converted to $CH_4$ by methanogens. Heidrich et al. [66] constructed a 120 L-volume MEC system, which consisted of six independent MEC modules using a stainless steel cathode and low-cost microporous membrane for domestic wastewater treatment. The MEC system produced virtually pure hydrogen gas (100 ± 6.4%) for more than 3 months with an average COD removal efficiency of 34% and hydrogen production rate of 0.015 L $L^{-1}$ $d^{-1}$.

Additionally, as the cathode potential of MECs can be controlled with the electricity supply, recalcitrant pollutants (such as nitrobenzene and 4-chlorophenol) can be reduced as electron acceptors at the cathodes. Compared with conventional electrochemical reduction, the removal of these pollutants in MECs consumes much less energy. Furthermore, electroactive microorganisms on the anode or cathode could greatly lower the overpotential of the electrochemical reactions, leading to higher removal efficiencies and rates.

## 3 Methane Production from Electromethanogenesis

### *3.1 Working Principle of Electromethanogensis*

In practice, MECs are usually inoculated with wastewater and sludge, as electrochemically active microorganisms are enriched in these environments. Coincidently, methanogens are also often enriched in wastewater and sludge, resulting in the production of $CH_4$ (rather than hydrogen) in MECs using biocathodes [21, 67, 68]. Although several approaches have been employed to inhibit the growth of methanogens in MECs [46], most of these approaches are ineffective or energy intensive. However, the production of $CH_4$ in bioelectrochemical systems possesses several advantages over hydrogen production. The storage requirements for $CH_4$ are not as restrictive as those for hydrogen. Moreover, $CH_4$ can be more easily integrated into the existing infrastructure. Furthermore, the standard potential of $CO_2/CH_4$ is higher than that of $H^+/H_2$ under neutral conditions, suggesting that bioelectrochemical $CH_4$ production is potentially more energy saving than hydrogen production in MECs.

Electromethanogensis (EM), a derivative of MEC, is a promising technology that can convert electric energy and $CO_2$ into $CH_4$ using microorganisms as biocatalysts. The configuration and working principle of EM are similar to that of MECs. Generally speaking, electrochemically active microorganisms adhering on the anode oxidize organic matter and transfer electrons to the anode. The electrons pass through the external circuit to the cathode with the assistance of a power source. On the cathode, microorganisms (mainly methanogens) attached on the surface utilize electrons from the cathode to reduce $CO_2$ to $CH_4$.

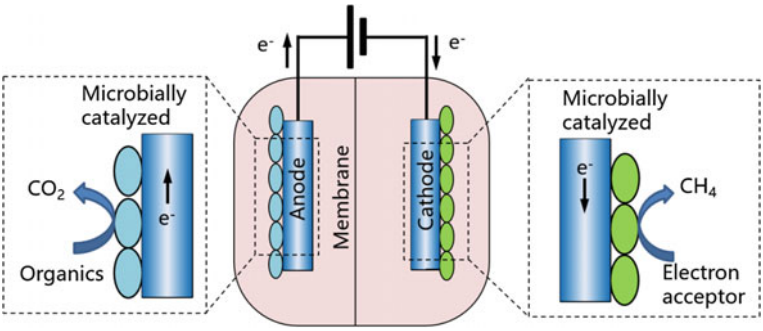


**Fig. 2** The working principle of electromethanogenesis

According to the Nernst equation, under neutral conditions, the standard potential of acetate/$CO_2$ (a typical anode reaction) and $CO_2$/$CH_4$ (cathode reaction) is −0.28 V and −0.24 V versus SHE, respectively. Thus, in theory, a $CH_4$-producing bioelectrochemical systems (BES) can be a spontaneous system based on a thermodynamic analysis. In practice, because of the overpotentials, ohmic losses, and concentration losses, a voltage of 0.6 V or more is required for efficient $CH_4$ production (Fig. 2).

## *3.2 Development of Electromethanogensis*

Park et al. reported on $CH_4$ production in a bioelectrochemical system for the first time and showed that methanogens could accept electrons through hydrogen or reduced neutral red to convert $CO_2$ to $CH_4$ [69]. In the following years, after hydrogen-producing biocathodes were first reported [34], researchers found that the produced hydrogen could be further converted into $CH_4$ by the hydrogenotrophic methanogens existing in the reactor. Cusick et al. developed a pilot-scale MEC using winery wastewater [21]. They found that the hydrogen produced at the cathodes was converted into $CH_4$. The conditions in the reactor that enriched the exoelectrogens and hydrogenogens were also suitable to promote the growth of the methanogens. Clauwaert et al. used an abiotic cathode of an MEC to produce hydrogen, which was further converted into $CH_4$ via anaerobic digestion in an external reactor [70]. Clauwaert and Verstraete were the first to use a biocathode to generate $CH_4$ as the main product in a single-chamber BES [45]. In these studies, hydrogen-mediated electron transfer from the cathode to the methanogens played the role of an electron shuttle. In other words, hydrogen was first produced on the biocathode and quickly consumed for hydrogenotrophic methanogenesis. As the heating value of hydrogen is much higher than that of $CH_4$, it was considered somewhat a pity to convert hydrogen to $CH_4$.

To avoid intermediate chemical transitions and to achieve a direct bioelectrochemical conversion of $CO_2$ to $CH_4$ at a biocathode, Cheng et al. used a two-chamber BES with $CO_2$ as the sole electron acceptor at the cathode. Although no significant hydrogen production was detected in this study, microbial- or abiotic-generated hydrogen may have acted as a mediating component between the cathode and methanogens [71]. Because of a relatively high cathode potential, the $CH_4$ production rate was much lower than that of a BES in which hydrogen mediated the electron transfer from the electrode to the methanogens [72]. Villano et al. indicated that bioelectrochemical $CH_4$ production can occur via both direct electron transfer and the intermediate production of hydrogen gas [73]. Fu et al. acclimated a biocathode that could produce $CH_4$ at a potential of −0.35 V versus SHE, suggesting that methanogens could directly accept electrons from the cathode surface without generating hydrogen.

## 3.3 *Mechanisms of Electron Transfer from the Electrode to the Methanogens*

Two major pathways have been proposed for the electron transfer from the electrodes to the methanogenic archaea (methanogen): Direct and indirect (mediated) electron transfer (Fig. 3; Pathways I and II) [73–76]. Until recently, however, these models could not be examined conclusively because of their experimental set-ups.

In most studies, environmental samples (such as anaerobic sludge and bioreactor effluents) were used as the inoculums [71, 72, 77, 78]. Thus, the resulting biocathodes generally contained multiple species of undefined metabolic abilities. It therefore was difficult to examine the roles of each microbial species on electromethanogenesis and the biocathode ecosystem. Moreover, ferredoxin and coenzyme F420, the central electron carriers of methanogens, have midpoint potentials in the range of −0.36 to −0.42 V versus SHE, which overlap with the redox potential of the small intermediates (such as hydrogen, −0.41 V vs. SHE, at a neutral pH) [79, 80]. Thus, the cathode potentials are negative enough to enable the

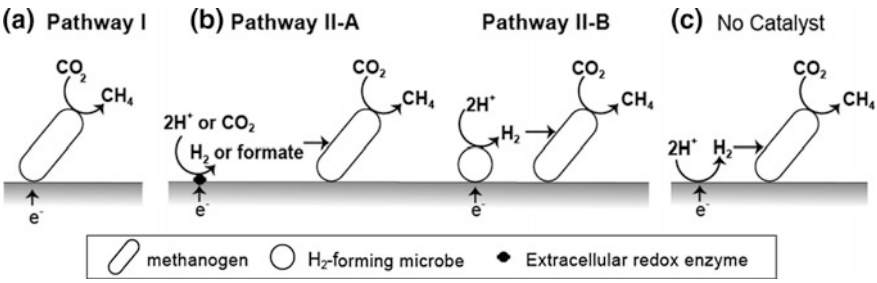


**Fig. 3** Electrons are transferred from the electrode to the methanogens via the direct electron transfer (Pathway I) and indirect electron transfer (Pathway II)

direct electron transfer to the redox-active components of the methanogens, and they can also facilitate the intermediate formations, making the discrimination of the electron-transfer pathways difficult.

Recently, several studies on electromethanogenic systems using defined species provided key insights into the electron transfer mechanisms by addressing the contributions of each pathway, as well as the role of each microbial species [81–84]. In this section, we describe the current knowledge about the electron transfer mechanisms at the electromethanogenic biocathode, particularly focusing on these studies.

### 3.3.1 Pathway I: Direct Electron Transfer from the Electrode to the Methanogens

Beese-Vasbender et al. reported electromethanogenesis by a pure culture of a biocathode inoculated with the strain IM1 [81]. The strain IM1 is an iron-corroding hydrogenotrophic methanogen closely related to *Methanobacterium* [85]. The authors employed a dual-compartment bioelectrochemical cell in which two bioreactors were connected via a salt bridge, and therefore, possible interferences from the anode side (such as the contamination of microbes, organic substrates, or reactive oxygen species from the anode compartments) to the cathode were minimized (Fig. 4).

A pure culture of the strain IM1 was inoculated onto a pre-sterilized cathode and incubated at the set potential of −0.4 V versus SHE. The IM1-inoculated biocathode started to produce $CH_4$ at 12 days postinoculation. The $CH_4$ production rate increased simultaneously with the increase in the current density and reached 3.5 mmol $m^{-2}$ $day^{-1}$ with a columbic efficiency of about 80% at 23 days postincubation. No appreciable $CH_4$ production or increase in the current density were observed at the cathode inoculated with *Methanococcus maripaludis* (another hydrogenotrophic methanogen) or the noninoculated control, suggesting that the strain IM1 had the ability of catalyzing electromethanogenesis at the cathode.

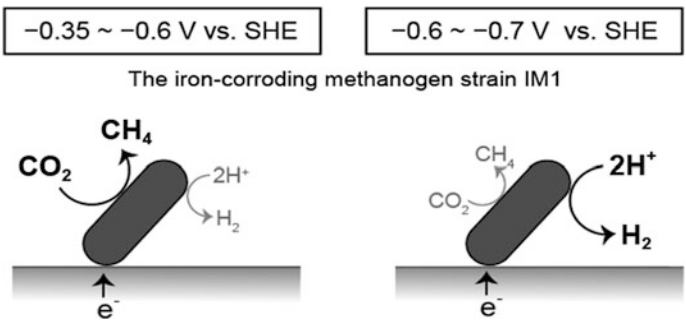


**Fig. 4** Electron transfer manner of the iron-corroding methanogen strain IM1

Cyclic voltammetry with the IM1-inoculated biocathode showed that the cathodic current increased when the cathode potential was lowered, indicating a facilitated electron transfer to the redox active components closely attached to the electrode's surface. On the cathode's surface, the strain IM1 cells were directly attached to the electrode's surface and relatively sparsely distributed. No obvious biofilm was observed, implying that soluble electron mediators (such as hydrogen) or conductive pili were likely not involved in the electron transfer from the electrode to the strain IM1. Taken together, these observations strongly suggest that the methanogens alone can take up electrons from the cathode's surface.

The $CH_4$ and current production by the IM1-inoculated biocathode showed a dependence on the set potential. At potentials around −0.3 V versus SHE, the $CH_4$ and current production rates were attenuated to the same level as the noninoculated cathode. At the potentials from −0.4 to −0.6 V versus SHE, the $CH_4$ production rate remained at similar levels, while the cathodic current density was increased as the cathode potential was lowered. However, at potentials more negative than −0.6 V versus SHE, the cathodic current density further increased and was accompanied by hydrogen evolution. The $CH_4$ formation rate was, on the contrary, significantly reduced. This hydrogen evolution (rather than methanogenesis) at lower potentials was likely due to the limited capacity of the enzyme system for methanogenesis and can be a protective mechanism of the strain IM1: By shuttling excess electrons to hydrogen evolution (i.e., hydrogenases), the strain IM1 can avoid the accumulation of negative charges close to the cell and maintain the electrochemical gradient across the cell membrane.

To understand the mechanism underlying the direct-electron transfer from an electrode to the strain IM1, it is crucial to identify the cell-surface-associated redox active component(s) of the methanogen, which serves as the entrance point of the electrons. As the strain IM1 is not genetically tractable, the detailed bioelectrochemical characterization of the outer surface of the cell in combination with proteomic approaches can provide further insights.

### 3.3.2 Pathway II: Indirect Electron Transfer Mediated via Diffusible Intermediates

Pathway II-A: Intermediate Formations Catalyzed by Extracellular Enzymes

At low redox potentials, diffusible molecules (such as hydrogen and formate) can be electrochemically formed on the electrode's surface. Such intermediates can be consumed rapidly by hydrogenotrophic methanogens [86], thereby mediating the electron transfer between the electrode and methanogen. Although the formation of these intermediates is thermodynamically favored at low potentials, the rates of these reactions at carbon electrodes (without catalysts) are too slow in comparison with the $CH_4$ formation rates at biocathodes [71, 73].

Recently, it has been suggested that the formation of intermediates can be catalyzed by extracellular redox enzymes, which are released from microbial cells and

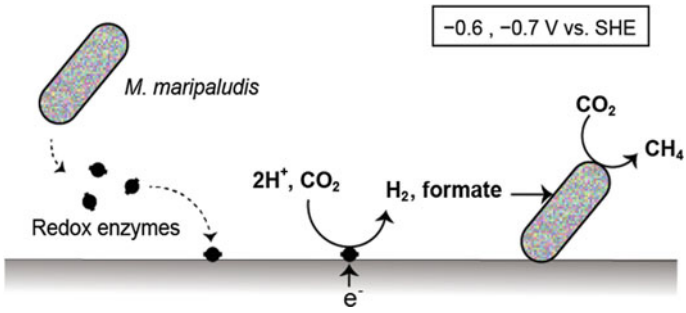


**Fig. 5** Formation of intermediates can be catalyzed by extracellular redox enzymes

adsorbed on the cathode's surface [82] (Fig. 5). Lohner et al. examined a cathode that was inoculated with a pure culture of *M. maripaludis* [84]. *M. maripaludis* is a hydrogenotrophic methanogen and genetically tractable [87, 88]. At a set potential of −0.6 V versus SHE, the *M. maripaludis* (the wild-type)-inoculated cathode produced $CH_4$ at a rate of ca. 11.4 mmol $m^{-2}$ $day^{-1}$ with a columbic efficiency of 70–80%. $CH_4$ formation was not detected in the absence of the low cathode potential (−0.6 V vs. SHE) or the inoculum. At the abiotic electrode, molecular hydrogen was produced at a rate of 1.2 mmol $m^{-2}$ $day^{-1}$, which was too low to account for the $CH_4$ production at the inoculated cathode. When a cathode was inoculated with the *M. maripaludis* strain MM1284, a mutant in which all genes encoding hydrogenases were knocked out, $CH_4$ was produced at the cathode potential of −0.6 V versus SHE. However, the $CH_4$ production rate was largely attenuated (ca. 10% of that of the wild-type-inoculated cathode). In the presence of 2-bromoethanesulfonate, a specific inhibitor of methylcoenzyme *M. reductase* (the key enzyme in the last step in methanogenesis) [89], the wild-type-inoculated cathode produced hydrogen and formate, whereas the cathode inoculated with the strain MM1284 produced only formate, suggesting that the hydrogenase(s) derived from *M. maripaludis* was responsible for the hydrogen formation.

Strikingly, the cell-free spent medium of the *M. maripaludis* culture could catalyze the formation of hydrogen as well as formate at a noninoculated cathode poised at −0.6 V versus SHE [82]. The rates of hydrogen and formate formation were sufficient to explain the $CH_4$ production rates at the inoculated cathode. The catalytic activity of the cell-free spent media was heat- and proteinase-sensitive, indicating that the enzymes were catalyzing these reactions. Moreover, the formation of formate (but not hydrogen) was facilitated by the cell-free spent medium of the mutant MM1284, suggesting that extracellular hydrogenase(s) released from *M. maripaludis* cells is responsible for the hydrogen formation. Furthermore, the current consumption at the cathode with the cell-free spent medium was higher than that in the control, even after several weeks of operation and medium exchanges, suggesting that the extracellular enzymes were relatively stable and tightly adsorbed onto the electrode.

These observations indicated that redox enzymes, such as hydrogenases and presumably formate dehydrogenases, are released from the cells of *M. maripaludis* and can utilize electrons from the cathode's surface, catalyzing the formation of intermediates (such as hydrogen and formate, respectively), which are rapidly consumed by the methanogens for methanogenesis (Fig. 5). The enzymes can be released from the cells by loss of the cellular integrity, which can be caused by nutrient starvation, physical stress (such as shearing by stirring), osmotic stress, and exposure to low redox potentials.

To complement these studies, proteomic approaches, as well as the bioelectrochemical characterization of the purified redox enzymes at an electrode, might be useful to further understand this pathway. It has been reported that the cell components of nonviable microorganisms (i.e., cell debris) can catalyze hydrogen formation at a cathode [90]. Thus, redox enzymes derived from microbes other than methanogens could contribute to the catalytic ability of the biocathode.

### Pathway II-B: Intermediate Formations Catalyzed by Microorganisms

At the mixed-culture biocathode, it is also possible that microorganisms (for example, bacteria other than methanogens) can take up electrons from the electrode, catalyzing the formation of diffusible intermediates (such as hydrogen), which are in turn utilized by methanogens for methanogenesis (Fig. 6). Previous studies have shown that bacteria can produce hydrogen at the cathode [42, 47, 91–93]. Thus, if present, methanogens can utilize the produced hydrogen for methanogenesis.

Deutzmann and Spormann examined a biocathode inoculated with a synthetic co-culture of the Fe(0)-corroding sulfate-reducing strain IS4 [85] and *M. maripaludis* [83] (Fig. 6). A cathode was first inoculated with a pure culture of the strain IS4. The IS4-inoculated cathode produced hydrogen upon the depletion of sulfate. At a poised potential of −0.4 V versus SHE, the hydrogen formation rate was of 96–120 mmol $m^{-2}$ $day^{-1}$. When the cathode potential was lowered to −0.5 V versus SHE (below the thermodynamic equilibrium potential of hydrogen formation), the hydrogen formation rate was significantly increased to 960–1680 mmol $m^{-2}$ $day^{-1}$. At −0.6 V versus SHE, however, hydrogen formation was not further enhanced. The coulombic efficiencies of the hydrogen formation were

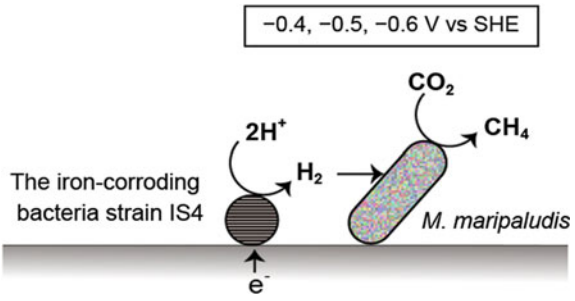


**Fig. 6** A synthetic co-culture of the Fe(0)-corroding sulfate-reducing strain IS4 and *M. maripaludis*

90–110% at the potentials examined (−0.4, −0.5, and −0.6 V vs. SHE). Cyclic voltammetry indicated that the hydrogen formation was reversible, and the overpotential for the reaction was significantly reduced to a practically unnoteworthy level (less than 5 mV). Thus, these observations indicated that the strain IS4 could effectively catalyze hydrogen formation by using electrons from the cathode.

The hydrogen-producing biocathode was further inoculated with a pure culture of *M. maripaludis*, resulting in the formation of $CH_4$. At −0.4 V versus SHE, the co-culture-inoculated biocathode produced $CH_4$ at a rate 24–33.6 mmol $m^{-2}$ $day^{-1}$. No accumulation of hydrogen was detected, indicating the produced hydrogen was rapidly consumed during methanogenesis. Moreover, the inoculation of the methanogens resulted in a small increase in the current consumption, which was likely due to this effective removal of hydrogen. When the cathode potential was lowered to −0.5 V (or −0.6 V) versus SHE, the $CH_4$ formation rate became 144–216 mmol $m^{-2}$ $day^{-1}$ (or 144–288 mmol $m^{-2}$ $day^{-1}$), responding to the potential changes with the responses of the hydrogen formation rate by the IS4-inoculated biocathode. Cyclic voltammetry showed that the electrochemical reaction at the co-culture-inoculated biocathode was irreversible, and no catalytic current was produced at potentials more positive than the thermodynamic equilibrium potential for proton reduction, further indicating the high efficiency of the interspecies hydrogen transfer. Moreover, the overpotential for electromethanogenesis by the co-culture biocathode was 4.2 V lower than that of the cathode inoculated with the *M. maripaludis* pure culture. Collectively, these observations indicate that a co-culture of strain IS4 and *M. maripaludis* can effectively catalyze methanogenesis at the cathode via multiple steps: The hydrogen formation uses cathodic electrons (by the strain IS4), and then the interspecies hydrogen transfer occurs, followed by hydrogenotrophic methanogenesis (by *M. maripaludis*).

In comparison with other defined-culture systems, the co-culture-inoculated biocathode showed a higher ability for catalyzing electromethanogenesis: At −0.4 V versus SHE, the methanogenesis rates were about one order of magnitude higher than those of the cathode inoculated with the methanogen strain IM1 (Pathway I). At −0.6 V versus SHE, the methanogenesis rates were about 20 times higher than those of the cathode inoculated with the pure culture of *M. maripaludis* (Pathway II-B). Thus, it has been proposed that such a co-culture system is a promising candidate for the industrial application of electromethanogenesis. To date, however, the mechanistic basis for the electron uptake by the hydrogen-forming microbes remains unknown. It would be useful to elucidate the mechanism of hydrogen formation at the biocathodes using genetically tractable model microbes (such as the *Shewanella* and *Geobacter* species). It has been reported that the *Geobacter* species transfer electrons to an electrode (anode) via nanowires and cytochromes [94–96]. Similarly, in *Shewanella oneidensis*, nanowire-like appendages (the outer membrane and periplasmic extensions) together with the outer membrane multi-heme cytochromes (MtrC and OmcA) transfer electrons from the bacteria to an anode [97–99]. It has been suggested that those components are also likely involved in the uptake of electrons from a cathode [100], whereas some components are required only for the electron uptake from the cathode [101].

## 3.4 Microbial Ecosystem at the Electromethanogenic Biocathodes

As described earlier, most studies on electromethanogenic biocathodes have been carried out using mixed microbial cultures. However, little is known about the microbial ecosystems developed on biocathodes. Although the microbial compositions of the acclimated biocathodes are rarely documented, it has been reported that hydrogenotrophic methanogens are commonly detected as the dominant archaea in biocathode microbiotas. The roles of the methanogens and other microorganisms in the biocathode's ecosystem can be speculated on based on the proposed electron-transfer mechanisms described earlier. It is likely that electromethanogenesis via all of the pathways can operate, depending on the cathode potential. Hydrogenotrophic methanogens with and without the ability to take up electrons from the cathode play central roles (Fig. 7).

*Methanobacterium* and, to a lesser extent, *Methanobrevibacter*, have previously been found to be the predominant genera in most of electromethanogenic biocathode microbiotas. Cheng et al. constructed a biocathode using the effluent of an existing bioanode as the inoculum [71]. The biocathode community was dominated by a methanogen closely related to *Methanobacterium palustre*, accounting for 86% of the total number of cells. In Marshall et al., a biocathode was developed by inoculating brewery wastewater sludge and incubating it at the set potential of −0.59 V versus SHE [77, 102]. The microbial community of the biocathode mainly consisted of methanogens related to *Methanobacterium* sp. (>93% in abundance) and *Methanobrevibacter* (∼5%). Similarly, *Methanobacterium* and *Methanobrevibacter* were highly enriched on cathodes inoculated with an anaerobic

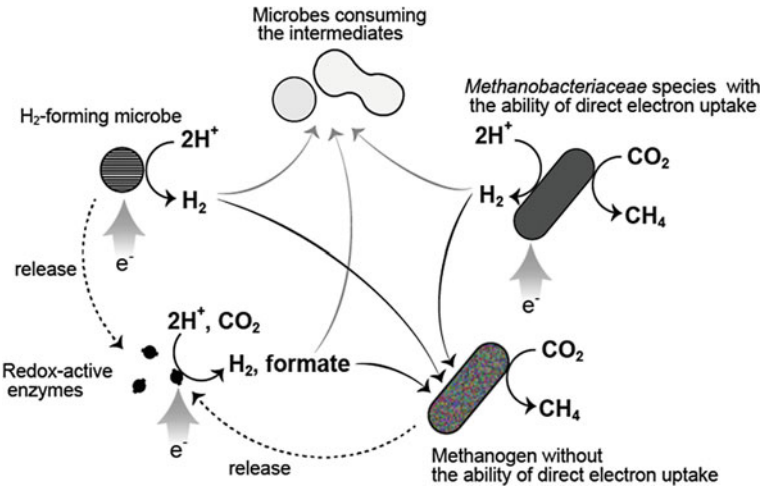


**Fig. 7** Roles of methanogens and other microorganisms in the biocathode ecosystem

bog sediment or anaerobic digester sludge [103, 104]. Moreover, Sigert et al. extensively investigated the microbial compositions of biocathodes made from 10 different materials (carbon brushes, plain graphite blocks, blocks coated with carbon black and Pt, stainless steel, nickel, ferrihydrite, magnetite, iron sulfide, and molybdenum disulfide) [78]. The cathodes were inoculated with anaerobic digester sludge and incubated at a set potential of −0.6 V versus SHE. The archaeal communities of all biocathodes, except those coated with Pt (a highly efficient hydrogen-forming catalyst), were dominated by *Methanobacterium* (a median of 97% in abundance of all archaea). In the Pt-coated cathode, the archaeal community was dominated by *Methanobrevibacter*. These two hydrogenotrophic genera were significantly enriched at the biocathodes, whereas the inoculum had contained primarily the genus *Methanosaeta*. The abundance of *Methanobacterium* and *Methanobrevibacter* in the cathode microbiotas increased 500-fold and 10,000-fold, respectively, after five fed-batch cycles. Moreover, because of the decrease in the numbers of bacteria on the cathode, the relative abundance of archaea in the total population increased 10-fold. These observations suggest that the genus *Methanobacterium* was primarily responsible for $CH_4$ production in those systems when cathodes lack efficient chemical catalysts for hydrogen formation. Additionally, in thermophilic biocathodes, which were inoculated with thermophiles derived from the formation water of a petroleum reservoir, the archaeal community mainly consisted of a thermophilic hydrogenotrophic methanogens closely related to *Methanothermobacter thermautotrophicus* [72, 105]. These genera, *Methanobacterium*, *Methanobrevibacter*, and *Methanothermobacter*, are close relatives and belong to the same family of *Methanobacteriaceae* of the *Methanobacteriales* order. It is unclear why, however, hydrogenotrophic methanogens of *Methanobacteraceae* are enriched in electromethanogenic biocathodes. Recently, it has been shown that direct interspecies electron transfer mediates the syntrophic interactions between electron-donating and -accepting microorganisms [106–109]. Several studies have indicated that acetoclastic methanogens, the *Methanosaeta* and *Methanosarcina* species, can accept electrons from their syntrophic partners (such as the *Geobacter* species). *Methanosaeta* and *Methanosarcina* belong to the order *Methanosarcinales*, all members of which have a broad substrate spectrum and contain cytochromes (although hydrogenotrophic methanogens lack cytochrome) [110]. Thus, it is possible that membrane-bound cytochromes may mediate the electron uptake. Yet, no acetoclastic methanogen has been shown to be predominant in electromethanogenic biocathodes. This is likely because acetoclastic methanogens generally have considerably higher threshold concentrations for hydrogen than hydrogenotrophic methanogens (which lack cytochromes), resulting in the inability of the acetoclastic methanogens to compete with the hydrogenotrophic methanogens for hydrogen [110]. Moreover, the ability to utilize formate is restricted to hydrogenotrophic methanogens [110]. Thus, the formation of hydrogen and formate as intermediates (Pathway II) can be a problem for acetoclastic methanogens with a high hydrogen threshold (>10 Pa) when they are in competition with other organisms (including hydrogenotrophic methanogens) with lower hydrogen thresholds (<10 Pa) in the biocathode microbiota.

However, the members of four orders, *Methanobacteriales*, *Methanopyrales*, *Methanococcales* (including the *Methanococcus* genus), and *Methanomicrobiales*, are all hydrogenotrophic methanogens lacking cytochromes. Those methanogens share low hydrogen thresholds, and some of them can also utilize formate for methanogenesis. Yet, hydrogenotrophic methanogens (including *M. maripaludis*) other than *Methanobacteriaceae* have rarely been detected as the predominant species at biocathodes [111]. Thus, it remains to be determined whether *Methanobacteriaceae* species have an advantage over other hydrogenotrophic methanogens in biocathode ecosystems. Because the strain IM1, which is closely related to *Methanobacterium*, can solely catalyze electromethanogenesis, it is tempting to speculate that some methanogens belonging to the *Methanobacteriaceae* family have the specialized ability to take up electrons from the electrodes. To this end, comparative genomic analysis between the members of the *Methanobacteriaceae* family (including the strain IM1) and other methanogens might provide insight into the genes responsible for such a specialized function. Yet this determination may be difficult to make, as no genetic system is currently available for *Methanobacteraceae* species.

Generally, wide varieties of anaerobic bacteria have been detected in biocathode microbiotas [71, 72, 77, 78, 102, 105, 111]. No dominant species, however, has been commonly identified for bacteria. In the study described previously, Sigert et al. showed a lack of bacterial clusters in the principal component analysis and the lack of a correlation between the bacterial cell numbers and biocathode performance, suggesting that specific bacteria were not directly involved in electromethanogenesis [78]. Because bacterial species still remain on the biocathodes after long-term operation (albeit in a lower abundance), they may play some role(s) in catalyzing electromethanogenesis or may have an advantage in biocathode ecosystems. Presumably, the bacteria or the redox enzymes released from them can catalyze the intermediate formations by taking up cathodic electrons. Because the methanogen strain IM1 not only catalyzed electromethanogenesis, but also promoted hydrogen formation at lower potentials [81], it is also possible that bacteria consume the produced hydrogen (as well as $CH_4$) for their metabolism. As studied in oxygen-reducing biocathodes [112], metagenomic approaches (including transcriptomic and proteomic analyses) may be useful to gain insight into the functions of each type of bacteria in biocathode ecosystems.

## 4 Remarks and Perspectives

Over the past 10 years, alternative fuels produced in bioelectrochemical systems have been intensively investigated. Among these alternative fuels, the hydrogen produced via MECs and $CH_4$ produced via electromethanogenesis are considered to be the most promising renewable fuels because of their high heating value and easy separation. However, many scientific, economic, and technical challenges still hinder the commercial application of these systems [113–115]. For example, until

now, there has been no research about the electron transfer characteristics in the cathode biofilm or about novel reactor configurations designed for microbial electrosynthesis cells. Further research is required to improve the application of bioelectrochemical systems. Researchers should focus on the bacterial communities on the biocathode, electron transfer manner from the cathode to the microorganisms, and the topography of the electrodes.

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