Chapter 18
Microbial Fuel Cells for Wastewater Treatment

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Abstract Microbial fuel cells (MFCs), which are the bioelectrochemical systems, have been developed rapidly over the past few decades and are considered as a promising technique to obtain renewable resources from wastewater. MFCs can be used to harness electricity from microorganisms during wastewater treatment. This chapter reviews recent literature on MFCs for wastewater treatment. We first introduce the concept of MFCs and summarize the materials and design of MFCs afterward. It shows that through innovative materials and design, the current density of MFCs has been greatly improved during the last decade. Microorganisms play a major role in the electricity production of MFCs and therefore, an in-depth discussion of the microbiology of MFCs was also included in this chapter. Extensive studies on exoelectrogenic bacteria and consortia are beginning to expose the mechanistic and ecological complexities of MFC biofilm communities. Yet, our understanding of electrochemically active microbes is still in its infancy, as the diverse communities have a multitude of undiscovered populations in different MFC applications. Further study is warranted to optimize design, materials, and microbiology to improve electricity recovery from MFCs.

18.1 Introduction

Wastewater treatment currently consumes substantial energy about 15 GW (McCarty et al. 2011), or accounts for approximately 3 % of the U.S. electrical energy load (EPA Office of Water 2006), and has similar level to that in other developed countries (Curtis 2010). However, there is abundant potential energy of approximately 17 GW of power (1.5×1011 kWh) contained in domestic,

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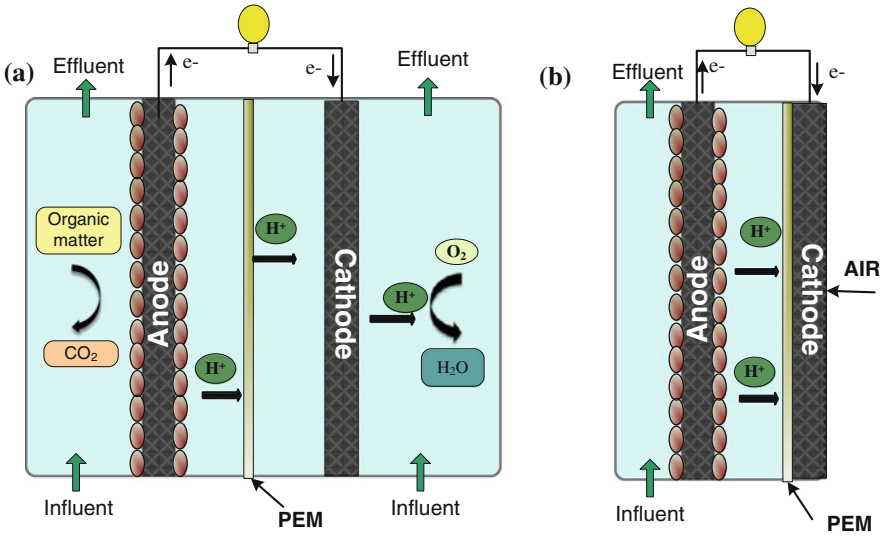

Fig. 18.1 Schematic diagrams of MFCs: **a** a two-chamber MFC; **b** a single-chamber MFC with open air cathode

industrial, and animal wastewater together (Logan 2004). Thus, capturing part of this energy would provide a new source of electrical power and would also compensate the consumption of energy for wastewater treatment.

Recently, microbial fuel cells (MFCs) (Allen and Bennetto 1993; Logan et al. 2006; Lovley 2006), which are the bioelectrochemical systems, are generally regarded as a promising and sustainable technology for their direct electrical power production from wastewaters (Rabaey and Verstraete 2005). A conventional MFC consists of a biological anode and a cathode (Fig. 18.1a), where exoelectrogenic microorganisms could catalyze electrochemical reactions through interaction with the electrodes (Logan et al. 2006; Rabaey et al. 2007; Clauwaert et al. 2008). The electrons available through the metabolism of the electron donors by microorganisms are transferred to the anode and then to the cathode through the circuit; in the cathode, the oxidant is reduced with the consumption of protons available through the membrane from the anode (Allen and Bennetto 1993).

In terms of potential applications, MFCs and related bioelectrochemical systems can be utilized for renewable energy generation and wastewater treatment, i.e., organic matter elimination and nitrogen removal (Logan and Regan 2006b; Clauwaert et al. 2007; Rozendal et al. 2008; Yu et al. 2011), for the potential production of valuable products, such as hydrogen, methane or hydrogen peroxide (Liu et al. 2005c; Rozendal et al. 2006, 2009), for bioremediation of recalcitrant compounds (Catal et al. 2008; Morris and Jin 2008), for desalination (Forrestal et al. 2012; Yuan et al. 2012; Feng et al. 2013a), and as biosensors for on-line monitoring of treatment processes (Kim et al. 2007b) and biological oxygen demand or toxic contaminants in wastewater (Kim et al. 2003; Chang et al. 2004; Kim et al. 2007b).

Recent investigations have shown that during the last 10 years, the current density of MFCs has been improved by 10,000-fold (Debabov 2008). Power densities of MFCs have increased from less than 1 W/m^3 to over 4000 W/m^3, which is the highest MFC power density reported up to date (Logan 2008; Biffinger et al. 2009). Despite their potential applications and continuously improved power, limited maximum power production by these systems impedes commercial applications of bioelectrochemical wastewater treatment, primarily because of high internal resistance including anode limitations and electrochemical losses. Improvements of power generation are also dependent on the materials and design of MFCs and capabilities of the microorganisms. Analysis of the community profiles of exoelectrogenic microbial consortia shows great diversity, ranging from primarily δ-Proteobacteria that dominate in sediment MFCs to communities composed of α-, β-, γ- or δ-Proteobacteria, Firmicutes, and uncharacterized clones in other types of MFCs. Much remains to be discovered about the physiology of these bacteria (collectively referred to as exoelectrogens) capable of exocellular electron transfer.

This chapter is intended to provide an overview of recent development and challenges in MFCs with a special focus on the materials, design, and microbiology of MFC research. Since microorganisms play a crucial role in the MFCs, comprehensive reviews focused on isolated exoelectrogens that have been identified to produce electricity, their mechanisms of exocellular electron transfer, and the microbial communities found in MFCs. In the end, the prospects for this emerging bioelectrochemical technology were discussed.

18.2 History of MFCs

Currently, MFCs have been recognized as a promising green technology for the generation of electricity through the microbial oxidation of biodegradable organic matters. The concept of generating electricity by bacteria was introduced more than 100 years ago. The electricity generated by microorganisms was firstly demonstrated in 1911 by Potter, a Professor of Botany Department at the University of Durham (Potter 1911). To examine the electricity producing capability of microorganism, he conducted his experiment using yeast and certain other bacteria in an apparatus consisted of a glass jar containing a porous cylinder. He observed that *Saccharomyces cerevisiae* and *Bacillus coli communis* (now called *Escherichia coli*) produced electric current when glucose was used as substrate. After that there was no important research on MFCs up to 1966 (Lewis 1966) and most studies on MFCs did not appear until the late twentieth century. However, experiments carried out by researchers used artificial electrochemical mediators to facilitate electron transfer between microbes and electrodes. Thurston and his colleagues used thionine as a redox mediator and *Proteus vulgaris* culture as catalyst in a two-chamber MFC to evaluate coulombic yield from glucose oxidation (Thurston et al. 1985). These chemicals were considered important for

obtaining a higher electron transfer rate and electron recovery between microbial cells and electrodes. In 1999, a breakthrough in MFCs was published by Kim and his colleagues, who showed that exogenous mediators were not necessary to be added to transfer electrons from bacterial cells to electrodes and they developed the first mediator-less MFC using a Fe(III)-reducing bacterium, *Shewanella putrefaciens* IR-1 (Kim et al. 1999). The cell suspension of *Shewanella putrefaciens* IR-1 was able to generate current without redox mediator in the presence of lactate as the main carbon source. Another important bacterium *Geobacter sulfurreducens* can transfer electrons to electrode in the absence of the mediators with high current generation (Bond and Lovley 2003) and has become an important issue on MFC research. After the discovery of mediator-less MFCs, scientists have become more interested to do research on MFCs, especially in wastewater treatment because mediator-less MFCs provide a more practical and promising approach to recover electricity from organic waste and wastewater through microbial systems (Liu and Logan 2004; Min and Logan 2004). Presently many research laboratories have been engaged in improving MFC technologies to enhance the electricity production and efficient removal of wastewater by designing different configurations of MFCs such as single chamber MFC, tubular MFC (Rabaey et al. 2005b), stacked MFC (Aelterman et al. 2006) and also membrane-less MFC (Feng et al. 2013b). The advancement of research on MFCs in the future may be the solution to energy scarcity and the clean-up of wastewater. Thus, MFCs have received a great deal of attention as a novel green technology for alternative energy generation and wastewater treatment.

18.3 Design and Operations of MFCs

An appropriate design and architecture is of great significance for improving performance in MFC systems (Du et al. 2007; Pant et al. 2010). The mode of operation and components of a typical two-chamber and a single-chamber MFC are shown in Fig. 18.1.

18.3.1 Two-Chamber MFC Systems

Traditional two-chamber MFCs consist of an anaerobic anode chamber and an aerobic cathode chamber separated by a proton exchange membrane (PEM) or sometimes a salt bridge, allowing proton transfer from anode to cathode and preventing oxygen diffusion to the anode chamber, as shown in Fig. 18.1a. Regardless of the problems in scale-up, the dual-chamber MFCs have remained the most popular devices for testing microbial activity and optimizing materials. There are a variety of designs and structures occurred based on the principles of two chamber MFC systems, e.g., the widely used and inexpensive H-type MFCs

(Min et al. 2005) and U-shaped MFCs (Milliken and May 2007) (Fig. 18.2a, b). In the H-configuration, the membrane is clamped in the middle of the tubes connecting the bottle. Although H-shape systems are usually available for basic parameter research, they generate low power densities. This may attribute to high internal resistance and electrode-based losses. Oh and colleagues demonstrated that the power densities had a close relationship with the relative sizes (cross sections) of the cathode to that of the anode and the membrane (Oh et al. 2004; Oh and Logan 2006).

Ringeisen and colleagues provided a miniature configuration of MFC (Mini-MFC) with a total volume of 1.2 cm^3 (Fig. 18.2c) (Ringeisen et al. 2006). As the result of its specific structure, the mini-MFC maintains a large surface area to volume ratio when graphite felt electrodes were used, enabling high power densities to be attained. Min and Logan (2004) designed a Flat Plate MFC (FPMFC) to treat domestic wastewater. The FPMFC was comprised of a single channel formed between two nonconductive (polycarbonate) plates that were separated into two halves by the electrode/PEM assembly (Fig. 18.2d). The anode electrode was a plain porous carbon paper (10×10 cm^2), while a carbon cloth combining a platinum catalyst (0.5 mg/cm^2 catalyst containing 10 % Pt) servers as cathode electrode. The wastewater was fed into the anode chamber and dry air could pass through the cathode chamber without any catholyte, both in a continuous flow mode. Average power density was obtained at 72 mW/m^2 (Min and Logan 2004).

Another reactor design, named upflow MFC (UMFC) working in continuous flow mode, was first tested by He et al. (2005) (Fig. 18.2e). Its configuration was improved by combining the advantages of upflow anaerobic sludge blanket system, which were operated in continuous mode. Another UMFC with a U-shaped cathode installed inside the anode chamber was developed based on the above configuration (He et al. 2006) (Fig. 18.2f). A U-shaped cathode compartment with a 2 cm diameter was constructed by gluing two tubes made from PEM into a plastic base connector. In addition to a practical configuration, UMFC achieved promising power outputs with a maximum volumetric power density of 29.2 W/m^3 with an overall internal resistance of 17.3 Ω (He et al. 2006). They suggested that the main limitation to power generation was the internal resistance. Overall, these systems seem to be more available for practical implementation as they are relatively easy to scale up.

18.3.2 Single-Chamber MFC Systems

In the single-chamber MFC (SCMFC), the cathode is exposed directly to the air by eliminating the cathodic compartment containing air-sparged solution (Park and Zeikus 2003; Liu and Logan 2004; Liu et al. 2004) (Fig. 18.1b). They typically possess only an anode chamber without the requirement of aeration in the cathode chamber. In comparison with dual chamber system, a SCMFC provides the simplified design, increased mass transfer to the cathode, cost savings and an overall decrease in reactor volume.

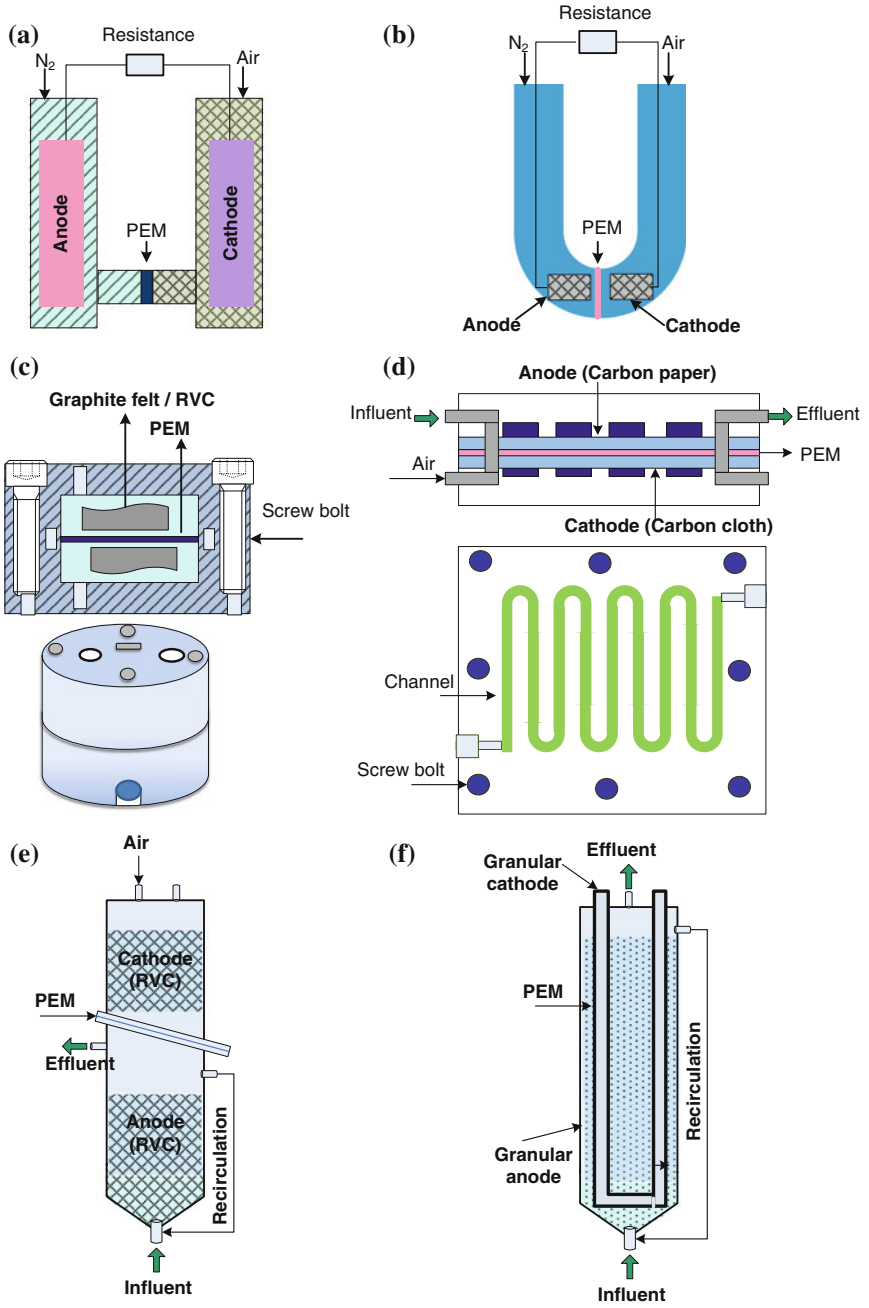

Fig. 18.2 Schematics of typical two-chamber MFCs: **a** H-type MFC with PEM or salt bridge; **b** U-shaped MFC; **c** Mini-MFC; **d** Flat plate MFC; **e, f** Up flow MFC with cylindrical shape

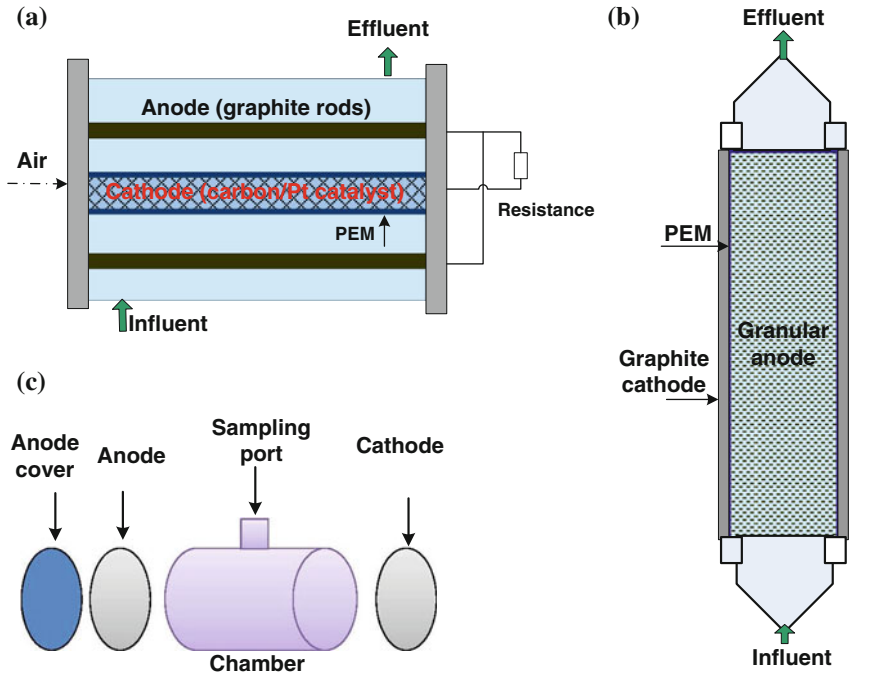

Fig. 18.3 Schematics of typical single-chamber MFCs: **a** The first SCMFC for domestic wastewater treatment; **b** Tubular MFC; **c** a lab-scale single-chamber MFC

Liu et al. (2004) first demonstrated that domestic wastewater could be used as the substrate in MFCs without actively feeding air into a cathode chamber. Their MFC consisted of a single chamber with eight graphite electrodes (anodes) and a single air cathode as shown in Fig. 18.3a. Most importantly, the promising idea of using MFC technology to reduce energy costs in wastewater treatment was initiated. A tubular MFC (TMFC), designed by Rebeay and colleagues (Rabaey et al. 2005b) was shown in Fig. 18.3b. The TMFC had a wet anode volume of 210 mL and generated a maximum volumetric power of 90 W/m^3 using graphite granules as the anode and a ferricyanide solution in the cathode chamber. A relatively low internal resistance of 4 Ω was achieved by sustaining a short distance between the anode and cathode electrodes and a large PEM surface area. Rabaey et al. (2005b) believed that the use of sustainable open air cathodes was a promising design for practical implementation.

It has been demonstrated that power output can further be increased in a single-chamber MFC by removing the PEM. Liu et al. (2004) found that there was a significant rise in power density by a factor of approximately 1.9 for glucose and 5.2 for wastewater through removing the PEM from a single chamber MFC (Fig. 18.3c). This increase was partly attributed to an enhancement of the proton flux from the anode to the cathode. The lack of a PEM substantially reduce the

expenditure on the materials needed to make a MFC and eliminated the disturbing biofouling of membrane. However, substantial oxygen diffusion into the anode chamber in the absence of the PEM could occur to reduce the fraction of electrons recovered as current.

18.3.3 Other MFC Configuration

Besides the above configurations, a series of variations on these basic designs have emerged in order to achieve different purposes, such as increase of power density, achieving continuous flow or nutrient removal. For example, to increase the overall system voltage, MFCs can be stacked or linked together in series (Aelterman et al. 2006). Another type of MFC, nitrifying and denitrifying MFC for decentralized wastewater treatment was reported by Feng et al. (2013b). Their MFC system was built on the basis of conventional anoxic/oxic wastewater treatment system and achieved the continuous flow mode by using a baffle with holes instead of PEM. An integrated photobioelectrochemical system was constructed by installing a MFC inside an algal bioreactor (Xiao et al. 2012). This system achieves the simultaneous removal of organics and nutrients from a synthetic solution, and the production of bioenergy in electricity and algal biomass through bioelectrochemical and microbiological processes.

18.4 Materials

MFCs are generally made of three major parts: anode, cathode, and PEM (if present). There are a variety of materials for their construction. Electrode materials play an important role both in the performance and cost of MFCs. A good anode material should have the following properties (Logan et al. 2006; Zhou et al. 2011): large surface area; excellent electrical conductivity, strong biocompatibility, chemical stability, appropriate mechanical strength and toughness. Up to now, various materials are used for electrodes including carbon materials, e.g., carbon paper (Liu et al. 2005a), carbon cloth, carbon felt (Chaudhuri and Lovley 2003) and reticulated vitreous carbon (RVC) (He et al. 2005; Rabaey et al. 2005b), graphite materials, e.g., graphite granules and graphite fiber brushes (Aelterman et al. 2006; He et al. 2006; Rinaldi et al. 2008), etc. Since different electrode materials vary obviously in their physical and chemical characteristics, they have impacts on microbial attachment, electron transfer, electrode resistance and the rate of electrode surface reaction. Thus, some strategies could be applied to boost the performance in terms of increasing the surface area and the biocompatibility. The anode materials could be fabricated with C/polyaniline (PANI) composites, carbon nanofibers, or nitric acid carbon activation (Scott et al. 2007), or integration of carbon nanotubes to PNAI (Qiao et al. 2007), etc.

Cathodes are made from the same materials as anodes, and catalysts are usually contained but not necessary. Because oxygen is the terminal electron acceptor in most cases, the high overpotential arising from oxygen reduction reaction causes the noncatalyst cathodes to be inefficient. Thus, catalysts and/or artificial mediators are generally required to improve performance. They are generally mounted on the cathodes with a binder such as Nafion (perfluorosulfonic acid) or polytetrafluoroethylene. Pt has become the most popular catalyst (Thurston et al. 1985), but its high cost and reduced activities due to formation of a PtO layer on the electrode surface restrict its practical application. For this reason non-Pt catalysts including nonabundant metals, e.g., Pd or Ru (Vante and Tributsch 1986; Fernández et al. 2005; Raghuveer et al. 2005) and nonprecious materials, e.g., Fe, Mn and Co (Park and Zeikus 2003) tend to be more appealing. They could exhibit essentially equal or slightly better performance than the more expensive Pt. Among the non-Pt catalysts, the most promising CoTMPP and iron (II) phthalocyanine (FePc) (Zhao et al. 2005) were proved to be inexpensive and efficient alternatives for MFC applications. Integration of noncorrosive metals (titanium and nickel) and carbon fibers can be used as cathode materials as well (Hasvold et al. 1997; Zhao et al. 2009). Additionally, catalysts are not required for catholyte cathodes, which use the redox mediators such as ferricyanide (Oh et al. 2004; Venkata Mohan et al. 2008) or permanganate (You et al. 2006). Using them as terminal electron acceptors could result in alternative cathodic reactions and further improve power output to 258 W/m^3 (Aelterman et al. 2006). These catholytes seemed to be impractical and unsustainable for practical application owning to the requirement of regeneration of the chemicals. On the basis of the above introduction, a large number of materials have been investigated to improve cathode performance. However, their long-term stability on the cathode should be further evaluated for future application.

The PEM is also an important component in the PEM-MFC configuration. It provides a separation between the anode and cathode chambers and allows for transport of positive charges to compensate the electron transport. Currently, the most widely used membrane material is Nafion™ (Park and Zeikus 2000; Bond and Lovley 2003), which has set the industry standard for PEM. Its properties have been extensively reviewed (Mauritz and Moore 2004). Obviously, Nafion™ was the predominant choices for current MFCs. Nevertheless, it has been recently found that the use of Nafion™ leads to some side effects such as pH imbalance and power reduction (Gil et al. 2003; Kim et al. 2004). In addition to Nafion™, polyether ether ketone (PEEK) is a promising polymer being actively studied by the MFC researchers to overcome the drawbacks of Nafion™ (Roziere and Jones 2003; Mecheri et al. 2006). In fact, membranes can be omitted from the bioelectrochemical configuration. The lack of a PEM could decrease the cost of the materials for a MFC, but substantial oxygen diffusion into the anode chamber in the absence of the PEM could reduce the fraction of electrons recovered as current.

18.5 Exoelectrogens

In nature, there are many microorganisms possessing the ability to transfer electrons derived from the metabolism of organic matters to the anode. Microorganisms capable of extracellular electron transfer are generally called "exoelectrogens". These microorganisms attain their required energy by oxidizing organic matter with the release of protons and electrons that are used in MFC to produce electricity. Marine sediment, soil, wastewater, fresh water sediment and activated sludge are rich sources for these microorganisms (Niessen et al. 2006; Zhang et al. 2006). In the beginning, it was considered that only a few types of bacteria were capable of producing electricity and most of them were gram negative Proteobacteria such as *Shewanella putrefaciens* (Park and Zeikus 2002), *Geobacter sulfurreducens* (Bond and Lovley 2003), etc. However, now gram positive bacteria also have been discovered to produce electricity, including *Clostridium butyricum* within the Firmicutes (Park et al. 2001). The capability to produce electricity generally depends on the nature of bacterial species and their ability to utilize different substrates. Power generation also depends on the optimal growth condition of bacteria, e.g., pH and temperature.

Up to now, most of isolated exoelectrogens are bacteria (Table 18.1) and were isolated from different MFCs using large varieties of substrates. Scientists are trying to discover new exoelectrogenic bacteria, which will have the capacity to achieve high power density. The pure strain *Geobacter sulfurreducens* operated in a two-chamber MFC with PEM and graphite electrode produced an electric current density of 65 mA/m^2 using acetate as the substrate (Bond and Lovley 2003). Other pure strains such as *Comamonas denitrificans* DX-4 and *Citrobacter sp.* SX-1 produced the highest power and current density of 35 mW/m^2 and 205 mA/m^2 using acetate and citrate as electron donors in MFC respectively (Xing et al. 2010; Xu and Liu 2011). One scientific report showed that power output in a MFC inoculated with a pure culture (*Geobacter metallireducens*) or a mixed culture (wastewater inoculums) was similar, with 40 ± 1 mW/m^2 for *Geobacter metallireducens* and 38 ± 1 mW/m^2 for the wastewater inocula (Min et al. 2005). However, *Rhodopseudomonas palustris* DX-1, isolated from an air cathode MFC, produced electricity at higher power densities (2720 ± 60 mW/m^2) than mixed culture in the same device using complex substrates including volatile acids, yeast extract and thiosulfate (Xing et al. 2008). In addition, some bacteria require exogenous redox compound to increase maximum power production. For example, *Shewanella putrefaciens* generated the maximum power density of 10.2 mW/m^2 when operated in the absence of exogenous electron acceptors in a single chambered MFC, but current production by *Shewanella putrefaciens* was enhanced 10-folds when an electron mediator, i.e., Mn^{4+} or neutral red was incorporated into the graphite anode (Park and Zeikus 2002).

Table 18.1 Some examples of isolated exoelectrogens

Class	Microorganism	Substrate	Mediator	Power or current density	References
α-Proteobacteria	*Rhodopseudomonas palustris* DX-1	Volatile acids, yeast extract	Mediator-less	2720 ± 60 mW/m^2	(Xing et al. 2008)
	Gluconobacter oxydans	Glucose	2-hydroxy-1,4-naphthoquinone	–	(Lee et al. 2002)
β-Proteobacteria	*Comamonas denitrificans* DX-4	Acetate	Mediator-less	35 mW/m^2	(Xing et al. 2010)
γ-Proteobacteria	*Shewanella oneidensis*	Lactate	Mediator-less	24 mW/m^2	(Ringeisen et al. 2006)
	Citrobacter sp. SX-1	Citrate	Mediator-less	205 mA/m^2	(Xu and Liu 2011)
	Shewanella putrefaciens	Sodium lactate	Mn $^{4+}$ or neutral red	10.2 mW/m^2	(Park and Zeikus 2002)
	Klebsiella pneumoniae	Glucose	2-hydroxy-1,4-naphthoquinone	126.7 ± 31.5 mW/m^2	(Rhoads et al. 2005)
	Actinobacillus succinogenes	Glucose	Neutral red	–	(Park and Zeikus 1999)
	Proteus mirabilis	Glucose	Thionin	–	(Thurston et al. 1985)
	Proteus vulgaris	Sucrose	Thionine	–	(Bennetto et al. 1985)
	Escherichia coli	Glucose	Neutral red	–	(Park and Zeikus 2000)
	Aeromonas hydrophila	Acetate	Mediator-less	–	(Pham et al. 2003)
	Pseudomonas aeruginosa KRP1	Glucose	Pyocyanin and phena-zine-1-carboxamide	–	(Rabaey et al. 2005a)
δ-Proteobacteria	*Geobacter metallireducens*	Acetate	Mediator-less	40 ± 1 mW/m^2	(Min et al. 2005)
	Geobacter sulfurreducens	Acetate	Mediator-less	65 mA/m^2	(Bond and Lovley 2003)
Clostridia	*Clostridium butyricum*	Glucose	Mediator-less	–	(Park et al. 2001)

18.6 Electron Transfer Mechanism of Exoelectrogens

The electron transfer mechanism is a key issue to understand the theory of how MFCs work. Numerous investigations were conducted to study how electrons were transferred from microbial cells to anode surface in the MFCs. There are generally two main mechanisms that are direct or mediator-less and indirect or mediated electron transfer (MET).

18.6.1 Direct or Mediator-Less Electron Transfer

Direct electron transfer (DET) requires a physical contact between the microbial cell membrane or a membrane organelle and the electrode surface. *Shewanella putrefaciens* (Kim et al. 2002), *Geobacter sulferreducens* (Bond and Lovley 2003), and *Geobacter metallireducens* (Min et al. 2005) can effectively transfer electrons directly to an electrode across the membrane. Some of DET bacteria transfer electrons through direct attachment of cell membrane to anode (Fig. 18.4a), while the rest use their pili or nanowires to transfer electrons to anode (Fig. 18.4b). Generally c-type cytochromes associated with bacterial outer membrane and conductive nanowires or pili can be used for DET (Peng et al. 2010).

18.6.2 Indirect or Mediated Electron Transfer

Although some bacteria can transfer electrons directly, many other microbes need redox-active chemical species or mediators to carry out electron transfer to anode; this type of mechanism is known as indirect or MET. In MET, direct contact between the bacterial cell membrane and the electrode surface is not required, but a redox mediator is essential. An electron mediator is a molecule that functions as an electron shuttle between microbes and an electrode. Mediators in the oxidized state are easily reduced by capturing electrons from within the bacterial cell membrane or the cytoplasm (Fig. 18.4c). The reduced mediators after passing across the membrane release their electrons to the electrode and become oxidized again in anode chamber and thus are reutilized. Generally chemical mediators are supplied from outside into the anode chamber of a MFC. Apart from externally provided mediators, some microorganisms are able to excrete their own mediators such as phenazine, 2-amino-3-carboxy-1,4-naphthoquinone and 2,6-di-tert-butyl-p-benzoquinone (Rabaey et al. 2005a; Freguia et al. 2009; Deng et al. 2010) that are used to transfer electron from cytoplasm to anode (Fig. 18.4d). In addition, there is another way by which some bacteria, especially fermentative bacteria, produce energy rich reduced metabolites such as H_2, ethanol or formate, which can be subsequently oxidized to provide electron to anode (Schroder 2007) (Fig. 18.4e). Furthermore, in

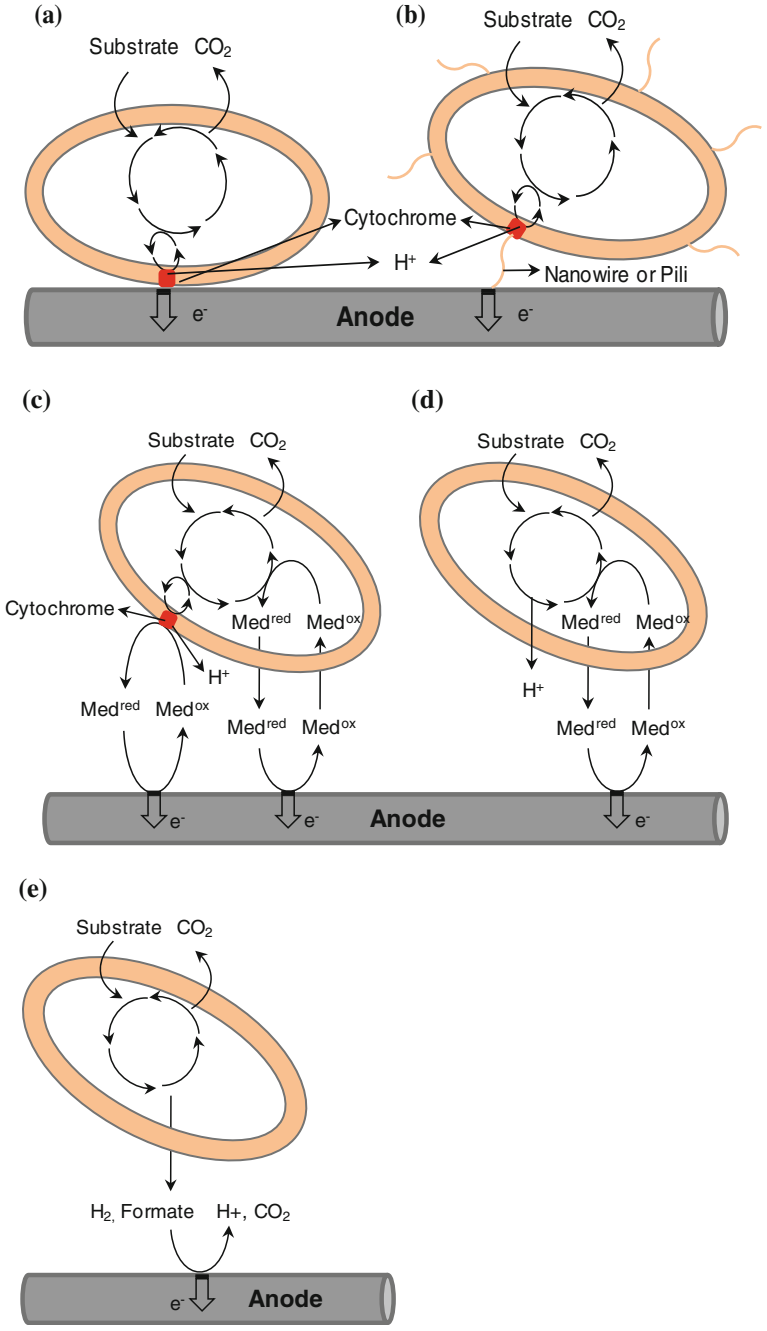

Fig. 18.4 Electron transfer mechanism of exoelectrogens: Direct electron transfer by **a** attachment of cell membrane or **b** nanowire; Indirect electron transfer by **c** exogenous mediators, **d** endogenous secondary metabolites or **e** primary metabolites

a synergistic biofilm consortium, it is likely that a nonelectrogenic microbe may secrete mediators that may help the electrogenic microbe to perform better electron transfer.

18.7 Microbial Community of Electroactive Biofilms

Biofilms more than ten micrometers in thickness are typically formed on the anode surfaces (Bond and Lovley 2003). They contain a complex microbial population (Kim et al. 2004; Rabaey et al. 2004), apart from the known electrogenic bacteria (*Geobacter, Shewanella*). Identifying members of the microbial community will be a valuable aid in terms of improving the performance of MFCs and a more comprehensive understanding of the key microbes required for exoelectrogenesis. Up to now, there are many publications associated with microbial communities in MFCs by means of PCR-amplified 16S rRNA gene fragments and sequencing such as denaturing gradient gel electrophoresis (DGGE) (Table 18.2). Analysis of the populations inhabiting such systems demonstrates that microbial communities are phylogenetically diverse in most MFCs. Microbial populations are affected by numerous factors, such as the substrate, cultivation mode, system architectures, anaerobiosis degree, as well as the conditions within the cathode chamber (Logan and Regan 2006a).

The composition of substrates has a close relationship with the microbial populations within the anode biofilms and MFC performance, as they serve as the carbon (nutrient) and energy source for the microbiological process. Commonly, the carbon sources contain pure compounds (acetate, glucose, lactic acid, etc.) (Chaudhuri and Lovley 2003; Liu et al. 2005b) and a variety of wastewaters (brewery, chocolate, meat packing and paper recycling wastewaters, etc.) (Feng et al. 2008; Huang and Logan 2008). The pure substrate inoculated systems are found to produce more power than those fed with wastewater perhaps as the result of different solution conductivity and buffer capacity (Pant et al. 2010). Based on 16S rRNA gene sequences, the dominant community members in the MFCs with pure substrate are more known exoelectrogens (*Geobacter* sp., *Desulfuromonas* sp., *Rhodopseudomonas* sp., etc.) and other bacteria with special function, such as *Clostridium* sp., which is useful for lignocellulose degradation in cellulose-fed MFCs (Cheng et al. 2011) (Table 18.2).

The highest power density of 4.31 W/m^2 was achieved using a mixed culture in a fed-batch MFC and glucose as the substrate in the reactor with a Coulombic efficiency (defined as the fraction of electrons recovered as current versus the maximum possible recovery) of 81 %. The analysis of the population using DGGE showed great phylogenetic diversity, with a complex mixture of bacteria (Firmicutes, γ-, β-, and α-Proteobacteria). Facultative anaerobic bacteria capable of hydrogen production (*Alcaligenes faecalis, Enterococcus gallinarum*) were predominant (Rabaey et al. 2004), probably owning to using a fermentable substrate with a mixed culture inocula (Debabov 2008). It was deduced that mediator production accounted for the

Table 18.2 Summary of dominant microbes present in bacterial community of the anode biofilm

Substrates	MFC	Technique	Dominant community members	Power density (mW/m^2)	Coulombic efficiency (%)	References
Acetate	Two-chamber	DGGE	*Geobacter sulfurreducens*	N/A	72	(Jung and Regan 2007)
	Single-chamber	Clone library	*Pelobacter propionicus*	835 ± 21	20	(Kiely et al. 2011b)
	Single-chamber	DGGE	*Rhodopseudomonas palustris*, *Geobacter sulfurreducens*, *Pseudomonas alcaligenes*	1797 ± 10	N/A	(Xing et al. 2009)
	Two-chamber	Clone library	*Thauera aromatica*, *Geobacter sulfurreducens*	64.3	72.3	(Chae et al. 2009)
	Two-chamber	Clone library	*Geobacter sulfurreducens*, *Pelobacter propionicus*	N/A	50	(Chae et al. 2008)
Ethanol	Single-chamber	Clone library	*Geobacter sulfurreducens*, *Pelobacter propionicus*	820 ± 24	11	(Kiely et al. 2011b)
	Two-chamber	RFLP	*Azoarcus* sp., *Desulfuromonas* sp.	40 ± 2	10	(Kim et al. 2007a)
Lactate	Single–chamber	Clone library	*Pelobacter propionicus*, *Desulfuromonas* sp.	739 ± 32.2	20	(Kiely et al. 2011b)
Propionate	Two-chamber	Clone library	*Bacillus* sp.	58	36	(Chae et al. 2009)
Butyrate	Two-chamber	DGGE	*Bacillus* sp.	N/A	46–67	(Freguia et al. 2010)
	Two-chamber	Clone library	*Dechloromonas* sp., *Geobacter* sp.	51.4	43	(Chae et al. 2009)
Formate	Two-chamber	Clone library	*Paracoccus* sp., *Geobacter* sp.	10	3–11	(Kiely et al. 2010)
	Two-chamber	DGGE	*Geobacter* sp.	N/A	5–6	(Ha et al. 2007)

(continued)

Table 18.2 (continued)

Substrates	MFC	Technique	Dominant community members	Power density (mW/m^2)	Coulombic efficiency (%)	References
Succinate	Single-chamber	Clone library	*Geobacter sulfurreducens*, *Pelobacter propionicus*	444 ± 12.5	16	(Kiely et al. 2011b)
Glucose	Two-chamber	Clone library	*Geobacter sulfurreducens*	156	15	(Chae et al. 2009)
	Single-chamber	DGGE	*Rhodopseudomonas palustris*, *Geobacter sulfurreducens*, *Clostridium* sp.	1000 ± 19	N/A	(Xing et al. 2009)
Cellulose	Single-chamber	Clone library	*Geobacter sulfurreducens*, *Clostridium* sp.	1070	25–50	(Xing et al. 2009)
	Two-chamber	Clone library	Clostridiales, Chloroflexi, Rhizobiales, Methanobacterium	10	N/A	(Chae et al. 2009)
	Single-chamber	DGGE	*Clostridium* sp.	331	4	(Wang et al. 2009)
Cysteine	Two-chamber	DGGE	*Shewanella* sp.	39	14	(Logan et al. 2005)
Organic wastewater	Two-chamber	DGGE	*Azoarcus* sp, *Thauera* sp.	N/A	N/A	(Kim et al. 2004)
Dairy manure wastewater	Single-chamber	Clone library	*Thauera aromatica*, *Clostridium* sp., *Geobacter* sp.	N/A	12	(Kiely et al. 2011a)
Potato wastewater	Single-chamber	Clone library	*Geobacter* sp., *Pelobacter propionicus*	N/A	21	(Kiely et al. 2011a)

excellent power generation, as large concentrations of highly colored mediators from this reactor were detected (Logan and Regan 2006a).

However, there are complicated organic matters in wastewater and complex metabolisms such as fermentation could get involved in MFCs. Molecular characterizations of anodic communities with complex wastewater sources revealed a high diversity of microbial species, dominant with α- (Phung et al. 2004), β- (Kim et al. 2004; Phung et al. 2004), and γ-Proteobacteria (Logan et al. 2005). For example, the characterization of anodic communities present in a two-chamber MFC treating chocolate wastewater showed a high percentage of β-Proteobacteria (51 %) (Patil et al. 2009). Whereas, microbial communities that developed by MFCs supplied with winery or potato wastewater, were a mixed consortia predominated by *Geobacter sulfurreducens*, representing 44 % and 60 % of 16S rRNA gene clones, respectively (Cusick et al. 2010; Kiely et al. 2011a). Most importantly, a large proportion of clones is uncharacterized in these mixed-culture systems, especially with complex wastewater sources. The lower frequency to detect known exoelectrogens implies a greater diversity of this phenotype than presently realized. The significance of the potential function of these dominant community members is still unknown.

Cultivation mode including fed-batch and continuous flow could affect microbial communities as well. In a continuous flow mode MFC supplied with acetate, the composition of anodic community revealed that the most dominant phyla were Proterobacteria (23–33 %), Bacteroidetes (17–40 %) and Chloroflexi (21–30%) on the basis of 454 pyrosequencing technique (Feng et al. 2013b). In an upflow system, a large number of methanogenic archaea in the mixed biomass appeared on the anode based on fluorescence in situ hybridization (He et al. 2005). Literature studies have demonstrated that δ-Proteobacteria (50–90 %) were dominant in the anode community of sediment MFC (Bond et al. 2002; Bond and Lovley 2003), while Cytophagales (up to 33 %), Firmicutes (11.6 %), and γ-Proteobacteria (9–10 %) were the minor components in the anodophilic consortia (Tender et al. 2002; Holmes et al. 2004).

18.8 The MFC's Full-Scale Applications

The development of MFC's practical application is still in the early stage. To date, most MFCs have been investigated in the bench-scale, generally less than 1 L and produced a maximum potential approximately 0.8 V. Apparently, the power density and MFC configuration have not yet reached a widely applicable level, remaining the challenging obstacle.

Sediment MFCs have been demonstrated at scales effective to be an alternative renewable power source in seawater applications (Bond et al. 2002; Lowy et al. 2006; Dewan et al. 2014). According to Fig. 18.5, in principle sediment MFCs consist of two electrodes made of conductive material. The anode is buried under

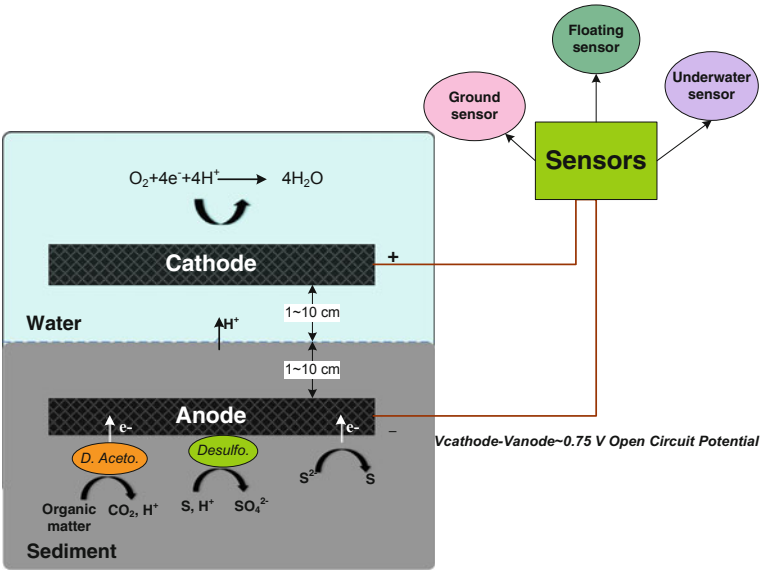

Fig. 18.5 Schematic representation of fundamental principle of the mediator less sediment MFCs used to provide energy for on-site sensors. Microorganisms colonizing the anode are most similar to *Desulfuromonas acetoxidans* (*D. Aceto.*), which could oxidize acetate in sediment and transfer electrons to the anode. *Desulfo.* represents the species in the *Desulfobulbus* or *Desulfucapsa* genera, which could oxidize anode generated S_0 to SO_4^{2-}

surface water or marine sediment and cathode is placed in the water above the sediment (Tender et al. 2002; Logan and Regan 2006b; Rezaei et al. 2007). The sedimentary organic carbon (Aller 1994) or sulfate compounds (Rabaey et al. 2006) present in the sediment are oxidized by microorganisms growing on the anode surface for production of electricity. There are several attempts to demonstrate the availability of sediment MFCs as power source for underwater (Donovan et al. 2013), ground (Donovan et al. 2008), and floating sensors (Nielsen et al. 2007; Tender et al. 2008; Donovan et al. 2011). The first demonstration of scale-up of MFC was used to power a weather buoy embedded with temperature and humidity sensors using two sediment MFCs that generated 24 mW and 36 mW (Tender et al. 2008). The sediment MFCs were deployed in the Potomac River, at Washington, DC and Tukerton, NJ, USA. Donovan et al. used sediment MFCs to operate a low-power (11 mW) and a high-power (2500 mW) wireless temperature sensors in a creek at Palouse, WA, USA. (Donovan et al. 2008; 2011). The average power generation to power a remote device via a sediment MFC ranges from 3.4 to 36 mW (Dewan et al. 2014). These studies illustrate that MFCs deployed in natural aquatic environment (i.e., rivers, lakes, or oceans) can produce enough energy to operate sensors requiring low power.

However, MFCs for wastewater treatment have faced a variety of restrictions in terms of practical implementation. First, the real wastewater contains complex

organics and diverse microorganisms such as methanogens. This may lead to an inferiority of electroactive biofilm due to methanogenic competition or metabolic diversity. The low ionic strength of real wastewater can limit the power output of MFCs as well (Rozendal et al. 2008). In addition, there are physical constraints with regard to linearly scaling up MFCs. Excessive pressure because of hydrostatic head could require variable permeability to regulate water loss and cathode hydration in the case of permeable membrane. Most importantly, the greatest hindrance lies in the increasing electrical losses and overpotentials with enlarged size (Oh et al. 2010). All of this means that innovative reactor designs are required for practically useful MFCs. As a consequence, after more than two decades of development, in which numerous studies have focused on MFC's application for wastewater treatment (Habermann and Pommer 1991), successful full-scale application is still relatively rare.

In view of the concept of MFCs with current wastewater treatment system, several types of MFCs have been proposed. In order to enhance the quality of effluent, Logan (2008) proposed an integrated bioprocess, which combined the post-treatment process, e.g., solids contact (SC) process or membrane bioreactor (MBR) with MFC system (Fig. 18.6a and b). However, performance of post bioreactor can be inhibited due to consumption of most organic matter in the preceding MFC. The MFC can be combined into the existing wastewater treatment facilities as well. Min and Angelidaki (2008) developed a submersible MFC by immersing an air-cathode MFC in an anaerobic reactor. Similarly, Cha et al. (2010) submerged a single chamber MFC into the aeration tank of the activated sludge process to optimize the cell configuration and electrode materials. The submersible MFC can be applied to the anaerobic (or aerobic) facility as an anode (or cathode) chamber without additional constructions (Min and Angelidaki 2008; Cha et al. 2010) (Fig. 18.6c, d). Yu et al. (2011 and Feng et al. (2013b) designed another configuration for decentralized wastewater treatment through immersing the anode into an anaerobic tank and the cathode into an aerobic tank of the A/O system, respectively (Fig. 18.6e). These types of configuration enable MFCs to be applied to existing wastewater treatment systems.

Meanwhile, the work on scaling up MFCs for wastewater treatment is moving forward. According to some information on the Internet or public literatures, there are at least two pilot-scale MFCs for wastewater treatment available for practical implementation. The first large-scale test of tubular MFCs was located at Foster's brewery in Yatala, Queensland (Australia) (http://www.microbialfuelcell.org). This system was constructed by the Advanced Water Management Center of the University of Queensland, led by Jurg Keller and Korneel Rabaey. MFCs consisted of 12 modules with an entire volume of 1 m^3. The anodes and cathodes are made of carbon fiber based on a brush design. Another pilot-scale multi-anode/cathode MFC (MAC MFC) was developed by researchers of University of Connecticut and their collaborators (Fuss and O'Neill, and Hydroqual Inc.) in the USA (Jiang et al. 2011). The MAC MFC contained 12 anodes/cathodes with a total volume of 20 L. The reactors contain graphite rods as the anode, with Cu-MnO_2 or Co-MnO_2 catalyzed carbon cloth cathodes. The systems are treating wastewater, achieving

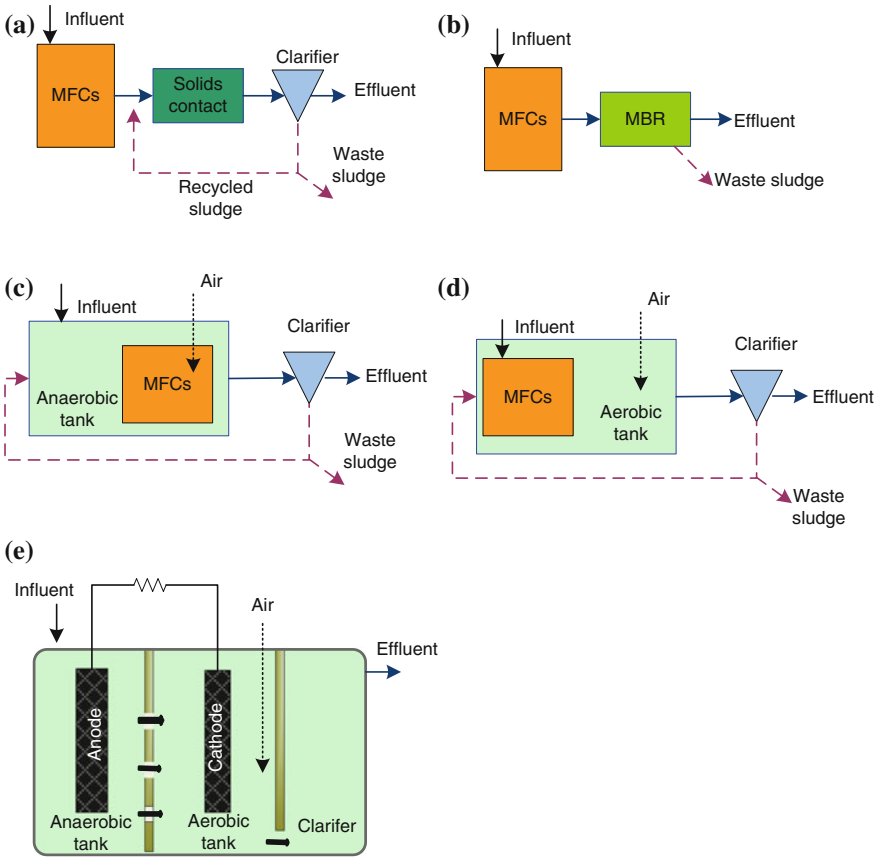

Fig. 18.6 Schematic diagram of MFCs combined wastewater treatment process: **a, b** MFC combined with a solids contact tank or a MBR; **c, d** MFC submerged into an anaerobic or aerobic tank of existing wastewater treatment process; **e** a decentralized wastewater treatment based on A/O system

80 % of contaminant removal at different organic loading rates (0.19–0.66 kg/m^3/d). The power density of MAC MFC reached 380 mW/m^2. In addition to the pilot scale MFCs, Ieropoulos et al. (2013) originally exploited a stack of small ceramic MFCs (6.25 mL) fed with real urine to power a mobile phone, which was previously considered impossible.

Therefore, tremendous efforts should be dedicated in terms of utilizing the voltage from MFCs in the near future. Dewan et al. (2014) pointed out that renewable energy sources tend to be applied to power remote sensors, due to the potential environmental risks and operational cost associated with batteries. More research is also required to focus on assessment of lifetime, reliability and renewability, which are of great significance in the process of promoting the MFCs widespread application.

18.9 The Conclusion and Perspective

Substantial efforts have been devoted to the development and improvement of MFC technology to reduce its operating cost, and to increase power output although MFC technology has not been widely scaled up for commercial application. MFC technology covers many distinct scientific disciplines, including material sciences, microbial ecology, and engineering design. Previous studies have proposed innovative designs of MFC reactors to improve the performance together with reduced capital costs. It has been demonstrated that different electrode materials exhibited different behaviors and electrode modification offers a good and effective approach for enhancing the performance. Development of the electrode with excellent proprieties and the reasonable price could be crucial for the practical application. Furthermore, appropriate integration or combination of MFCs with present wastewater treatment technologies should be taken into consideration.

MFCs provide us with a model system to study the different microbial populations present in the exoelectrogenic biofilms, and it would be an important research area in understanding how the microbial ecology of electricity producing communities develops and shifts over time. Extensive studies on exoelectrogenic bacteria and consortia begin to expose the mechanistic and ecological complexities of MFC biofilm communities. Yet, our understanding of electrochemically active microbes is still in its infancy, as the diverse communities have a multitude of undiscovered electrochemical capabilities that can be exploited in different MFC applications. Discovery of the potential exoelectrogenic bacteria is important in understanding the function of anodic microbial communities and to improve the electron transfer efficiency of MFCs.

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