Microbial Fuel Cells (MFCs) - a novel source of energy for new millennium

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Continued use of petroleum fuels is now widely recognized as unsustainable because of their depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment. Renewable, carbon neutral, transport fuels are necessary for environmental and economic sustainability. A microbial fuel cell (MFC), a novel form of microbial respiration has recently been discovered, it is a bioreactor that converts chemical energy present in the organic compounds (in the form of chemical bonds) to electrical energy through catalytic reactions of microorganisms under anaerobic conditions. These organisms, termed electricigens, Microbial fuel cells (MFCs) provide new opportunities for the sustainable production of energy from biodegradable compounds. MFCs function on different carbohydrates and also on complex substrates present in wastewaters and renewable biomass. Biomass, especially organic waste, is being considered as a valuable candidate. The use of biomass, in the case of waste organics, is environment friendly and regarded as a renewable energy source. The construction and analysis of MFCs requires knowledge at both scientific and engineering fields, ranging from microbiology and electrochemistry to materials and environmental engineering. Therefore unfolding MFC systems involves an understanding of these diverse scientific and engineering principles. At present, microbial fuel cells are not commonly considered a part of the energy portfolio for the future, is that microbial fuel technology is not yet sufficiently well developed to produce substantial quantities of power in a cost-effective manner. In this chapter, we provide a review of the different materials and methods used to construct MFCs, techniques used to analyse system performance, and recommendations on what information to include in MFC studies in the new millennium.

1. Introduction:

The use of fossil fuels, especially oil and gas, for all human needs in recent years has accelerated and this triggers the global energy crisis. Renewable bioenergy is viewed as one of the ways to decrease the current global warming crisis. It is well known that fuels, such as ethanol, butanol, methane and hydrogen can produce by microorganisms. But the electricity production using microbes, which is known as microbial fuel cells (MFCs), is recent development in energy biology and highly attracting area. Microbial fuel cells put forward the possibility of harvesting electricity from organic waste and renewable biomass. These are attractive sources of energy because they are 'carbon-neutral'. [1, 2, 3].

Microbial fuel cells are significantly different from that of the better known conventional fuel cells, i.e., abiotic hydrogen and methanol driven fuel cells, 1. Abiotic fuel cells require expensive catalysts to promote oxidation of the electron donors, whereas in MFCs naturally occurring microorganisms catalyse the oxidation of the fuels. 2. Abiotic fuel cells need high temperatures for their operation, but microbial fuel cells can be operated at room temperature and could potentially be designed to function at any temperature at which microbial life is possible. 3. The fuels for abiotic fuel cells are highly explosive or toxic and have to be highly purified to avoid poisoning the catalysts. By contrast, the microorganisms that power microbial fuel cells can oxidize a diverse range of 'dirty' fuels that are often of little perceived value, such as organic waste and the organic matter in soils and sediments. 4. The ubiquitous and harmless properties of fuels for microbial fuel cells alleviates the need for the complex and highly regulated distribution systems that are required for hydrogen and methanol. Therefore, microbial fuel cells might be particularly attractive power sources in remote locations and regions of developing countries that are not served by well-developed, centralized power grids [3].

MFCs are also different from the well developed enzymatic fuel cells in which electricity is generated through enzymes or cell extracts rather than whole cells [4]. Enzymatic fuel cells can produce high levels of power for their size and are well suited to applications such as sensors. However, enzymatic fuel cells typically only harvest a small percentage of the electrons available in organic fuels, because incorporating the full complement of enzymes necessary to completely oxidize organic fuels to carbon dioxide is not yet technically feasible. Microbial fuel cells offer the possibility of extracting over 90% of the electrons from organic compounds, and can be self-sustaining and renewing when populated with microorganisms that conserve energy from electron transfer to electrodes.

Over the past 40 years researchers have been suggested that microbial fuel cells might be developed for a wide range of applications, including serving as household electrical generators and powering items such as small portable electronic devices boats, automobiles, electronics in space and self-feeding robots [5-8]. Another interesting area is developing large-scale microbial fuel cells for the conversion of sewage and other organic waste to electricity and the bioremediation of contaminated environments [9–11]. However, none of these applications is yet practical. At present, microbial fuel cells can produce enough current to power small electronic devices for short periods or to trickle-charge

capacitors for applications with higher power demands. However, the size of these microbial fuel cells precludes their incorporation into the electronic devices where they can supply power.

The purpose of this chapter is to summarize our present knowledge of the microbiology of electricity production. Many microorganisms can contribute to electricity production in microbial fuel cell. Recently researchers have discovered a new metabolic type of electricity-producing microorganisms that has indicated that a wide diversity of organic compounds can be effectively converted to electricity in self-sustaining microbial fuel cells. These organisms, known as electricigens, can completely oxidize organic compounds to carbon dioxide, with an electrode serving as the sole electron acceptor, and conserve energy to support growth from this electron transfer. The known physiology and ecology of electricigens, their potential mechanisms for electron transfer to electrodes and present concepts for optimizing their performance are reviewed.

2. What is microbial fuel cell?

Microbial fuel cells (MFCs) are devices which convert organic matter to energy (electricity or hydrogen) using microorganisms as catalysts. Generally bacteria are used in MFCs to generate electricity while accomplishing the biodegradation of organic matters or wastes [12, 13]. Figure (1) shows a schematic diagram of a typical MFC for producing electricity. It consists of anodic and cathodic chambers partitioned by a proton exchange membrane (PEM) [14, 15]. The anode compartment is typically maintained under anoxic conditions, whereas the cathode can be suspended in aerobic solutions or exposed to air. Electrons flow from the anode to the cathode through an external electrical connection that typically includes a resistor, a battery to be charged or some other electrical device.

Microbes in the anodic chamber of an MFC oxidize added substrates and generate electrons and protons in the process. Carbon dioxide is produced as an oxidation product. However, there is no net carbon emission because the carbon dioxide in the renewable biomass originally comes from the atmosphere in the photosynthesis process. Unlike in a direct combustion process, the electrons are absorbed by the anode and are transported to the cathode through an external circuit. After crossing a PEM or a salt bridge, the protons enter the cathodic chamber where they combine with oxygen to form water. Microbes in the anodic chamber extract electrons and protons in the dissimilative process of oxidizing organic substrates [15].

Typical electrode reactions are shown below using acetate as an example substrate.

Anodic reaction: $CH_3COO^- + 2H_2O \xrightarrow{\text{Microbes}} 2CO_2 + 7H^+ + 8e^-$

Cathodic reaction: $O_2 + 4e^- + 4H^+ \longrightarrow 2H_2O$

The overall reaction is the break down of the substrate to carbon dioxide and water with a concomitant production of electricity as a by-product. Based on the electrode reaction pair above, an MFC bioreactor can generate electricity from the electron flow from the anode to cathode in the external circuit.

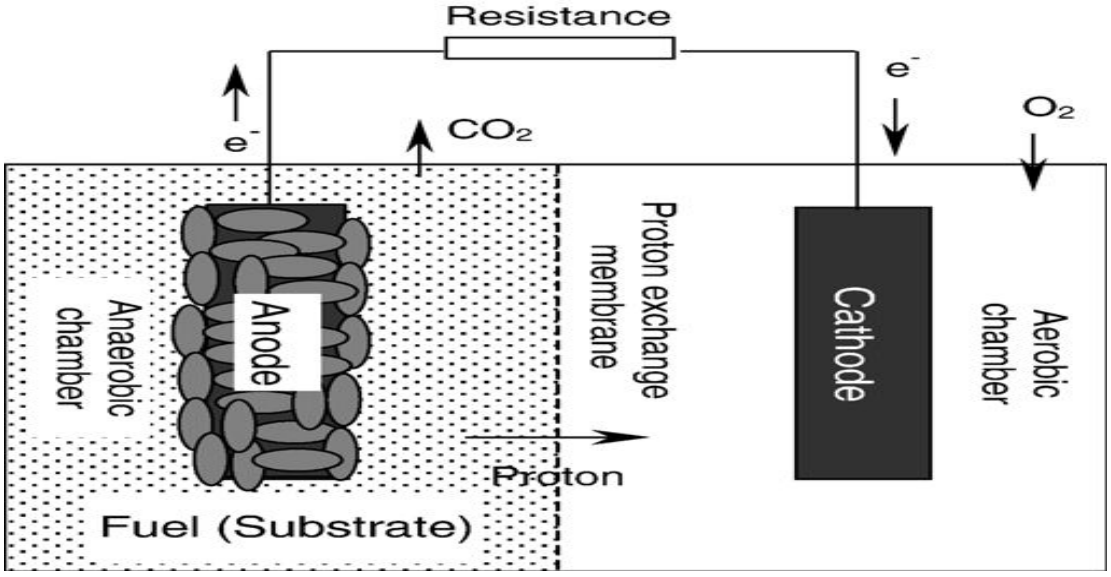

Fig 1: Schematic diagram of typical two-chamber microbial fuel cell
(Figure redrawn modifications after Du et al 2007)

3. How microbes liberate electrons from organic matter:

As we discussed in previous section anaerobic metabolism is essential to convert organic matter to electricity in a effective manner. Fermentation is a well-known mechanism for anaerobic metabolism of organic matter and many microbial fuel-cell studies till now relied solely on fermentative microorganisms [6]. However, fermentation alone cannot be a approach for efficient conversion of organic matter to electricity. The products produced during fermentation do not readily react with electrodes. Effective anaerobic oxidation of complex assemblages of organic matter, such as those found in most wastes and biomass, requires the fermentation products from the metabolism of sugars, amino acids and related compounds, in addition to other constituents, such as aromatic compounds and long-chain fatty acids, to be oxidized with electron transfer to an electron acceptor. The closest analogues to electrodes for microbial metabolism in natural environments are probably Fe^{3+} oxides, because both electrodes and Fe^{3+} oxides are insoluble and extracellular electron acceptors. The oxidation of organic matter, coupled to the reduction of Fe^{3+} oxides in sedimentary environments, requires the co operation of a consortium of fermentative micro organisms and Fe^{3+}-reducing microorganisms (Fig 2). Fe^{3+}-reducing microorganisms metabolize the fermentation products and the organic compounds that fermentative microorganisms do not readily metabolize, oxidizing them to carbon dioxide, with Fe^{3+} oxides serving as the electron acceptor [16]. It seems likely that, in order to effectively convert organic matter to electricity, similar cooperative consortia and pathways are required, with the exception that an anode serves as the final electron acceptor.

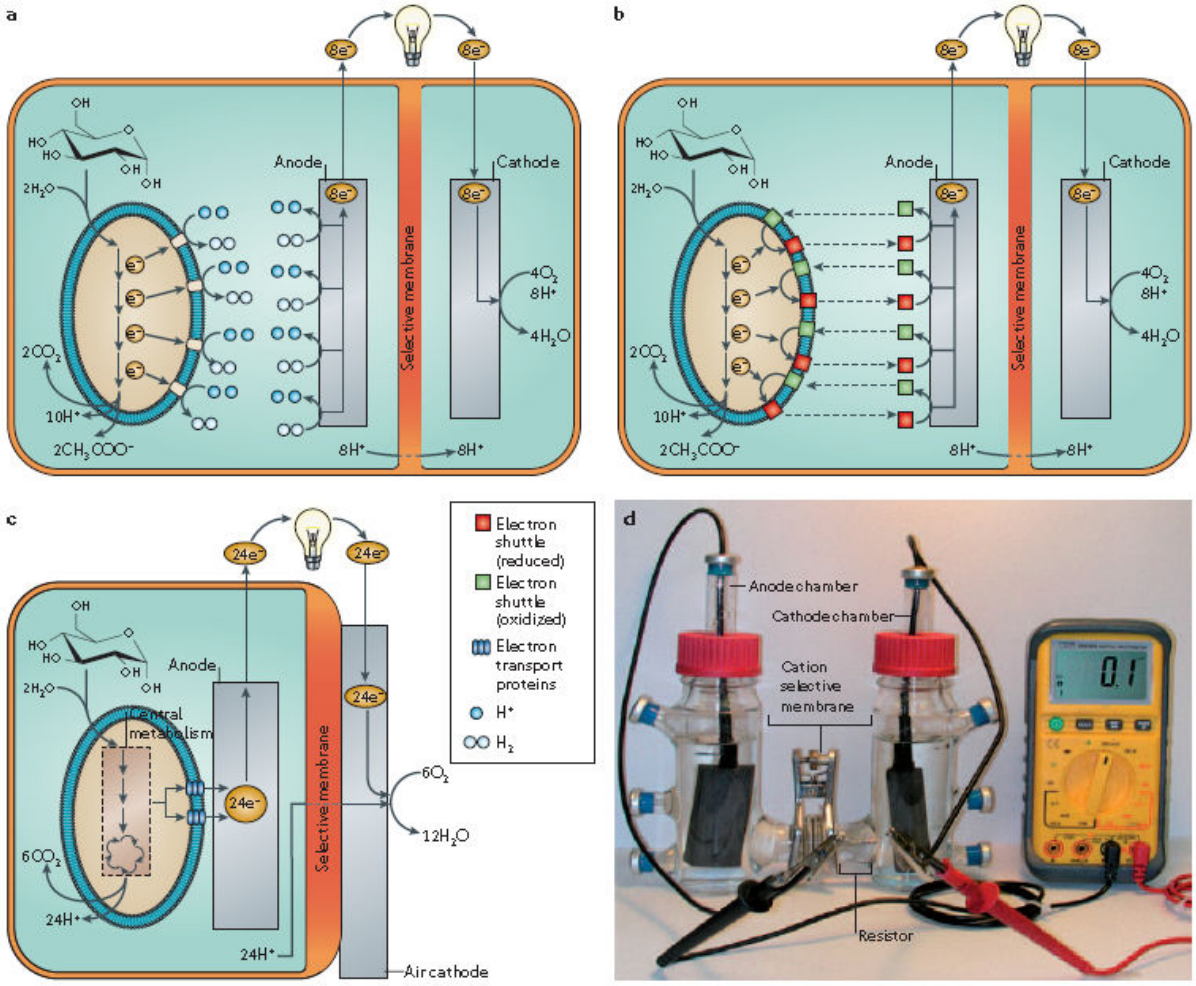

Figure 2: Examples of microbial fuel cells producing electricity through different mechanisms of electrontransfer to the anode. A. An indirect microbial fuel cell. B.| A mediator-driven microbial fuel cell. C. The oxidation of glucose to carbon dioxide with direct electron transfer to the electrode surface. D. A two-chambered microbial fuel cell.
(source from Lovely 2006b)

4. Mechanisms for electron transport to electrodes:

In microbial fuel cells, the electrons liberated from the organic matter are transferred to electrodes and generates the electricity. So this is the key mechanism one has to understand for the efficient conversion of waste to electricity generation. There are four primary mechanisms are proposed for microorganisms to transfer electrons to electrodes.

4. 1. Indirect electron transport by reduced products:

In the earliest days of microbial fuel cell research investigators used fermentation microorganisms like yeast for power generation and they dont have the well defined mechanisms that understand the power generation. It was implied that reduced products of microbial fermentation were abiotically oxidized at the anode surface to provide electrons (Fig. 2a). These products might include hydrogen, alcohols or ammonia [3, 17–19]. However, there were no studies that actually documented this mechanism or directly quantified which reduced products were oxidized at the anode. Such systems for electricity production are inherently inefficient because many fermentation products, including organic acids, react very slowly with electrodes, if at all. Although it is possible to modify the composition of anodes to increase their reactivity with some metabolic end products these electrodes were tend to foul with oxidation products [20].

4. 2. Electron transport by artificial mediators:

In this proposed mechanism electrons are transported by artificial mediators, sometimes referred to as electron shuttles. This chemical materials offer the possibility for microorganisms to generate reduced products that are more electrochemically active than most fermentation products (Fig. 2b). These electron shuttles are typically capable of crossing cell membranes, accepting electrons from one or more electron carriers within the cell, exiting the cell in the reduced form and then transferring electrons onto the electrode surface [2, 5]. Mediators are important in microbial fuel cells which use microorganisms such as *Escherichia coli*, *Pseudomonas*, *Proteus*, and *Bacillus* species that are unable to effectively transfer electrons derived from central metabolism to the outside of the cell [3]. The Commonly used electron shuttles include, thionine, benzylviologen, 2,6-dichlorophenolindophenol, 2-hydroxy-1,4-naphthoquinone and various phenazines, phenothiazines, phenoxoazines, iron chelates and neutral red. Detailed investigations on the action of neutral red, which seems to be one of the most effective mediators, have demonstrated that it can accept electrons from NADH and can be enzymatically reduced by a hydrogenase, and possibly formate dehydrogenase [12, 21-24]. The mediators should posses the following characters for efficient electron transportation (1) able to cross the cell membrane easily; (2) able to grab electrons from the electron carries of the electron transport chains; (3) possessing a high electrode reaction rate; (4) having a good solubility in the anolyte; (5) non-biodegradable and non-toxic to microbes; (6) low cost.

4. 3. Electron transport through microorganism's own mediator:

It is also known that some microorganisms can produce their own mediators to promote extracellular electron transfer. This was first proposed as a mechanism to facilitate electron transfer to Fe^{3+}in *Shewanella oneidensis* [25]. Other organisms, such as *Geothrix ferementans* [26] and *Pseudomonas* species also produce electron shuttles [27]. Biosynthesizing an electron shuttle is energetically expensive and therefore an electron shuttle must be recycled many times in order to recoup this energy investment. For this reason, microorganisms that produce electron shuttles are expected to be at a competitive disadvantage in open environments in which the shuttle will rapidly be lost from the site of release. This might explain why species from the *Geobacteraceae* predominate over other species under Fe^{3+}-reducing conditions in many sedimentary environments [28]. Electron shuttles were produced in a microbial fuel cell that was sequentially fed glucose over time, but without substantial medium replacement [29]. *Pseudomonas* species isolated from this fuel cell, and *Pseudomonas aeruginosa*, produce phenazine electron shuttles that could aid in electron transfer to electrodes [27]. Significant limiting factor in electricity production by several microorganisms that produce an electron shuttle is that they only incompletely oxidize their organic fuels.

4. 4. Direct electron transfer:

It was first proposed that microorganisms might be able to transfer electrons to an electrode surface when it was discovered that cultures of *Shewanella putrefaciens* produced electricity while metabolizing lactate. However, this was prior to the discovery, discussed above, that *Shewanella* species produce an electron shuttle, which could account for the electron transfer to the electrode. It was proposed that electrons might be directly transferred from the cell to the electrode through outer-membrane c-type cytochromes48, but no direct evidence for this was provided. Furthermore, it is now recognized that outer-membrane cytochromes are important in electron shuttle reduction in *Shewanella* [30].

In summary, the studies described in this section demonstrate the potential for microbial cultures to generate electricity and have greatly advanced understanding in this field. However, none of the types of metabolism documented in this section affords the possibility for complete oxidation of a wide variety of organic compounds coupled with electron transfer to an electrode that, as outlined above, is considered to be necessary for effective

conversion of organic matter to electricity. Furthermore, microbial growth that is fuelled by the generation of energy derived from electron transfer to electrodes has not been shown, which is an important consideration in the long-term sustainability of microbial fuel cells.

5. Microbes used in Microbial Fuel Cells:

Many microorganisms possess the ability to transfer the electrons derived from the metabolism of organic matters to the anode. A list of them is shown in Table (1) together with their substrates [31]. Marine sediment, soil, wastewater, fresh water sediment and activated sludge are all rich sources for these microorganisms. A number of recent publications discussed the screening and identification of microbes and the construction of a chromosome library for microorganisms that are able to generate electricity from degrading organic matters [32, 33]. The anodic electron transfer mechanism in MFC is a key issue in understanding the theory of how MFCs work.

Table 1 : Microbes used in microbial fuel cells (MFCs)

Microbes	Substrate	Applications
Actinobacillus succinogenes	Glucose	Neutral red or thionin as electron mediator
Aeromonas hydrophila	Acetate	Mediator-less MFC
Alcaligenes faecalis, Enterococcus	Glucose	Self-mediate consortia isolated from MFC with a maximal level of 4.31 W m−2.
gallinarum, Pseudomonas aeruginosa	Starch, glucose,	Fermentative bacterium
Clostridium beijerinckii	Starch, glucose,lactate, molasses	Fermentative bacterium
Clostridium butyricum	Starch, glucose,lactate, molasses	Sulphate/sulphide as mediator
Desulfovibrio desulfuricans	Sucrose	Ferric chelate complex as mediators
Erwinia dissolven	Glucose	Ferric chelate complex as mediators
Escherichia coli	Glucose sucrose	Mediators such as methylene blue needed.
Geobacter metallireducens	Acetate	Mediator-less MFC
Geobacter sulfurreducens	Acetate	Mediator-less MFC
Gluconobacter oxydans	Glucose	Mediator (HNQ, resazurin or thionine) needed
Klebsiella pneumoniae	Glucose	HNQ as mediator biomineralized manganese as electron acceptor
Lactobacillus plantarum	Glucose	Ferric chelate complex as mediators
Proteus mirabilis	Glucose	Thionin as mediator
Pseudomonas aeruginosa	Glucose	Pyocyanin and phenazine-1-carboxamide as mediator
Rhodoferax ferrireducens	Glucose, xylose, sucrose, altose	Mediator-less MFC
Shewanella oneidensis	Lactate	Anthraquinone-2,6-disulfonate (AQDS) as mediator
Shewanella putrefaciens	Lactate, pyruvate, acetate, glucose	Mediator-less MFC but incorporating an electron mediator like Mn(IV) or NR into the anode enhanced the electricity production
Streptococcus lactis	Glucose	Ferric chelate complex as mediators

6. Developments in Design of Microbial Fuel Cells:

6.1. MFC components

A typical MFC consists of an anodic chamber and a cathodic chamber separated by a PEM as shown in Fig. 1. One-compartment MFC eliminates the need for the cathodic chamber by exposing the cathode directly to the air. Table (2) shows the MFC components and the materials used to construct them [2, 32]. There are may types of MFCs have developed in advancement of technology to improve the power density: 1. Two-compartment MFC systems 2. Single-compartment MFC systems 3. Up-flow mode MFC systems 4. Stacked microbial fuel cell

Table 2: Basic components of microbial fuel cells

Items	Materials	Remarks
Anode	Graphite, graphite felt, carbon paper,carbon-cloth, Pt, Pt black, RVC	Necessary
Cathode	Graphite, graphite felt, carbon paper,carbon-cloth, Pt, Pt black, RVC	Necessary
Anodic Chamber	Glass, polycarbonate, Plexiglas	Necessary
Cathodic Chamber	Glass, polycarbonate, Plexiglas	Optional
Proton Exchange system	Proton exchange membrane: Nafion, Ultrex, polyethylene.poly,(styrene-co-divinylbenzene); salt bridge, porcelain septum, or solely electrolyte	Necessary
Electrode catalyst	Pt, Pt black, MnO2, Fe3+, polyaniline, electron mediator immobilized on anode	Optional

(Source from Du et al 2007)

7. Factors affecting the MFCs efficiency:

7.1. Electrode Material:

Type of material used in electrode preparation will show vital effect on MFCs efficiency. Better performing electrode materials usage will always improve the performance of MFC because different anode materials result in different activation polarization losses. Pt and Pt black electrodes are superior to graphite, graphite felt and carbon-cloth electrodes for both anode and cathode constructions, but their costs are much higher. Schroder et al. [21] reported that a current of 2–4 mA could be achieved with platinumized carbon-cloth anode in an agitated anaerobic culture of E. coli using a standard glucose medium at 0.55 mmol/L. Pt also has a higher catalytic activity with regard to oxygen than graphite materials. MFCs with Pt or Pt-coated cathodes yielded higher power densities than those with graphite or graphite felt cathodes [34, 35].

7.2. pH Buffer and Electrolyte:

If no buffer solution is used in a working MFC, there will be an obvious pH difference between the anodic and cathodic chambers, though theoretically there will be no pH shift when the reaction rate of protons, electrons and oxygen at the cathode equals the production rate of protons at the anode. The PEM causes transport barrier to the cross membrane diffusion of the protons, and proton transport through the membrane is slower than its production rate in the anode and its consumption rate in the cathode chambers at initial stage of MFC operation thus brings a pH difference [36]. However, the pH difference increases the driving force of the proton diffusion from the anode to the cathode chamber and finally a dynamic equilibrium forms. Some protons generated with the biodegradation of the organic substrate transferred to the cathodic chamber are able to react with the dissolved oxygen while some protons are accumulated in the anodic chamber when they do not transfer across the PEM or salt bridge quickly enough to the cathodic chamber. It was possible that the buffer compensated the slow proton transport rate and improved the proton availability for the cathodic reaction. This again suggests that the proton availability to the cathode is a limiting factor in electricity generation. Increasing ionic strength by adding NaCl to MFCs also improved the power output [37] possibly due to the fact that NaCl enhanced the conductivity of both by anolyte and the catholyte.

7. 3. Proton Exchange System:

Proton exchange system can affect an MFC system's internal resistance and concentration polarization loss and they in turn influence the power output of the MFC. Nafion (DuPont, Wilmington, Delaware) is most popular because of its highly selective permeability of protons. However, side effect of other cations transport is unavoidable during the MFC operation with Nafion. But its usage is better in the sense of charge balance between the anodic and cathodic chambers. Hence Nafion as well as other PEMs used in the MFCs are not a necessarily proton specific membranes but actually cation specific membranes. The ratio of PEM surface area to system volume is important for the power output. The MFC internal resistance decreases with the increase of PEM surface area over a relatively large range [38]. Membranes and Kaolin septum are prone to fouling if the fuel is something like municipal wastewater. Membrane-less MFCs are desired if fouling or cost of the membrane becomes a problem in such applications.

7. 4. Operating conditions in the anodic chamber:

Substrate type, concentration and feed rate are important factors that impact the performance of an MFC. Power density varies greatly with different substrates using same a given microbe or microbial consortium. Electricity generation is dependent on substrate concentration both in batch and continuous-flow mode MFCs. Usually a higher substrate concentration yields a higher power output in a wide concentration range. Park and Zeikus (2003) [25] reported that a higher current level with lactate (as substrate) concentration increased until it was in excess at 200 mM in a single-

compartment MFC inoculated with *S. putrefaciens*. Gil et al. (2003) [36] found that the current increased with a wastewater concentration up to 50 mg/L in their MFC. Moon et al. (2006) [35] investigated the effects of substrate concentration on the performance of an MFC and showed that the power density was increased with the increase in substrate concentration. Interestingly, the electricity generation in an MFC often higher at a relatively low level of feed rate before heading downward. This may be because a high feed rate promoted the growth of fermentative bacteria faster than those of the electrochemically active bacteria in a mixed culture [35, 39]. However, if microbes are growing around the electrodes as biofilms, the increased feed rate is unlikely to affect the flora. Another possible reason is that the high feed rate brings in other alternate electron acceptors competing with the anode to lower the output.

7. 5. Operating conditions in the cathodic chamber:

Oxygen is the most commonly used electron acceptor in MFCs for the cathodic reaction. Power output of an MFC strongly depends on the concentration level of electron acceptors. Several studies [34, 36] indicated that DO was a major limiting factor when it remained below the air-saturated level. Surprisingly, a catholyte sparged with pure oxygen that gave 38 mg/L DO did not further increase the power output compared to that of the air-saturated water (at 7.9 mg/L DO) [34] . Rate of oxygen diffusion toward the anode chamber goes up with the DO concentration. Power output is much greater using ferricyanide as the electron acceptor in the cathodic chamber. So far, reported cases with very high power outputs such as 7200 mW/m^2, 4310 mW/m^2 and 3600 mW/m^2 all used ferricyanide in the cathodic chamber [34, 22, 40], while less than 1000 mW/m^2 was reported in studies using DO regardless of the electrode material. This is likely due to the greater mass transfer rate and lower activation energy for the cathodic reaction offered by ferricyanide. Using hydrogen peroxide solution as the final electron acceptor in the cathodic chamber increased power output and current density according to [41].

Surely changing operating conditions can improve the power output level of the MFCs. The bottlenecks responsible for the low power out put are 1. low rate of metabolism of the microbes in the MFCs, 2. The biotransformation rate of substrates to electrons has a fixed ceiling which is inherently slow. To improve the MFCs efficiency one should be focused on how to break the inherent metabolic limitation of the microbes for the MFC application. As we know high temperature can accelerate nearly all kinds of reactions including chemical and biological ones. Use of thermophilic species might benefit for improving rates of electron production, however, to the best of our knowledge, no such investigation is reported in the literature. Therefore this is probably another scope of improvement for theMFC technology fromthe laboratory research to a real applicable energy source.

8. Applications of MFCs:

MFCs have very broad range of application including
1. Electricity generation 2. Bio-hydrogen production 3. Waste water treatment 4. Biosensors 5. Bioremediation

9. Conclusions and Future Perspectives:

At present the field of MFCs is in its infancy and also this is an exciting time in microbial fuel cell research. The MFCs technology has evolve to compete with well advanced methanogesis technology where biomass is used as substrate. In contrast to methanogenesis MFCs are capable to convert biomass to electricity at low temperatures and substrate concentration. The discovery and usage of new anodophilic microbes that vastly enhance the electron transport rate from the biofilm covering an anode to the anode are much needed to improve the power density output in MFCs. Mutagenesis and rDNA technology can conceivably be used in the future to obtain some “super bugs” for MFCs. Microbes may be used as a pure culture or a mixed culture forming a synergistic microbial consortium to offer better performance. One type of bacterium in a consortium may provide electron mediators that are used by another type of bacterium to transport electrons more efficiently to an anode. It is possible in the future that an optimized microbial consortium can be obtained to operate an MFC without extraneous mediators or biofilms while achieving superior mass transfer and electron transfer rates.

Furthermore, there are many microorganisms yet to be discovered that might be beneficial for electricity production. The well coordination efforts of different scientific fields like electrochemists, materials scientists, engineers and microbiologists is well require in the development of the several potential practical applications of microbial fuel cells. Even if the generation of high levels of electricity from microbial fuel cells is a long way off, an understanding of the coupling of organic matter oxidation to electron transfer to electrodes is likely to yield important insights into the diversity of microbial respiratory capabilities and might lead to as-yet-unforeseen applications in nano-electronics.

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