

PROTOTYPE DESIGNING AND OPERATIONAL ASPECTS OF MICROBIAL FUEL CELL – REVIEW PAPER

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ABSTRACT: *The looming energy crisis due to both prevailing and prospective exhaustion of non-renewable energy resources such as coal, oil and gas has gripped the whole world. As a result, renewable sources of energy such as wind, solar, Hydrogen, biomass, etc are being harnessed and developed for their utilization as the alternate energy sources to cover for the depleting energy sources. This research review paper is based on the comparison of studies that involve the designing and fabrication of microbial fuel cell (MFC) as a means of producing sustainable production of electric power. The main aspects of MFC research are to reduce the cost of treatment as well as simplifying operational or functional conditions. MFCs can be the next generation of fuel cell technology and thus might play an important role in energy conservation, electricity generation, bio-hydrogen production, biosensors and wastewater treatment as well as in alternate fuel utilization using microorganisms to generate electricity.*

Keywords: *Microbial Fuel cell, Design, Fabrication, Microorganisms, organic matter*

INTRODUCTION

Renewable energy in the near future will probably consist of a large portion of global energy production and usage [1-7]. Current predictions for the global energy have led to the search for alternate energy resources [8-16]. The non-renewable resources of energy are depleting at a faster rate in the present scenario [17-29], implying that there is a need for high efficient energy transformation technologies and ways to utilize alternate renewable energy sources. Microbial fuel cell (MFC) technology represents a new way of renewable energy production by generating electricity from what would otherwise be considered as a waste material [30-35]. This technology uses mostly anaerobic bacteria which may already be present in wastewater and that they work as catalysts to generate electricity while simultaneously treating wastewater [36-39]. Microorganisms have potential to generate electricity from a wide variety of organic wastes while oxidizing the wastes to less harmful forms [40-42]. Although MFCs generate lower amount of power than hydrogen fuel cells, combination of both electricity production and wastewater treatment could reduce the cost of treating primary effluent wastewater [43-44]. Currently, most of the research performed on MFCs is concerned with increasing the power density of the system with respect to the peripheral anode surface area, while little research has been done on determining the effects of voltage output in comparison to varying fuel cell components. The main aspect of fuel cell research is to reduce the treatment cost and simplifying process implementation conditions [45]. Much of the current relevant research is focused on the development of ways and means to convert chemical energy stored in biomass to electricity [46-47]. The energy transformation from burning of biomass (chemical energy to heat) and subsequent utilization of heat for different purposes is very less energy-efficient [25-31]. Since most of the rural population has to depend on subsidized yet scarce electricity supply, a technology such as MFCs can convert the energy stored in chemical bonds in organic compounds to electrical energy via enzymatic reactions associated with the activity of microorganisms has generated considerable

interest in the last decade [12,3]. Two different types of MFCs can be utilized viz. Single chambered or Double chambered MFCs having both the anodic as well as cathodic compartments. Microorganisms in the anodic compartment utilize the biomass for growth forming electrons and protons [48-49]. These electrons can be transported out of cells using electron mediators [4, 14] or some microorganisms have the tendency to expel electron for reducing the substrates which can be absorbed by electrodes [12, 50]. The protons or H⁺ ion can be oxidized to water in cathode chamber, with no other byproducts being formed as the fuel cells are reported to have efficiency higher than that of conventional electricity generation devices [10-18]. MFCs systems are very adaptable and hold much promise to provide energy in a sustainable fashion. Mostly mixed cultures are preferred over single medium as they would probably have a wide range of substrates. Sulphates and sulphides mediated systems have a major role to play in power generation, as most of the time sludge is rich in these compounds [7]. Thus large scale reactors can be synthesized to use sludge and sewage sources as substrates for electricity generation [8-11].

In this review paper, designing and functional aspects of double chambered MFC are discussed including microorganisms and electron transfer mechanism, use of mediators and effect of operational conditions.

MFC DESIGNING

The standard design of most MFCs consists of an anaerobic anode chamber containing a feed source that is inoculated with a mixed microbial culture, and an anode chamber which contains an oxidizing agent such as dissolved oxygen [7-9, 51]. Among the basic components of MFCs which are essential in their construction include electrodes, wirings, glass cell and salt bridge that play an important role in their designing. Induction of proton exchange membrane (PEM) in the fuel cells though enhances the designing cost but gives an increased efficiency of the system [52-59]. Though many different configurations are possible for MFCs, but a widely used and inexpensive design is a two-chamber MFC built in

a traditional "H" shape, consisting usually of two bottles connected by a tube containing a separator such as Nafion (12, 13) or Ultrex (18), or a plain salt bridge (17-23), which a membrane that allows protons to pass between the chambers. In the H-configuration, the membrane is clamped in the middle of the tubes connecting the bottle. As long as the two chambers are kept separated, they can be pressed up onto either side of the membrane and clamped together to form a large surface. The bottles can be joined by means of a glass tube that is inserted through the lid of each bottle filled with agar and salt to serve the same purpose as does CEM.

Single chambered MFC

Single chambered microbial fuel cells (Fig.1) are comprised of a simple anode compartment with no definitive cathode compartment and may not contain proton exchange membranes as well [2-9, 17]. Porous cathodes form one side of the wall of the cathode chamber utilizing oxygen from air atmosphere and letting protons diffuse through them. They are quite simple to scale up than the double chambered MFCs and thus have been extensively used for the relevant research studies. The anodes are normal carbon electrodes whereas the cathodes are either porous carbon electrodes or PEM bonded with flexible carbon cloth electrodes. Cathodes are often covered with graphite in which electrolytes are poured in steady fashion, which behave as catholytes and prevent the membrane and cathode from drying. Thus water management or better fluid management is an important issue in single chambered MFCs [11-15].

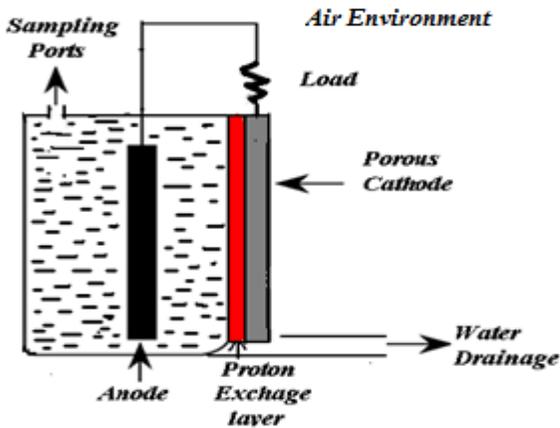


Fig.1: Schematic design of single chambered MFC.

Double chambered MFC

A double chamber microbial fuel cell (DCMFC) consists of two chambers cathode and anode that are housed in separate compartments or chambers, connected via PEM or salt bridge [14]. PEM or salt bridge mainly function as a medium for transfer of protons to make the circuit complete as shown in Fig.1A. This not only completes the reaction process but also prevents anode to come in direct contact with oxygen or any other oxidizers. DCMFCs are mostly run in batches and can be used for the production of higher power output [53-57]. DCMFCs can be suitably designed for scale up to treat large volumes of wastewater and other sources of carbon [60-65]. Such type of DCMFC is particularly referred to as up-flow mode of MFC as shown Fig.1B. The operation of

is usually a cation exchange membrane (CEM), also known as PEM. The key to this design is to choose

such type of DCMFCs is without the use of mediators and sometimes even without using membranes but they can be used for large scale reduction of electricity from the wastes [15,16].

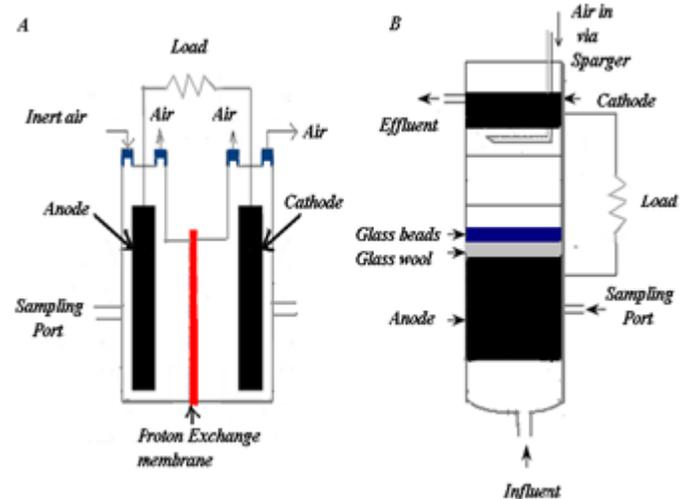


Fig.2: Schematic designs of, (A) Double chambered MFC, and (B) Cylindrical membrane-less MFC.

MICROORGANISMS AND ELECTRON TRANSFER MECHANISM IN AN MFC

Microorganisms are the backbone of MFC technology, as their activity inside an MFC is a direct source of energy generation. Microbes used in an MFC catalyze the conversion of organic matter into electricity [51]. When bacteria oxidize organic matter present in wastewater, electrons are shuttled to the electrode and protons produced are diffused through water to the counter electrode or cathode giving the electrode positive characteristics [3, 25]. Normal microbial catabolism entails substrate initially being oxidized anaerobically, when its electrons are released by enzymatic reactions [61]. They oxidize the substrate and produce electrons and protons in the anode chamber, which are then transported to the cathode chamber by an external circuit, while the protons diffuse through PEM internally. Electrons are stored as intermediates such as Nicotinamide adenine dinucleotide and quinones which become reduced and are then used to provide the living cell with energy. In addition, electrons and protons are consumed in the cathode chamber by reducing oxygen, usually forming water [52, 61]. There are three categories of microbes that can be used in MFCs; one, that can directly transfer electrons to anode using anode as terminal electron acceptor [47-50]; marine sediments, wastewater, fresh water sediments, mining dumps are the main sources of such microorganisms. Two, that can't directly induce the electron transfer, but use mediators to carry out this function with comparatively higher efficiency [3-5]. And third, that can accept electrons from cathode chamber itself. Electron transfer happens due to shuttling of electrons already present in the soluble form in

bulk solution of extracellular matrix [40-50]. Terminal electron acceptors are the oxides in which electrons are transported by direct contact with microorganisms. Figure 3 [53] illustrates the phenomenon of the transfer of electrons via the function of a microbe in the anodic compartment obtained from an electron donor or any organic compound to the anode electrode. The mechanism involved in this electron transfer process may consist of either via direct contact, nano-wires, or mobile electron shuttles, which are small size spheres that represent as the final membrane associated shuttle. During the process of electron production protons are also produced in excess, which migrate through PEM into the cathode chamber [54].

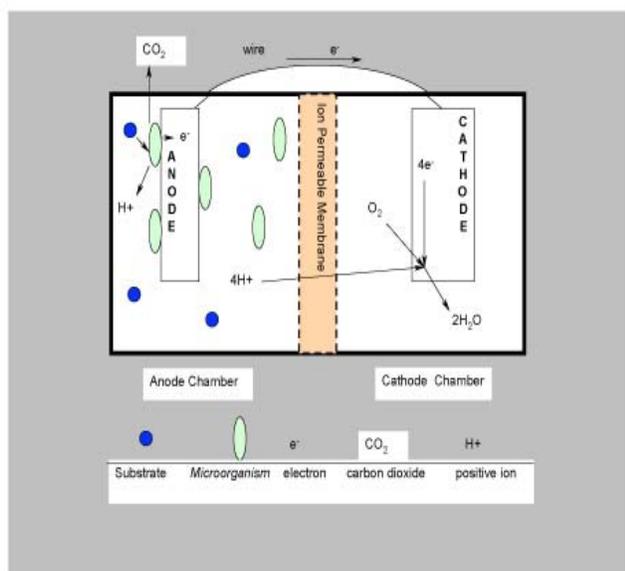


Fig. 3: Flow of electrons in a working MFC.

The outer layers of the majority of microbial species are composed of non-conductive lipid membrane, peptidoglycans and lipopolysaccharides, which stop the facilitation of electron transfer to the anode [4]. When these microbes metabolize food to provide them with energy, they are tapping the energy of oxidation of energy-rich or electron-rich substances, liberated from carbohydrates [52]. The chemiosmotic hypothesis states that electron transfer chains of bacteria are coupled to the translocation of protons across the membranes which are in turn linked to the synthesis of adenosine triphosphate (ATP) by the proton electrochemical potential across the energy transducing membrane. The bacterial cell membrane functions as an energy transducing membrane operating according to the chemiosmotic principle. The translocation of protons towards the outside of the membrane results in the establishment of a proton electrochemical gradient. The pH gradient adds up to this membrane potential and results in the proton motive force. The re-entry of these protons across the ATP-synthase enzyme is accompanied by ATP synthesis. The ATP thus synthesized is used by the bacteria for their survival [53].

USE OF MEDIATORS FOR ELECTRON TRANSFER

MFC concept was demonstrated as early as 1910 when *Escherichia coli* and *Saccharomyces sp.* were used to generate electricity using platinum electrodes [21, 51]. This idea remained in its infancy till early 1980s when the concept of MFC was boosted with the advent of the use of electron mediators to enhance electricity generation [52, 6]. Mediators play an important role that of a shuttle between anode and electron carriers inside the cell. Since most microbial cells are electrochemically inactive, electron transfer from microbial cells to the electrode is facilitated by the help of mediators such as thionine, methyl viologen, humic acid, and so on [66]. Mediators then transfer across the membrane and release the electrons to the electrode and become oxidized again in anodic chamber and thus are reutilized. Mediators can divert electrons from the respiratory chain by entering the outer cell membrane, becoming reduced, and then leaving in a reduced state to shuttle the electrons to the electrode [61]. Microorganisms transfer electrons to the anode electrode in three ways: one via exogenous mediators, which are external to the cell, such as potassium ferricyanide, thionine, or neutral red; two, using endogenous mediators that are produced by the bacteria; and third, via direct transfer of electrons from the respiratory enzymes e.g. cytochromes, to the electrode [6, 62].

Good and efficient mediators should be permeable in a cell membrane and have higher electron affinity than the electron carriers of the electron transport chains. They should also possess a high electrode reaction rate, be soluble as well as completely non-biodegradable and non-toxic to microbes [54]. In contrast, lower redox potential mediators are theoretically better than higher redox potential mediators in terms of high affinity for electrons [55, 6]. In addition, microbes are also known to use naturally occurring compounds including microbial metabolites or endogenous mediators such as humic acids, anthraquinone, and oxyanions of sulphur, sulphate and thiosulphate [56-60]. Mediators are best prepared as a concentration solution of 5-10 mM in water and then added to the anolyte suspension of microorganisms from a syringe to give the required final concentration [54].

Direct electron transfer requires a physical contact between the bacterial cell membrane and the cathode electrode surface, and electrons from the electrode are directly received by the outer membrane redox macromolecules such as cytochromes (Fig. 4A). While some bacteria perform direct electron transfer, some microorganisms can excrete redox-active compounds to carry out indirect electron transfer with electrodes. This mechanism does not need a physical contact between the bacterial cell membrane and the cathode electrode surface (Fig. 4B).

It has been suggested that in MFCs with naturally occurring microbial communities, extracellular substances are always involved in the electron transfer between microbes and electrodes [61]. While the addition of exogenous mediators may be beneficial for the electron transfer between cathode electrode and microorganisms, in view of operation, it contributes additional cost and many of these mediators are toxic, short-lived, and unsustainable [62].

OPERATIONAL CHARACTERISTICS AND PERFORMANCE EVALUATION OF MFC

MFC parameters such as electrode materials, PEMs or salt bridge along with operational conditions of anode and cathode chambers have vital effect on MFC operation [5-7]. The electrode material determines the diffusivity of oxygen in MFCs in case when electrodes are relatively more porous allowing oxygen diffusion to anode chamber as well as the loss of power incurred in MFCs due to internal resistance [19, 66]. PEMs, though costly, but are very important in terms of performance enhancement of MFCs but might need to be best fitted in order to limit the dangers of clogging and drying [12-15]. Their presence makes the MFC assembly very robust and thus is usable in practical conditions [20]. The ratio of membrane

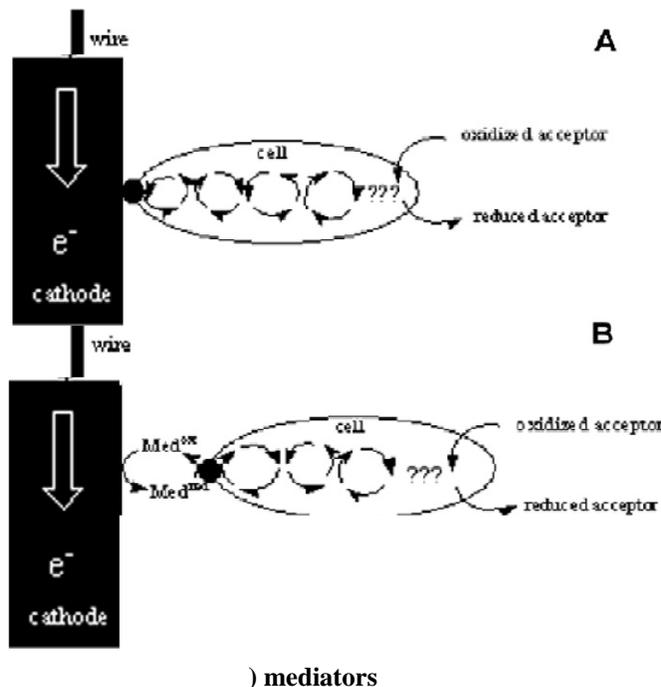


Fig. 4. Schematics for electron transfer mechanism (ETM) via membrane bound cytochromes:

- (A) **indirect ETM via added (exogenous) mediators, and**
 (B) **direct ETM via secreted (endogenous [55]).**

surface area to system volume is critical to the system performance [16-18]. Dissolved Oxygen (DO) profile is an important parameter for the operation of MFCs with lower DO being used by the anode component as compared to higher DO used by the cathode compartment [13, 15]. In addition, higher DO facilitates diffusion of more oxygen into anode compartment through the porous membrane. Fuel or substrate concentration is another important factor in the successful operation of MFCs with maximum yield of power generation can be accrued usually with higher than normal concentration of the substrate [5-10]. Thus optimum current generation during the course of MFC operation depends on several factors such as pH, resistance, electrolyte used, and dissolved oxygen concentration in the cathode compartment. Table 1 shows the updated results obtained using MFCs in

terms of its varying operational characteristics. The performance of the reactors is highlighted in terms of power generation in Watts per m³ of anode compartment volume used. The data in Table 1 shows that the relationship between MFC current and power is not always unequivocal. The overall MFC performance can be expressed by plotting the polarization curve, which shows the effect of operational parameters such as fuel concentration, fuel-feeding rate and temperature [78]. Korneel and Verstraete (2005) have referred to the fact that power density until now was used as a representative performance index for MFCs; however, it cannot clearly indicate the performance as it can be substantially varied subject to the determination method of electrode area. In MFCs, only a part of the electrode surface area is involved in the electrode reaction rather than total surface area including internal surface area as in typical chemical fuel cells such as PEM fuel cells. Thus, for electrode material with internal structure, the total area of electrodes including the internal surface or apparent area is considered for computing of power density in MFCs. Whereas, volumetric power can also be used as an alternative performance index to power density in a large volume process such as wastewater treatment.

APPLICATIONS OF MFCs

The most obvious use of MFCs is the generation of electricity via their operation. The latest research done in this regard suggests that MFCs have been found as efficient devices when run on the large scale with a conversion efficiency of fuel to electricity in the range of 70-92% [13, 43]. The electric power thus produced can also best be preserved by storing the same in rechargeable batteries. In addition, low-power wireless systems can be adequately powered as such with MFCs [25]. MFC technology is highly suitable for wastewater treatment as during the course of the treatment, electricity can be harnessed from the decomposition of wastewater contents in addition to wastewater depollution [33-41]. The process of MFC operation generates minimum of solid waste from the process, whereas the electricity produced can be used for aerating the sludge [43-48]. MFCs can also be used for the production of bio-hydrogen from the cathode section of MFCs, though the process may not be thermodynamically feasible, still hydrogen can be produced at the cathode if a potential is applied to overcome the energy barrier [42-52]. A potential of 110 mV can be applied to produce hydrogen, which is far less than 1210 mV required to break water into hydrogen and oxygen via electrolysis [33]. MFCs can also be used to produce sensors to detect the level of pollutants by measuring the voltage as well as oxygen demand of the waste material [26]. However, their overall applicability and potential to operate them at ambient temperatures is still largely unexplored. Efficient designing and operation of MFCs can make them a platform technology, which could be applicable in diverse fields without substantial modification. MFCs can prove to be a sustainable core technology adaptable to a wide variety of applications converting a wide array of electron donors with effective energy generation at low and moderate temperatures, even

when the electron donor is provided at low concentrations [79].

CONCLUSIONS

There is a wide scope for the development of microbial fuel cells due to their evolution of being a simple yet robust treatment technology. This can particularly be feasible in the field of wastewater treatment, where middle term application can be expected at the market value prices. Induction of suitable microorganisms is the main player in the production of higher number of electrons at the anode section. Materials of construction for MFCs need to be studied in depth to bring down the internal resistance of the system as well as to

minimize the corrosion problems. MFCs can also be applied to power up medical implants and hand-held appliances in addition to supply power to remote surveillance and communication gears that are used in unmanned stations. The implementation and operational cost of MFCs can also be lowered with better designs in case of single chamber fuel cells.

Table 1: Performance of MFCs based on both axenic (single culture) and mixed culture systems in terms of current generation [78].

	Substrate	Electrode type	I, mA	P, W/m ³
Axenic Cultures				
<i>Proteus vulgaris</i>	Glucose	Glassy carbon	0.8	18
<i>Erwinia dissolvens</i>	Glucose	Woven graphite	0.7	n.a.
<i>Shewanella putrefaciens</i>	Lactate	Woven graphite	0.04	0.08
<i>Geobacter sulfurreducens</i>	Acetate	Plain graphite	0.4	0.35
<i>Rhodospirillum rubrum</i>	Glucose	Plain graphite	0.2	0.25
		Woven graphite	0.57	1.7
		Graphite foam	0.45	0.96
<i>Pseudomonas aeruginosa</i>	Glucose	Plain graphite	0.1	8.8
<i>Escherichia coli</i>	Lactate	Woven graphite	3.3	7.6
		Plain graphite	2.6	3.6
Mixed cultures				
Mixed, saltwater	Acetate	Plain graphite	0.23	n.a.
	S ₂ -/acetate	Plain graphite	60	n.a.
Mixed consortium, batch	Glucose	Plain graphite	30	216
Activated sludge	Wastewater	Woven graphite	4.85	1.6
		Woven graphite	11	34
		Plain graphite	2.6	32
	Glucose	Woven graphite	0.9	13
	Sucrose	Granular graphite	6.2	47
Mixed consortium, Continuous	Glucose	Granular graphite	5.4	37
	Acetate	Carbon paper	1.27	13
	Butyrate	Carbon paper	0.46	7.6
n.a. = data not available				

REFERENCES

- [1] Potter M. C., Electrical effects accompanying the decomposition of organic compounds, *Proc. R. Soc. Ser.* **84**:260–276(2003).
- [2] Allen R. M. and H. P. Bennetto, Microbial fuel-cells: electricity production from carbohydrates, *Appl. Biochem. Biotechnol.*, **39**(40):27–40(2004).
- [3] Rabaey K. and W. Verstraete, Microbial fuel cells: novel biotechnology for energy generation, *Trends Biotechnol.*, **23**:291–298(2003).
- [4] Davis F. and S. Higson, Biofuel cells-recent advances and applications, *Biosens. Bioelectron.*, **22**: 1224–1235(2007).
- [5] Ieropoulos I. A., Greenman J., Melhuish C and J. Hart, Comparative study of three types of microbial fuel cell, *Enzyme Microb Tech.*; **37**:238–245(2006).
- [6] Park D. H. and J. Zeikus, Electricity generation in microbial fuel cells using neutral red as an electronophore, *Appl. Environ Microb.* **66**:1292–1297(2000).
- [7] Tender L., Gray S., Groveman E., Lowy D., Kauffma P., Melhado R., Tyce R., Flynn D., Petrecca R., and J. Dobarro, The first demonstration of a microbial fuel cell as a viable power supply: Powering a meteorological buoy, *J. Power Source*, **179**: 571–575(2008).
- [8] Lovley D. R., Dissimilatory metal reduction, *Annu. Rev. Microbial.*, **47**: 263–290(2003).

- [9] Kim B. H., Kim H. J., Hyun M. S. and D. H. Park, Direct electrode reaction of Fe(III)-reducing bacterium, *Shewanella putrefaciens*, *J. Microbiol. Biotechnol*, **9**: 127–131(1993).
- [10] Kim H. J., Park H. S., Hyun M. S., Chang I. S., Kim M. and B. H. Kim, A mediatorless microbial fuel cell using a metal reducing bacterium *Shewanella putrefaciens*, *Enzyme Microb. Tech*, **30**:145–152(2002).
- [11] Bond D. R. and D. Lovley, Electricity production by *Geobacter sulfur-reducens* attached to electrodes, *Appl. Environ. Microbiol*, **69**: 1548–1555(2003).
- [12] Min B., Cheng S. and B. E. Logan, Electricity generation using membrane and salt bridge microbial fuel cells, *Water Research*, **9**: 1675–1686(2005).
- [13] Chaudhuri S. K. and D. R. Lovley, Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells, *Nat. Biotechnol*, **21**: 1229–1232(2003).
- [14] Niessen J., Schroder U. and F. Scholz, Exploiting complex carbohydrates for microbial electricity generation—a bacterial fuel cell operating on starch, *Electrochem. Commun*, **6**: 955–958(2004).
- [15] Ringeisen B. R., Henderson E., Wu P. K., Pietron J., Ray R. and B. Little, High power density from a miniature microbial fuel cell using *Shewanella oneidensis* DSP10, *Environ. Sci. Technol*, **40**: 2629–2634(2006).
- [16] He Z., Minteer S. D. and L. Angenent, Electricity generation from artificial wastewater using an upflow microbial fuel cell, *Environ. Sci. Technol*, **39**: 5262–5267(2005).
- [17] Jang J. K., Pham T. H., Chang I. S., Kang K. H., Moon H. and K. S. Cho, Construction and operation of a novel mediator-and membrane-less microbial fuel cell, *Process Biochem*, **39**: 1007–1012(2004).
- [18] Park D. H. and J. G. Zeikus, Improved fuel cell and electrode designs for producing electricity from microbial degradation, *Biotechnol. Bioeng*, **81**: 348–355(2003).
- [19] Aelterman P., Rabaey K., Pham H. T., Boon N. and W. Verstraete, Continuous electricity generation at high voltages and currents using stacked microbial fuel cells, *Environ. Sci. Technol*, **40**: 3388–3394(2006).
- [20] Oh S. E. and B. E. Logan, Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies, *Water Research*, **39**: 4673–4682(2005).
- [21] Rozendal R. A., Hamelers H. V. and C. J. Buisman, Effects of membrane cation transport on pH and microbial fuel cell performance, *Environ. Sci. Technol*, **40**: 5206–5211(2006).
- [22] Grzebyk M. and G. Pozniak, Microbial fuel cells (MFCs) with interpolymer cation exchange membranes, *Sep. Purif. Technol*, **41**: 321–328(2005).
- [23] Oh S. E., Min B. and B. E. Logan, Cathode performance as a factor in electricity generation in microbial fuel cells, *Environ. Sci. Technol*, **38**: 4900–4944(2004).
- [24] Rosenbaum M., Schroder U. and F. Scholz, Investigation of the electrocatalytic oxidation of ethanol at platinum black under microbial fuel cell conditions, *J. Solid State Electrochem*, **10**: 872–878(2006).
- [25] Ieropoulos I., Greenman J. and C. Melhuish, Imitation metabolism: energy autonomy in biologically inspired robots, Proceedings of 2nd International Symposium on Imitation of Animals and Artifacts, 191–194(2003).
- [26] Liu H., Grot S. and B. E. Logan, Electrochemically assisted microbial production of hydrogen from acetate, *Environ. Sci. Technol*, 4317–4320(2003).
- [27] Gong M., Liu X., Tremblay J. and C. Johnson, Sulfur-tolerant anode materials for solid oxide fuel cell application, *J. Power Source*, **168**: 289–298(2007).
- [28] Kim I. S., Chae K. J., Choi M. J. and W. Verstraete, Microbial fuel cells: recent advances, bacterial communities and application beyond electricity generation. *Environ. Eng. Res*, **13**(2): 51–65(2008).
- [29] Kim J. R., Min B. and B. E. Logan, Evaluation of procedures to acclimate a microbial fuel cell for electricity generation, *Appl. Microbiol. Biotechnol*, **68**: 23–30(2006).
- [30] Lee J., Phung N. T., Chang I. S., Kim B. H. and H. C. Sung, Use of acetate for enrichment of electrochemically active microorganisms and their 16S rDNA analyses, *Microbiol. Lett*, **223**: 185–191(2003).
- [31] Liu H., Cheng S. and B. E. Logan, Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell, *Environ. Sci. Technol*, **39**: 658–662(2005).
- [32] Pham T. H., Jang J. K., Chang I. S. and B. H. Kim, Improvement of cathode reaction of a mediator-less microbial fuel cell, *J. Microbiol. Biotechnol*, **14**: 324–329(2004).
- [33] Ragauskas A. J., Williams C. K., Davison B. H., Britovsek G., Cairney J., Eckert C. A., Frederick Jr. W. J., Hallett J. P., Leak D. J., Liotta C. L., Mielenz J. R., Murphy R., Templer R. and T. Tschaplinski, The path forward for biofuels and biomaterials, *Science*, **311**: 484–489(2006).
- [34] Song C., Fuel processing for low-temperature and high-temperature fuel cells, Challenges and opportunities for sustainable development in the 21st century, *Catal. Today*, **77**: 17–49(2002).
- [35] Aelterman P., Rabaey K., Pham H. T., Boon N. and W. Verstraete, Continuous electricity generation at high voltages and currents using stacked microbial fuel cells, *Environ. Sci. Technol*, **40**(10): 3388–3394(2006).
- [36] He Z., Minteer S. D. and L. T. Angenent, Electricity generation from artificial wastewater using an upflow microbial fuel cell, *Environ. Sci. Technol*, **39**: 5262–5267(2005).
- [37] Liu H. and B. E. Logan, Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane, *Environ. Sci. Technol*, **38**: 4040–4046(2004).
- [38] Liu H., Ramnarayanan R. and B. E. Logan, Production of electricity during wastewater treatment using a

- single chamber microbial fuel cell, *Environ Sci Technol*, **38**(7): 2281-2285(2004).
- [39] Min B., Kim J. R., Oh S. E., Regan, J. M. and B. E. Logan, Electricity generation from swine wastewater using microbial fuel cells, *Water Research*, **39**: 4961-4968(2005).
- [40] Moon H., Chang I. S. and B. H. Kim, Continuous electricity production from artificial wastewater using a mediator-less microbial fuel cell, *Bioresour. Technol.* **97**: 621-627(2006).
- [41] Oh S. and B. E. Logan, Proton exchange membrane and electrode surface areas as factors that affect power generation in microbial fuel cells, *Appl. Microbiol. Biotechnol.* **70**: 162-169(2006).
- [42] Rabaey K., Lissens G., Siciliano S. D. and W. Verstraete, A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency, *Biotechnol. Lett.*, **25**: 1531-1535(2003).
- [43] Rabaey K., Boon N., Ho M. and W. Verstraete, Microbial phenazine production enhances electron transfer in biofuel cells, *Environ. Sci. Technol.*, **39**: 3401-3408(2005).
- [44] Rozendal R. A., Hamelers H. V. M. and C. J. N. Buisman, Effects of membrane cation transport on pH and microbial fuel cell performance, *Environ. Sci. Technol.*, **40**: 5206-5211(2006).
- [45] Min B., Kim J., Oh S., Regan J. M. and B. E. Logan, Electricity generation from swine wastewater using microbial fuel cells, *Water Research*, **39**: 4961-4968(2005).
- [46] Kim J. R., Zuo Y., Regan J. M. and B. E. Logan, Analysis of ammonia loss mechanisms in microbial fuel cells treating animal wastewater. *Biotechnol. Bioeng.* **99**: 1120-1127(2008).
- [47] He Z., Shao H. and L. T. Angenent, Increased power production from a sediment microbial fuel cell with a rotating cathode, *Biosens. Bioelectron.*, **22**: 3252-3255(2007).
- [48] He Z., Minter S. D. and L. T. Angenent, Electricity generation from artificial wastewater using an upflow microbial fuel cell, *Environ. Sci. Technol.*, **39**: 5262-5267(2005).
- [49] Scafer H. and G. Muyzer, Denaturing gradient gel electrophoresis in marine microbial ecology, *Methods in Microbiology*, Paul J. Ed. Academic Press London, pp 425-468(2001).
- [50] Liu H., Cheng S. and B. E. Logan, Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration, *Environ. Sci. Technol.*, **39**: 5488-5493(2005).
- [51] Bond D. R. and D. R. Lovley, Electricity production by *Geobacter sulphur-reducens* attached to electrodes, *Applied and Environmental Microbiology*, **69**: 1548-1555(2003).
- [52] Cheng S., Liu H. and B. E. Logan, Increased power generation in a continuous flow MFC with advective flow through the porous anode and reduced electrode spacing. *Environmental Science & Technology*, **40**: 2426-2432(2006).
- [53] Henslee B. E., Gehres P. D., Bettin C. C., Stokes R. C., Frew, B. A., Weber A. V., Nazareth M. And S. A. Marcus, Biological Fuel Cell: Modeling, Design, and Testing, *Final Report for ASAE's G.B. Gunlogson Student Environmental Design Competition. Ohio State University, Columbus, Ohio.*, 2004.
- [54] Bennetto, H. P., Electricity generation by microorganisms, *Biotechnology Education*, **4**: 163-168(1990).
- [55] Liping H., John M. R. And Q. Xie, Electron transfer mechanisms, new applications, and performance of biocathode microbial fuel cells, *Bioresource Technology*, **102**: 316-323(2011).
- [56] Du Z., Li H. and T. A. Gu, state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy, *Biotechnology Advances*: **25**: 464-482(2007).
- [57] Viridis B., Rabaey K., Yuan Z. and J. Keller, Microbial fuel cells for simultaneous carbon and nitrogen removal, *Water Research*, **42**: 3013-3024(2008).
- [58] You S. J., Identification of denitrifying bacteria diversity in an activated sludge system by using nitrite reductase genes, *Biotechnol. Lett.*, **27**: 1477-1482(2006).
- [59] Bond D. R., Holmes D. E. and L. M. Tender, Electrodereducing microorganisms that harvest energy from marine sediments, *Science*, **295**: 483-485(2002).
- [60] Clauwaert P., Vander H. D. and N. Boon, Open air biocathode enables effective electricity generation with microbial fuel cells, *Environ Sci Technol*, **41**: 7564-7569(2007).
- [61] Watanabe K., Manefield M., Lee M. And A. Kouzuma, Electron shuttles in biotechnology, *Curr. Opin. Biotechnol.* **20**: 633-641(2009).
- [62] Huang L. and I. Angelidaki, Effect of humic acids on electricity generation integrated with xylose degradation in microbial fuel cells, *Biotechnol. Bioeng.* **100**: 413-422(2008).
- [63] Liu H and B. E. Logan, Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane, *Environ Sci Technol*, **38**: 4040-4046(2004).
- [64] Liu H., Ramnarayanan R. and B. E. Logan, Production of electricity during wastewater treatment using a single chamber microbial fuel cell, *Environ Sci Technol*, **38**: 2281-2285(2004).
- [65] Logan B. E., Cheng S. A. and V. Watson, Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells, *Environ Sci Technol*, **41**: 3341- 3346(2007).
- [66] Geun-Cheol G., In-Seop C., Byung H. K., Mia K., Jaeyung J., Hyung S. P. And J. K. Hyung, Operational parameters affecting the performance of a mediator-less microbial fuel cell, *Biosensors and Bioelectronics*, **18**: 327-334(2003).

- [67] Min B., Cheng S. and B. E. Logan, Electricity generation using membrane and salt bridge microbial fuel cells, *Water Research*, **39**: 1675–1686(2005).
- [68] Rabaey K., Clauwaert P. and P. Aelterman, Tubular microbial fuel cells for efficient electricity generation, *Environ Sci Technol*, **39**: 8077–8082(2005).
- [69] Schroöder U., Niessen J. and F. Scholz, A generation of microbial fuel cells with current outputs boosted by more than one order of magnitude. *Angew Chem Int Ed*, **42**: 2880–2883(2003).
- [70] You S., Zhao Q. and J. Zhang, A microbial fuel cell using permanganate as a cathodic electron acceptor, *J Power Sources*, **162**: 1409–1415(2006).
- [71] You S., Zhao Q. and J. Zhang, A graphite-granule membrane-less tubular air-cathode microbial fuel cell for power generation under continuously operational conditions, *J Power Sources*, **173**: 172–177(2007).
- [72] Zhao F., Harnisch F. and U. Schroder, Application of pyrolyzed iron (II) phthalocyanine and CoTMPP based oxygen reduction catalysts as cathode materials in microbial fuel cells, *Electrochem Commun*, **7**(12): 1405–1410(2005).
- [73] Bond, D. R. and D. R. Lovley, Electricity production by *Geobacter sulfurreducens* attached to electrodes, *Appl. Environ. Microbiol*, **69**: 1548–1555(2003).
- [74] Nevin, K. P., Richter H., Covalla S. F., Johnson J. P., Woodard T. L., Orloff A. L., Jia H., Zhang M. and D. R. Lovley, Power output and columbic efficiencies from biofilms of *Geobacter sulfurreducens* comparable to mixed community microbial fuel cells, *Environ. Microbiol.*, **10**: 2505–2514(2008).
- [75] Kim N., Choi Y., Jung S. and S. Kim, Effect of initial carbon sources on the performance of microbial fuel cells containing *Proteus vulgaris*, *Biotechnol. Bioeng*, **70**: 109–114(2004).
- [76] Lu N., Zhou S. G. Zhuang L., Zhang J. T. and J. R. Ni, Electricity generation from starch processing wastewater using microbial fuel cell technology, *Biochem. Eng. J.*, **43**: 246–251(2009).
- [77] Ren Z., Steinberg L. M. and J. M. Regan, Electricity production and microbial biofilm characterization in cellulose-fed microbial fuel cells, *Water Sci. Technol*, **58**: 617–622(2009).
- [78] Korneel R. and W. Verstraete, Microbial fuel cells: novel biotechnology for energy generation, *Trends in Biotechnology*, **23** (6): 292-298(2005).
- [79] Hyunsoo M., In Seop C. and B. H. Kim, Continuous electricity production from artificial wastewater using a mediator-less microbial fuel cell, *Bioresource Technology*, **97**: 621–627(2006).