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Recent advances in microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) for wastewater treatment, bioenergy and bioproducts

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Abstract

Bioenergy is a renewable energy that plays an indispensable role in meeting today's ever increasing energy needs. Unlike biofuels, microbial fuel cells (MFCs) convert energy harvested from redox reactions directly into bioelectricity. MFCs can utilize low-grade organic carbons (fuels) in waste streams. The oxidation of the fuel molecules requires biofilm catalysis. In recent years, MFCs have also been used in the electrolysis mode to produce bioproducts in laboratory tests. MFCs research has intensified in the past decade and the maximum MFCs power density output has been increased greatly and many types of waste streams have been tested. However, new breakthroughs are needed for MFCs to be practical in wastewater treatment and power generation beyond powering small sensor devices. To reduce capital and operational costs, simple and robust membrane-less MFCs reactors are desired, but these reactors require highly efficient biofilms. Newly discovered conductive cell aggregates, improved electron transport through hyperpilation via mutation or genetic recombination and other advances in biofilm engineering present opportunities. This review is an update on the recent advances on MFCs designs and operations.

Keywords: microbial fuel cells; biofilm; bioenergy; wastewater treatment; electron transfer; microbial electrolysis cell

INTRODUCTION

The world is facing an energy crisis as petroleum reserves are being depleted faster than new discoveries are made. New emerging economies are using more and more energy resources. There is also a growing awareness and concern over the global warming effect caused by increased use of fossil fuels. World governments are pushing to conserve energy use and to expand non-fossil-fuel energies. The general consensus is that no single energy solution is sufficient. A multi-faceted approach is needed to alleviate the energy crisis. Solar, wind, geothermal, nuclear and bioenergy will all play a role. Bioenergy uses renewable resources to produce ethanol, butanol, biodiesels, biohydrogen and even bioelectricity directly (by using MFCs).

MFC is an emerging technology that uses biofilms as catalysts to convert chemical energy in organic (and some inorganic) matter directly into electricity.[1,2] MFC has a distinct advantage in that it can utilize low-grade biomass or even wastewater, which is otherwise not utilized, to produce bioelectricity. Tremendous advances have been made in the past decade. Research activities and the number of publications in this area have exploded in recent years. Du *et al*.[2] presented a comprehensive review on MFC mechanisms and reactor configurations. Additional MFC reactors were reviewed by Yang *et al*.[3] Zhou *et al*.[4] and Wei *et al*.[5] discussed various MFCs electrodes. Li *et al*.[6] reviewed different materials used to partition anodic and cathodic chambers such as various membranes and salt bridges. Pant *et al*.,[7] Huang *et al*.[8] and Huang *et al*.[9] summarized substrates used in MFC operations including artificial media and various wastewaters. Micro-sized MFCs were recently studied by Wang *et al*.[10] and Qian *et al*.[11] Although MFCs have an attractive potential for alternative green energy production, major technical hurdles remain for their practical deployment. This present work discusses various important aspects of MFC configurations and operations. This review emphasizes advances in the last 5 years.

MFC MECHANISMS

Basic cell setup

A typical MFC is a dual-chamber MFC consisting of an anodic chamber and a cathodic chamber separated by a proton exchange membrane (PEM). In the anodic chamber, an anaerobic biofilm

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Table 1. Some electrogenic microbes for MFCs and their electron transfer mechanisms

Microbes	Mechanism	Reference
In anodic biofilm		
Aeromonas hydrophila	DET	72
Geobacter metallireducens	DET	73
Rhodoferax ferrireducens	DET	74
Shewanella putrefaciens	DET	75
Actinobacillus succinogenes	MET	76, 77
Alcaligenes faecalis	MET	78
Enterococcus gallinarum	MET	78
Proteus vulgaris	MET	79
Shewanella oneidensis	MET	80
In cathodic biofilm		
G. sulfurreducens DL1	DET	81
Geobacter sulfurreducens	DET	82
Acidithiobacillus ferrooxidans	DET	83
Shewanella putrefaciens	DET	84
Desulfovibrio vulgaris	DET	85
D. vulgaris	DET	86
Clostridium beijerinckii	MET	87
Pseudomonas spp	MET	88
S. oneidensis	MET	89
Acinetobacter calcoaceticus	MET	90

oxidizes a substrate, producing electrons and protons. Protons migrate from the anode region to the cathode in the aqueous solution through the PEM. The electrons are donated by a biofilm on the anode to the electrode. The electrons flow through an external circuit from the anode to the cathode and in the process drive an external load. The electrons are subsequently used to reduce electron acceptor in the cathodic chamber.[1,12] A large number of substrates, including various artificial and real wastewaters and lignocellulosic biomass, have been explored as feed for MFCs.[7,12,13]

Electron transfer methods

Some microbes are electrochemically active, capable of accepting electrons from an external source or donating electrons to an external object such as an electrode. These microbes are known as electrogenic microbes.[1] Not all microbes are electrogenic, but non-electrogenic microbes may still be part of a synergistic electrogenic biofilm consortium because they perform other functions such as providing certain organic nutrients to the electrogenic microbes in the consortium.

Microbial cells are generally non-conductive because their cell membranes mostly contain non-conductive materials such as polysaccharides, lipids and peptidoglycans. Electron transfer between microbes and electrodes rely on two mechanisms, namely direct electron transfer (DET) and mediated electron transfer (MET).[14] Table 1 shows a list of some electrogenic microbes reported in the literature for MFC applications. It should be noted that some electrogenic microbes, such as some microbes in biofilm consortia in activated sludge, have yet to be characterized although such uncharacterized mixed-culture biofilms have been used widely.

DET requires direct physical contact between the microbial cell membrane or a membrane organelle and the anode electrode surface, without the need for any diffusional redox species in the

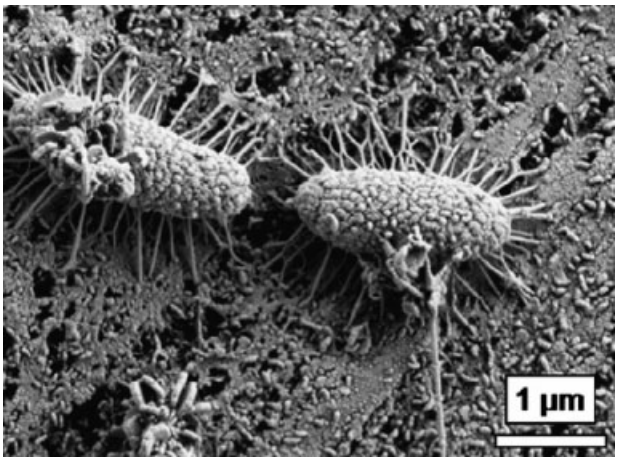

Figure 1. Extensive pilus network linking two sulfate reducing bacterial cells with an iron surface when the cells were starved of organic carbon (reprinted from Sherar *et al.*[17] with permission from Elsevier).

electron transfer process. Both c-type cytochromes associated with bacterial outer membrane (OM) and conductive nanowires or pili[15] can be used for DET. Pili can be formed on demand[16] to facilitate electron transfer between microbial cells and a solid surface. In Fig. 1, two sulfate reducing bacteria (SRB) cells formed numerous pili linking the cell walls to a steel surface. These pili were absent when the culture medium contained organic carbon.[17] In the absence of organic carbon, the SRB cells formed pili to transport electrons from iron oxidation for reduction of sulfate in the SRB cytoplasm. This redox reaction provides maintenance energy for survival in the absence of organic carbon.[18] Iron is as energetic as lactate (a favored organic carbon for many SRB cells) because their standard reduction potentials are very close (−447 mV vs. −430 mV). Gorby *et al.*[19] suggested that formation of pili may be a common strategy used by electrogenic bacteria for efficient electron transfer and energy distribution. For MFC operations, this means that some microbes purposefully develop a network of pili to facilitate electron donation to an anode or electron acceptance from a biocathode in MFC operations. After all, allowing the electron flow enables respiratory metabolism that benefits the biofilms bioenergetically.

While some microbes perform DET, other microbes need redox-active chemical species (mediators) to carry out indirect electron transfer; this is known as MET. Apart from externally supplied mediators, some microorganisms are able to excrete their own mediators such as phenazine, 2-amino-3 carboxy-1, 4-naphthoquinone, 1,2-dihydroxynaphthalene and 2,6-di-tert-butyl-p-benzoquinone.[12,20−22] An electron mediator is a molecule that functions as an electron shuttle between microbes and an electrode. In MET, direct contact between the bacterial cell membrane and the electrode surface is not required. In a synergistic biofilm consortium, it is possible that a non-electrogenic microbe may secrete mediators that help the electrogenic microbe perform better.

It was generally believed that only a monolayer of electrogenic sessile cells in a body of biofilm are directly responsible for electron transfer, especially in DET.[8] This means that only the monolayer of cells are directly responsible for electricity generation. However, in recent years, more experimental evidence was obtained for multilayer conductive cells either through pili networking, mediators, or interspecies hydrogen transfer. Summers *et al.*[23] discovered that a laboratory evolution of a coculture consisting of

Geobacter sulfurreducens and *Geobacter metallireducens* grown on ethanol formed cell aggregates that were electrically conductive as a whole. They suspected that a likely mechanism for electron exchange between the two microbes is that c-type cytochrome OmcS of *G. sulfurreducens* is able to accept electrons from *G. metallireducens* via the c-type cytochromes on the outer surface that are either localized on the *G. metallireducens* cell or along pili. This overcomes the inability of *G. sulfurreducens* to use molecular hydrogen for electron exchange. When mediators are used, it is possible that more than one monolayer of sessile cells can perform electron transfer.

Evaluation of MFC performances

COD removal from wastewater reflects the total energy harvested from the organic matters. The COD removal efficiency (η_{COD}) is calculated from the equation

$$\eta_{COD} = (COD_{inf} - COD_{eff}) / COD_{inf} \times 100\% \quad (1)$$

where COD_{inf} and COD_{eff} are the influent and effluent COD (mg L^{-1}), respectively.

Not all the energy harvested from bioconversion of an organic matter is converted to electricity. Some energy is utilized by the biofilm as maintenance energy that is necessary for its survival and health. Some energy is wasted due to overpotentials, namely activation overpotential, concentration (or mass transfer) overpotential near an electrode, and ohmic loss due to internal resistance. The wasted energy is released as unrecoverable low-grade heat. The actual closed circuit potential output of an MFC is much less than the theoretical open circuit potential. The actual closed circuit potential is calculated from standard potentials as

$$U_{output} = E_{cathode} - E_{anode} - \sum \eta_j + I \bullet R_i \quad (2)$$

where $\sum \eta_j$ is the sum of activation and concentration overpotentials for the anode and cathode. R_i is the internal resistance and I is the current flow. The electrode potentials ($E_{cathode}$ and E_{anode}) are calculated based on the Nernst equation, which depends on standard potentials and activities and partial pressures (for gaseous chemicals such as hydrogen gas). The various overpotentials and the internal resistance all contribute to the Coulombic efficiency loss.[1] Coulombic efficiency reflects the ratio of the number of electrons passing through the external load R (ohms), which generates electricity, to the number of electrons removed from the substrate during bioconversion. It is calculated from the equation below for batch MFC operation with an air cathode:[24]

$$CE = \frac{\left(\int_{t_1}^{t_2} Udt\right)/R}{F \cdot b\,(\Delta COD)\,V} \cdot MW \quad (3)$$

where U is the output voltage as function of t (time), R the external load in ohms, F Faraday's constant (96 485 C mol^{-1}), b the number of electrons exchanged per mole of O_2, equal to 4, ΔCOD is the removal of COD, V the wastewater volume (L) in the anodic chamber, and MW the molecular weight of O_2. The integral in Equation (3) is for a time duration (t_1 to t_2) during which electricity is harvested through the external load.

MFC power density output based on an electrode surface area (P_A) and power density based on the liquid volume in the anodic chamber or the cathodic chamber (P_v) are readily calculated from the equations

$$P = IU \quad (4)$$

$$P_A = P/A \quad (5)$$

$$P_v = P/V \quad (6)$$

where A is the surface area of an electrode, and V the liquid volume in the anodic or the cathodic chamber. MFC performance is typically measured using power density based on the anode or cathode surface area. Table 2 shows some outstanding performances reported in the literature.

These power density figures are still 3 to 4 orders of magnitude lower than that produced by a typical proton exchange chemical fuel cell.[25] However, this kind of comparison is inherently biased and impractical because chemical fuel cells use high energy-density fuels while MFCs typically use low-grade fuels in wastewaters.

NEW MFC REACTOR CONFIGURATIONS

Various MFC configurations were reviewed by Du *et al.*[2] Figure 2 shows a summary of reactor types based on different classification criteria.

Some recently reported innovative MFC configurations

An overflow-type wetted-wall MFC (WWMFC) was constructed with two coaxial cylindrical tubes.[26] Its carbon-cloth anode was positioned in the anode chamber between two glass tubes. Its carbon-cloth cathode was bonded to the inner surface of the inner tube. The culture medium in the anode chamber overflowed into the cathode chamber through a gap between the inner tube and the top Plexiglas cover on the cathode surface as a liquid film. The reactor achieved a maximum power density of 18.2 W m^{-3} using 1000 mg L^{-1} acetate as substrate at a flow rate of 25 mL min^{-1}. However, further increasing the flow rate restrained the oxygen diffusion from the gas phase to the electrode, which resulted in a poor performance and thus limited the time-spatial treatment efficiency.

Cheng *et al.*[27] designed a rotatable bio-electrochemical contactor (RBEC) that consisted of an array of rotating electrode disks, each of which had its upper semi-circle exposed to air and its lower side submerged in water. Intermittent rotation allowed each half to act as anode and cathode alternately. The COD removal rate was increased by 15% by allowing electron flow from the lower to the upper half of the disk. The reactor is more energy-efficient than conventional activated sludge processes since the COD removal rate was comparable while the required energy input per COD removed was less. Moreover, the performance could be achieved without aeration and wastewater pH adjustment. However, efforts on electrode modification were required to reduce overpotential of the cathodic oxygen reduction.

Zhang and Angelidaki[28] designed a self-stacked submersible MFC (SSMFC). As shown in Fig. 3, each electrode (anode and cathode) was hot-pressed together with a PEM. The SSMFC with two sandwich-type electrodes had an open circuit voltage of 1.12 V that was rather high. It produced a maximum power density of 0.294 W m^{-2}. Several reactors could be stacked together to improve sediment MFC performances. Voltage reversal could be

Table 2. Some upper-end performance data reported in the literature for some MFC reactors

MFC types	Fuel(s)	η_{COD} (%)	CE (%)	Power density (W m^{-2})	Reference
flow-through anode; air-cathode	acetate (8.92 mg L^{-1} – 1.98 mg L^{-1})	–	88 ± 5.7%	3.65	90
air–cathode single chamber	swine wastewater (60000 mg L^{-1} COD, 670 mg L^{-1} P)	76–91%	37–47%	1–2.3	91
sludge MFC	cyanide laden cassava mill wastewater (COD 16000 mg L^{-1}, 86 mg L^{-1} cyanide)	28%–72%	20%	1.77	92
air–cathode	amino acids (720 mg L^{-1} TOC)	> 91%	13 ± 3% – 30 ± 1%	0.556–0.768	93
submersible MFC	domestic wastewater	–	–	0.832	73

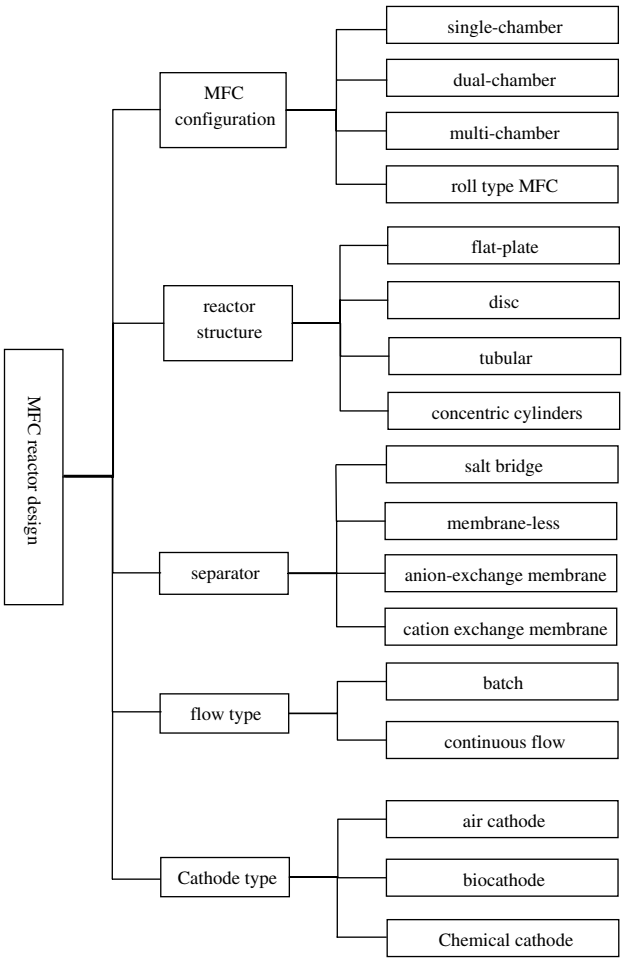

Figure 2. MFC reactors based on different classification criteria.

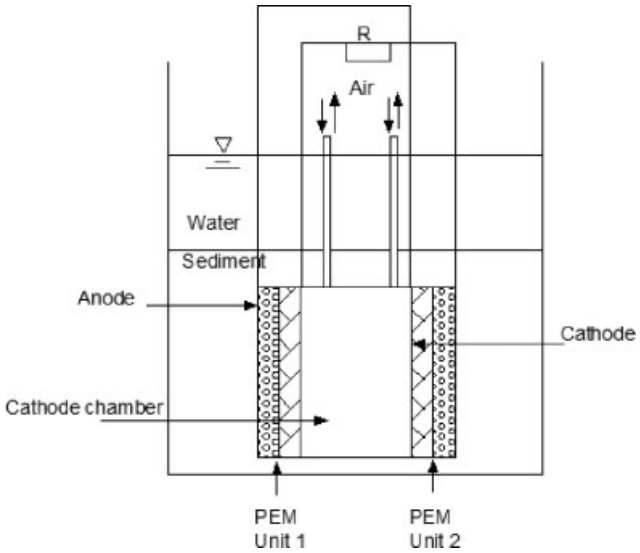

Figure 3. Self-stacked submersible MFC (figure redrawn after Zhang and Angelidaki[28]).

eliminated by using capacitors. Like all MFCs using PEM, membrane fouling could be a major issue in field applications.

Biocathode MFCs

Oxygen reduction at the cathode of an MFC requires catalysis. This is often achieved by using an electrode with catalytic materials such as platinum, which can be prohibitively expensive for practical applications. Efforts are being made by many researchers to create new catalytic electrode materials for cost reduction, such as manganese oxides,[29] polypyrrole (Ppy),[30] Fe3+ cathode made with ferric sulfate,[31] and activated carbon.[32] Another solution is to use biofilms on cathodes for catalysis. In addition to aerobic biofilms that catalyze oxygen reduction,[33] anaerobic biofilms can also be used to reduce a non-oxygen oxidant such as sulfate and nitrate that may be present in some wastewaters.[34]

Oxygen reduction is a very slow reaction on the cathode without catalysts that tend to be quite expensive. Biofilms can be used to perform the reduction reaction on the cathode by accepting the electrons from the anode via an external circuit. This kind of biocathode can lower the costs.

A three-chamber MFC with two cathodic chambers sandwiching one anodic chamber in the middle was recently developed by Zhang *et al.*[35] The two cathodic chambers used graphite granules covered by a mixed-culture biofilm consortium. This multi-anode design aimed at reducing the distance between the anode and the two cathodes. It achieved a maximum power density of 8.15 ± 0.20 W m^{-3} using dairy manure. Because two PEM membranes are needed to partition the symmetric cathodic chambers from the anodic chamber in the middle, large-scale operations will likely encounter membrane fouling.

Although biocathodes are attractive because they allow the use of inexpensive non-catalytic electrode materials and they can also treat a second wastewater stream, the voltage output of an MFC with a biocathode can be much lower than that using an oxygen or air cathode. The oxygen reduction potential is far more positive than the reduction potentials of sulfate, nitrate, etc. Figure 4 illustrates that the potential energy of a brick depends on the floor level. This analogy can be used to explain MFC voltage output using oxygen cathode vs. a biocathode.

Table 3. Standard reduction potential $E^{o'}$ under physiological pH 7 and number of electrons involved for some biocathodes in MFCs

Redox couple	n	$E^{o'}$ (mV)
CO_2/CH_4	8	−244
SO_4^{2-}/HS^-	8	−217
NO_2^-/NH_3	6	330
NO_3^-/NH_3	8	360
NO_3^-/NO_2^-	2	430
$2NO_3^-/N_2$	10	760
$O_2/2H_2O$	4	818

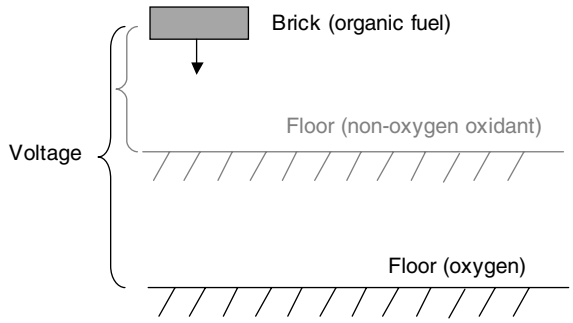

Figure 4. Theoretical MFC voltage output using oxygen cathode or a biocathode using brick falling analogue.

Table 3 (data taken from Thauer *et al.*[36]) shows that the standard reduction potential of $O_2/2H_2O$ is 0.818 V, which is much more positive than the standard reduction potentials of sulfate/bisulfide (−0.217 V), nitrate/ammonia (0.360 V), nitrate/nitrite (0.430 V). Only nitrate/nitrogen reduction potential (0.760 V) is close. Nitrate is usually not available in a typical unpolluted water stream. However, wastewater contaminated with agricultural run-off may contain a significant amount of nitrate from leftover fertilizers.[37] When an oxidant with a low reduction potential such as sulfate is used, the actual MFC voltage output might be too small after deducting overpotentials. This kind of inherent limitation hinders the applicability of some biocathodes.

System integration

To promote MFC performances or fulfill specific needs such as a certain voltage demand, MFC system integration is a hot research area. Basically, there are three integrated strategies. One is the combination of multi-anode/cathode or multiple cells of MFCs. The second is to integrate with some other physical/chemical process, and the third is to extend MFCs with other biological processes.

Strategy one

A multi-anode/cathode MFC system was presented by Jiang *et al.*[38] They inserted multiple graphite rods in a bed filled with activated carbon granules as anode to pair with a single cathode. For an MFC with four anodes and one cathode, they obtained a power density of 1.18 W m^{-3}, which was 2.2 times higher than using only a single-anode. This kind of design can only improve the performance of an MFC in which anodic reaction rather than cathodic reaction is limiting.

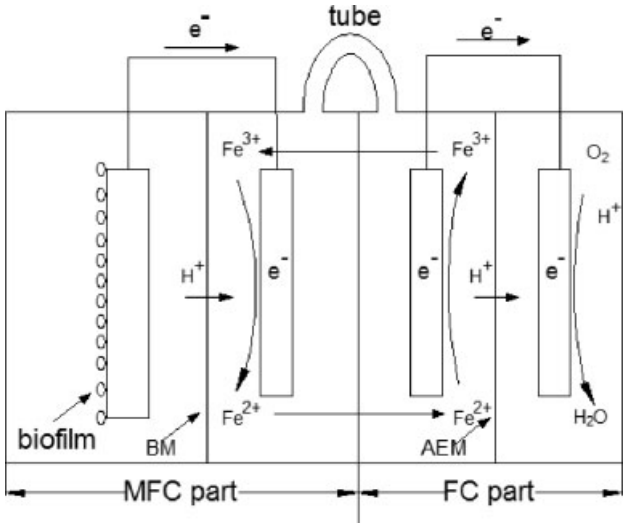

Figure 5. Hybrid MFC and FC system (figure redrawn after Eom *et al.*[40]).

Xie *et al.*[39] published a design for an MFC system that combined an oxic-biocathode MFC (O-MFC) with an anoxic-biocathode MFC (A-MFC). It was capable of simultaneous removal of carbon and nitrogen. The anode of each MFC was sandwiched between two cathodes. The COD in the influent was mostly removed in the two anode chambers. Ammonium was oxidized in the O-MFC's cathode chambers to nitrate. Its effluent containing the nitrate was fed into the cathode chambers of the A-MFC for denitrification by the biofilms on the cathodes. It achieved power densities of 14.0 W m^{-3} NCC (net cathodic compartment) and 7.2 W m^{-3} NCC for O-MFC and A-MFC, respectively. The maximum NH_4^+-N, total nitrogen and COD removal rates were 97.4%, 97.3% and 98.8%, respectively. This rather complicated reactor design is more expensive to build and more difficult to maintain stable operations.

Strategy two

Figure 5 shows a hybrid MFC and fuel cell (FC) system (M2FC reactor).[40] Carbon cloth was used for all electrodes except the FC's cathode electrode, which used a platinum catalyst. Oxygen in dissolved air was the FC's cathodic electron acceptor. An anion-exchange membrane was used for the FC and a bipolar membrane for the MFC. The MFC's cathode chamber was connected to the FC's anode chamber via a tube for electrolyte circulation. The M2FC reactor produced a time-averaged power density of 0.65 W m^{-2}, which was approximately six times higher than that for the corresponding MFC system. However, Pt catalyst is too expensive for practical systems and membrane costs and fouling are also major concerns.

Strategy three

A new sediment microbial fuel cell (SMFC), which has two cathodes was presented by Chen *et al.*[41] One of the cathodes was in the rice rhizosphere and another at the air–water interface. This work proved that the excreted oxygen from the rice rhizosphere could serve as a biocathode that is comparable in efficiency with an air cathode. An advantage of this biocathode is that it is directly in the soil, which means it can be placed very close to the anode. It remains to be seen whether this design can be scaled up.

MFCs can also be converted to microbial carbon capture cells (MCCs) for CO_2 sequestration utilizing photosynthetic algae.[42]

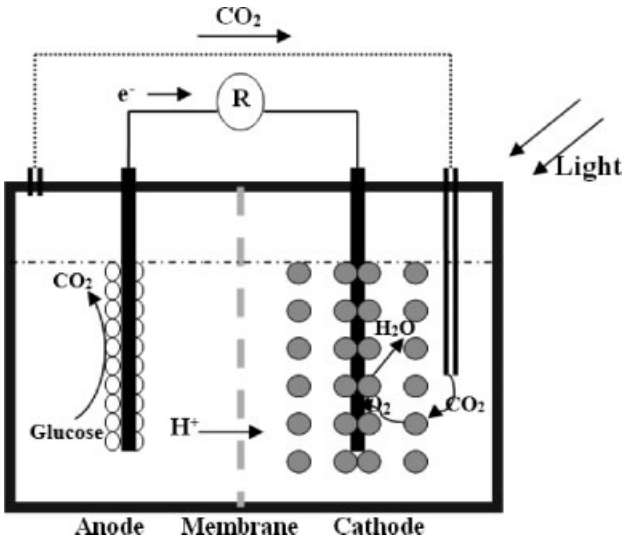

Figure 6. MCC utilizing immobilized algae cells (figure redrawn after Zhou *et al.*[43]).

The CO_2 off-gas generated from oxidation reaction on the anode was fed into the catholyte solution for utilization by suspended *Chlorella vulgaris* cells in the cathode, which converted it into biomass through photosynthesis. The oxygen generated by *C. vulgaris* cells served as electron acceptors for the cathodic electrons supplied by the anode with the catalysis of the *C. vulgaris* cells attached to the biocathode. A maximum power density of 5.6 W m^{-3} was obtained. As an improvement, Zhou *et al.*[43] immobilized microalgae (*C. vulgaris*) in alginate calcium beads (Fig. 6), which benefited both microalgae separation and MFCs performance. Their reactor achieved a maximum power density and Coulombic efficiency 88% and 57.7%, respectively, greater than those without beads. This kind of biocathode design requires light to penetrate the reactor wall and the catholyte liquid efficiently, and the presence of algae can hinder mass transfer.

'SUPER BUGS' FOR IMPROVED MFC PERFORMANCE

MFC power output is still much lower than that needed for practical power generation beyond powering small sensors, despite recent advances in reactor design. Complicated reactor designs may improve power output, but the cost could be prohibitive for practical applications. Although better biofilms have been isolated from activated sludge and other natural sources, a further breakthrough in biofilm performance is needed. Genetic modifications can create 'super bugs' (i.e. high-performance sessile cells) with MFC performance enhancement properties.[44]

The following improvements may be targeted: *(1) Type IV pili and redox-active cytochromes on the cell surface*. To increase electrogenic capacity of two metal reducing *Shewanella* and *Geobacter*, bacterial geneticists could over-express genes such as the *pilA* (encoding conductive pilin 'nanowires') gene product or, in *Shewanella* spp., overexpress the decaheme cytochromes MtrA/F[45,46] and two other decaheme proteins, MtrC[47] and OmcA[48] on the cellular surface, thereby increasing the likelihood of electron flow to an electrode (Fig. 7).

(2) Increase production of electron-conductive, extracellular mediators including phenazines and flavins. Many bacteria secrete soluble redox-active mediators that can donate electrons to conductive surfaces and independent of bacterial surface attachment. These include pyocyanin and pyorubrin in *Pseudomonas aeruginosa*[12] and riboflavin in *Shewanella*.[49]

(3) Increase biofilm formation. Surface appendages including flagella,[50] type IV pili (specifically twitching motility),[50] and quorum sensing[51] are among a myriad of factors that influence biofilm formation in a variety of bacteria.

(4) Prevent dispersion of biofilm bacteria from anodes and/or cathodes. The last of five steps in bacterial biofilm development, dispersion, is an active exit of viable cells. The intracellular concentration of bis-(3'-5')-cyclic dimeric GMP (c-di-GMP), mediated by diguanylate cyclases (DGCs) and phosphodiesterases (PDEs).[52] In *P. aeruginosa*, *BdlA* (biofilm-dispersion locus) is required for dispersion from pre-formed biofilms.[53]

(5) Limit production of nutrient-restrictive biofilm matrix. The matrix surrounding biofilm bacteria can limit nutrient access to bacteria. Thus, genes involved in dispersion from biofilms are activated when *Pseudomonas* putida are starved for nutrients.[54] Sporulation in B. subtilis is activated within biofilm bacteria.[55] Thus, limiting the production and secretion of complex, thick polysaccharides (e.g. alginate in *P. aeruginosa*[56]), and others known as Pel (for pellicle formation[57]) and *Psl* (polysaccharide locus[58]) would optimize nutrient access to biofilm bacteria in MFCs.

(6) Increase the rate of respiration and carbon source oxidation. Enhanced metabolic rate by bacteria in MFCs is critical for optimal MFC performance. These can include (i) reducing $FADH_2$/NADH levels to trigger demand for cellular ATP or (ii) uncoupling natural processes of oxidation and phosphorylation. Recently, Izallalen *et al.*[59] used an IPTG-regulatable hydrolytic, F1 portion of the membrane-bound H^+ F0F1-ATP synthase in *G. sulfurreducens* that resulted in increased metabolic rate because reducing power in the forms of FADH2 and NADH resulting from increase TCA cycle activity caused a ~50% reduction in cellular ATP. Uncouplers (thermogenin, UCPs[45–47]), compounds that dissipate the proton gradient, increase substrate oxidation rates and power generation.

To facilitate the discovery, fast and efficient screening of a large number of electrogens is needed. It may be achieved by using dedicated miniature devices that analyze multiple samples at once. Hou *et al.*[60] invented a 24-well MFC array specifically for the evaluation of MFC performance using different biofilm communities simultaneously.

A low-cost simplistic tubular membrane-less MFC reactor powered by super bugs was envisioned by Zhou *et al.*[61] for wastewater treatment. Figure 8 shows a modified version of such a reactor. It shows a tubular reactor with two anodes and two cathodes. The electrodes are cartridges that can be removed for replacement or maintenance. They can also be cartridges with different biofilms to process different organic substrates or oxidants. The cartridges may contain granules such as inexpensive graphite granules packed in a cage. Apart from using air cathode, a different oxidant such as nitrate can be supplemented if there is lack of oxidant in the upstream. When a non-gaseous oxidant is used, there is no need for upward flow (to benefit air flow) that requires a pump. In this design, a sufficiently high convective flow rate prevents any externally supplied oxidant from entering the upstream anode region, thus eliminating the need for PEM partitioning the anodic region and the cathodic region. Obviously, such a high flow rate requires a very large biofilm surface area and high biofilm metabolic rates to digest the utilizable organic carbons. Current microbes used in MFC are still inadequate to run such a reactor. A breakthrough in engineering super bugs is needed.

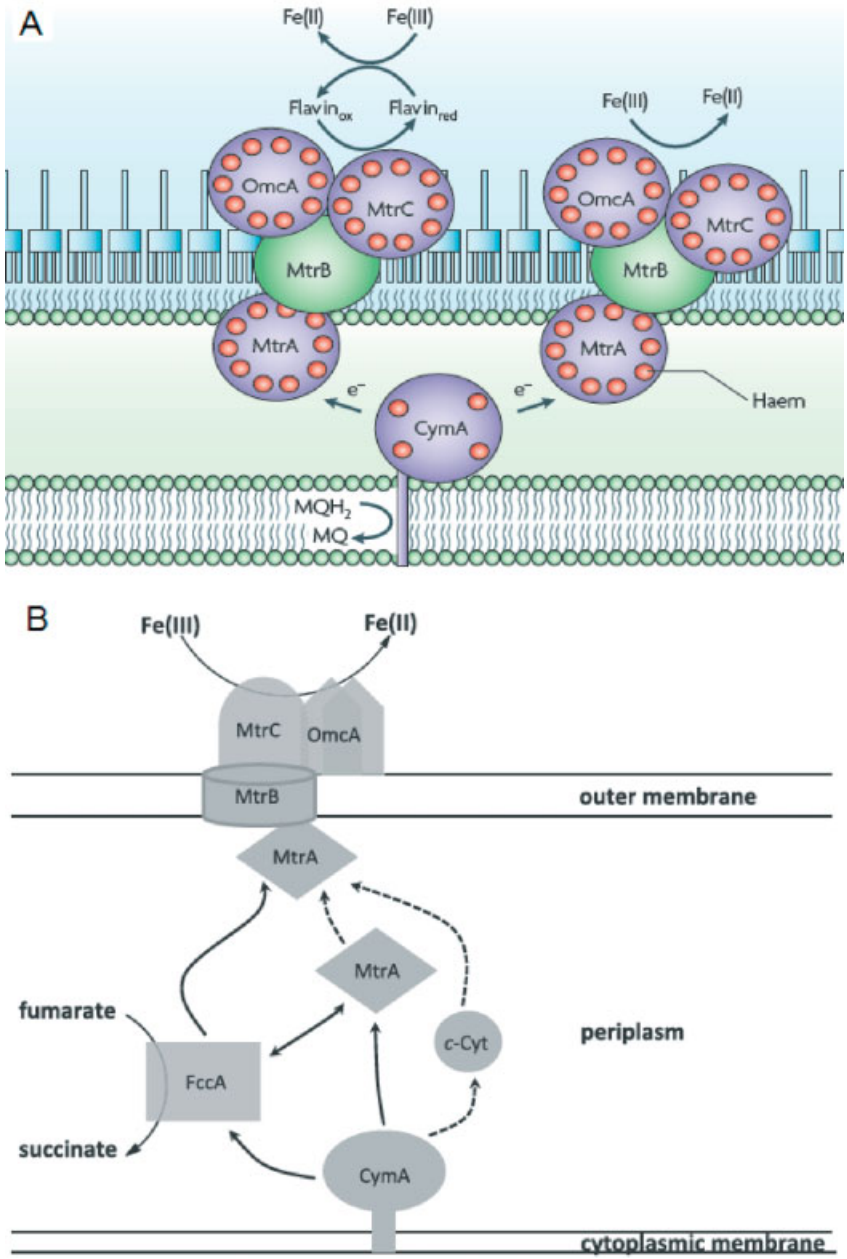

Figure 7. Shematic diagram of cytochrome-mediator and mediatorless electrogenesis in *S. oneidensis* (reprinted from Fredrickson *et al*.[99] and Richter *et al*.[100] with permissions).

MICROBIAL ELECTROLYSIS CELLS (MECS)

MEC mechanisms

MECs oxidize organic matters electrochemically using microbial biofilms in an anodic chamber to yield protons and electrons, which are subsequently used in a reduction reaction to produce value-added products such as hydrogen and methane. The electrogenic biofilm on the anode acts as a biocatalyst to push the anodic reaction forward. Electrons donated by the anodic biofilm to the anode reach the cathode via an external electrical circuit, where they reduce H_2O and proton to produce OH^- and H_2, which is released from the cathode compartment. An externally supplied voltage is required because the coupled redox reaction is a thermodynamically unfavorable. Less power is needed for the process than in water electrolysis because degradation of organic carbon in an MEC supplies part of the needed energy. In addition to biohydrogen, other products such as methane can also be produced if something other than proton or water is reduced on the cathode.

Products from MECs

Table 4 shows some reported value-added products from MECs including H_2, methane, and H_2O_2. Hydrogen gas generation in the cathodic chamber of microbial electrolysis cells has two pathways. One pathway is H_2O reduction, the other proton reduction. Methanogenesis through oxidation of H_2 by methanogens in an anodic biofilm community is a side reaction that compromises hydrogen productivity of hydrogen by MECs, especially when a membrane-less MEC is used. Without a membrane partition, some

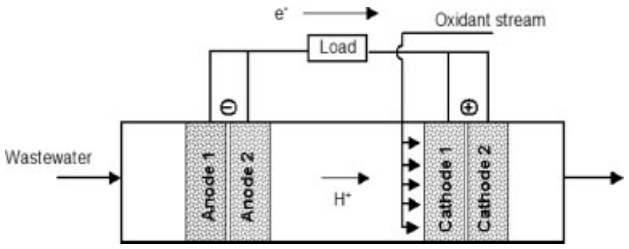

Figure 8. Convective-flow membrane-less MFC with dual anodes and dual cathodes for wastewater treatment (figure redrawn after Zhou *et al.*[61] with modifications).

Table 4. MECs with different bioproducts

Product	Power supply	Recovery rate ($L\,L^{-1}\,d^{-1}$)	Energy efficiency (%)	Ref.
H_2	1.06 V	0.3	–	94
	0.5 V	0.02	169	95
	0.6 V	0.53	204	64
	0.4 V	0.2	267	
	Anode poised at +0.5 V	0.018	57	96
CH_4	0.8 V	0.17–0.75	240–84	97
	0.9 V	0.12	67	68
	Anode poised at 0.0 V	0.53	70	98
H_2O_2	0.5 V	1.25 ± 0.13	83.1 ± 4.8	95

hydrogen gas generated in the cathode region can escape to the anodic region where methanogens can consume it.

Reactor configurations of MECs

Double-chamber MECs

A dual-chamber MEC reactor designed by Kyazze *et al.*[62] consisted of two concentric tubular Plexiglas chambers. The inner tube was radially perforated on one side of the tube and inserted into the larger outer tube. The inner tube contained an anode electrode assembly rolled several times around a plastic inner rod. A cation exchange membrane was wrapped around the outer surface of the inner tube to cover the perforations, thus forming a partition between the internal volumes of the two tubes. The cathode electrode assembly was wrapped around the cation exchange membrane. The highest hydrogen production rate was obtained at an applied voltage of 850 mV. The Coulombic efficiency and cathodic hydrogen recovery were 60% and 45%, respectively. Hydrogen yield was up to 1.1 mol for each mole of acetate converted, corresponding to a 30.5% COD reduction. The efficiency should be further improved before practical applications are attempted.

Single-chamber MECs

To cut cost and simplify reactor construction, single-chamber MECs have also been investigated. Since oxygen is not produced in an MEC, thus there is no need to prevent the gas produced at the cathode from entering the anode chamber. A membrane-less single-chamber MEC was developed by Call and Logan[63] with improved hydrogen production. Hu *et al.*[64] also used a membrane-free single-chamber MEC to reduce the potential loss due to mass transfer resistance exerted by a membrane. This system with an applied voltage of 0.6 V had a hydrogen production rate of $0.53\,m^3\,m^{-3}\,d^{-1}$ based on liquid volume (or $0.11\,m^3\,m^{-2}\,d^{-1}$ based on electrode area). These membrane-less designs are attractive because in practical applications, the costly membranes can be deformed and fouled. The hydrogen production rate of $0.53\,m^3\,m^{-3}\,d^{-1}$ is rather low. It should be further improved by increasing electrode surface area and operating the reactor in continuous flow mode instead of batch mode.

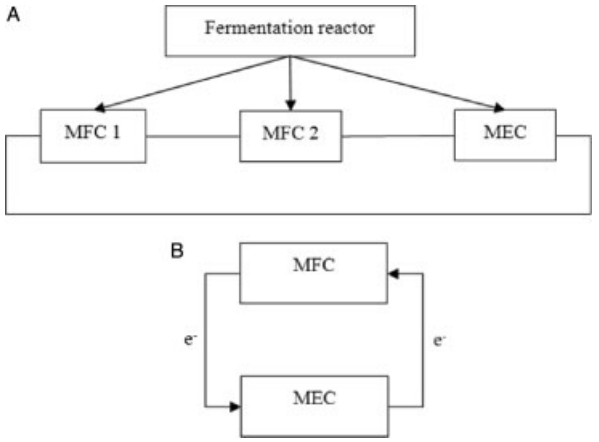

Figure 9. Integrated MEC systems.

Continuous flow MECs

A membrane-less MEC with continuous flow employing a gas-phase cathode was presented by Tartakovsky *et al.*[65] Its anode was made from carbon felt and its cathode was a gas diffusion cathode. The two electrodes were partitioned using a J-cloth. This MEC achieved a hydrogen production rate of $6.3\,L\,L^{-1}\,d^{-1}$, which is much higher than the $0.53\,m^3\,m^{-3}\,d^{-1}$ value achieved in batch mode by Hu *et al.*[64] Despite the absence of a membrane partition, methane concentration remained below 2.1% in the gas collection chamber. Wang *et al.*[66] developed a membrane-less single-chamber up-flow biocatalyzed electrolysis reactor (UBER). It reduced toxic chemicals in the continuous feed to less- or non-toxic products in its cathode zone. The influent entered from the cathode zone at the bottom of the UBER and then reached the anode zone near the top. The reactor was able to convert nitrobenzene (>99%) into aniline as a major product with an external voltage of 0.5 V. One drawback for upward flow is that it requires a pump for wastewater transport.

Integrated MEC systems

To explore the scalability of MECs, Rader *et al.*[67] constructed a continuous flow MEC with multiple electrodes. The maximum current of the multiple-electrode MEC was 181 mA ($1.18\,A\,m^{-2}$), and the maximum hydrogen production rate $0.53\,L\,L^{-1}\,d^{-1}$ with an energy efficiency of 144% relative to its electric energy input.

Figure 9 shows that a hydrogen-producing MEC could be integrated with an electricity-assisting MFC.[68] Hydrogen was produced in the MEC utilizing the electricity generated from the MFC. The system was regulated using loading resistors connected in series. By using the loading resistors the MEC electricity requirement was lowered.

Integration of MECs with MFCs instead of using a conventional power source inherently complicates process design and operation, making scale-up more difficult. Electro-hydrolysis could be applied directly to dark fermentation of volatile fatty acids

to produce H_2 because the protons produced by a volume-based fermentation are reduced on an electrode surface to form hydrogen without the need for a biofilm.[69] The use of MECs for bioproduct production during wastewater treatment is a relatively new concept. Cost of reactor construction and difficulties in operation and maintenance must be substantially reduced before they can be considered for practical applications.

PILOT STUDIES

Despite intensive laboratory investigations of MFCs and MECs, published pilot studies have been very scarce. A pilot-scale MFC study was conducted by the Advanced Water Management Center of the University of Queensland.[70] This reactor was fed with brewery wastewater from a Foster's Group brewery in Yatala, Queensland, Australia. It consisted of 12 vertical tubular reactor modules (each 3 m tall) with a combined liquid volume of 1 m^3. Carbon fiber was used for anodes and cathodes. The feed was pumped upward in each module inside the tube through the anode carbon fiber brush and it flew downward along the outside of the tube where the carbon fiber cathode was situated. The reactor operation suffered from low conductivity of the feed solution that resulted in low COD removal and biofouling of the cathodes. The power density output was 8 W m^{-3}, far below the desired level for practical applications.[61] The upward fluid transport itself would consume significant power. To improve the power generation, electrode surface area could be increased using different electrode materials and designs, but the cost would also increase. For this reason, larger MFCs like the one above are underdesigned compared with small MFCs, resulting in performance losses.

Recently, Cusick *et al*.[71] discussed the results of their 1000 L pilot-scale continuous flow MEC fed with winery wastewater. at the Napa Wine Company located in Oakville, California, USA. Their reactor was operated with 144 electrode pairs in 24 modules with an externally applied voltage of 0.9 V. Acetate enrichment was needed as well as an elevated wastewater temperature of $31 \pm 1\ ^{\circ}C$ to achieve soluble chemical oxygen demand removal rate of $62 \pm 20\%$. The maximum current generation was measured at 7.4 A m^{-3} after 100 days when the test ended. The total gas production rate of 0.19 ± 0.04 L L^{-1} d^{-1} was quite low compared with laboratory studies under continuous flow or even batch flow condition. Although the target product was hydrogen gas, the majority of the gas produced ($86 \pm 6\%$) by volume was converted to methane. Methanogenesis was obviously a big problem. This problem points to the need to create robust electrogenic biofilms to inhibit methanogens. Cusick *et al*. also noticed that enrichment of the biofilm took up to 60 days, much longer than that needed under laboratory conditions. This again suggests the need for developing better biofilms that readily establish themselves in practical applications. The pilot MEC had the advantage that no membrane partitioning was used, but it could also have contributed to the hydrogen diffusion into the anode zone where methanogenesis could occur. For an MEC that produces mostly methane, it will have to compete with methane digesters that are volume based and cheaper to operate. Thus, methanogenesis must be minimized during hydrogen production.

SUMMARY AND PERSPECTIVES

The current worldwide energy crisis requires concerted efforts from researchers to search for all possible energy solutions. MFCs can potentially be an attractive part of bioenergy because they can utilize low-grade organic carbons in wastewater. One should never expect the power density output of an MFC to reach that of a chemical fuel cell because the latter uses energy intensive fuels such as hydrogen and methanol, while MFCs typically use low-grade organic matter in wastewater. By using MFCs for wastewater treatment, a significant energy saving may be achieved. New developments in MFC research have found more uses of MFCs in the form of MECs for production of biomaterials apart from biohydrogen. The various MFC reactor types and operating conditions reviewed in this work were aimed at enhancing MFC performance while lowering costs. Super-bug biofilm consortia, engineered through mutation or genetic engineering, increase the possibilities of practical MFC deployment beyond powering small sensors. It is likely that any practical deployment of MFCs for locally distributed power generation or wastewater treatment will be membrane-less because a membrane poses a major mass transfer resistance and a significant cost factor in reactor design and maintenance. Despite the major advances made in the past decade, MFCs and MECs still face considerable challenges for large-scale real-world applications.

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