Chapter 13
Rice Paddy-Field Microbial Fuel Cells: Fundamental and Recent Progress

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Abstract Microbial fuel cells (MFCs) are devices that exploit living microbes for the conversion of organics into electricity. Bioreactor-type MFCs have been extensively examined in the laboratory for applying them to processes that convert organic wastes into electric power. It is also possible to exploit MFCs at the interface between water and sediment in the aquatic environment, and these MFCs are termed sediment MFCs (S-MFCs). One option of S-MFCs is rice paddy field MFCs (RP-MFCs), in which anodes are set in the rhizosphere of rice plants, whereas cathodes are placed in flooded water. Studies have attempted to optimize electrode structures to enhance power outputs, and recent work has reported power outputs as high as 140 mW m^{-2} (based on the projected area of the anode). In addition, studies have been conducted to gain insights into microbes involved in power generation in RP-MFCs, and analyses using metabarcoding of PCR products and metagenomics have suggested that *Geobacter* relatives occur at anodes and contribute to the current generation. This chapter describes the fundamentals and recent signs of progress of RP-MFCs that are expected to serve as on-site power sources contributing to sustainable agriculture.

Keywords Energy harvest · Microbial fuel cell · Microbial solar cell · Exoelectrogen · Electrochemically active bacteria · Extracellular electron transfer

13.1 Introduction

Electric power is essential for human society, and a large portion of electric power is currently generated in power plants with the expense of fossil fuels. Two concerns are associated with this power generation; first, fossil fuels are finite resources, and it has been predicted that most of these will be depleted within the twenty-first century.

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Second, the combustion of fossil fuels emits carbon dioxide, the major causative agent in global warming. For the sustainable development of human society, renewable resources, such as solar energy, water energy, and biomass, must be more extensively used for power generation. Furthermore, it is eagerly anticipated that as yet unexploited energy sources will be discovered.

Recently, it has been suggested that the natural environment harbors massive amounts of as-yet unexploited energy sources, and researchers in the field of sustainable developments search for technologies that harvest unexploited natural energy for electricity generation (Priya and Inman 2009). A variety of technologies have been proposed for this purpose (Priya and Inman 2009), and these include rice paddy field microbial fuel cells (RP-MFCs). RP-MFCs are on-site power generators that can serve as energy sources for wireless monitors for measuring environmental parameters in paddy fields, such as, water and atmospheric temperatures, humidity, and solar irradiation (Kouzuma et al. 2014).

Microbial fuel cells (MFCs) are devices that use living microbes for the conversion of organics into electricity (Logan et al. 2006; Watanabe 2008). Bioreactor-type MFCs have been extensively examined in the laboratory for their application to processes that convert organic wastes onto electric power (Santoro et al. 2017). It is also possible to set up MFCs at the interface between water and sediment in the aquatic environment (termed sediment MFCs, S-MFCs), and researchers have examined S-MFCs at marine sediments and riverbeds (Tender et al. 2002; Reimers et al. 2006; Donovan et al. 2008). In S-MFCs, anodes are buried in sediments, while cathodes are floated in water immediately above the sediments, and electricity is generated primarily with the aid of microbes at anodes. RP-MFC is a type of S-MFC that is set in a rice paddy field (Kaku et al. 2008). Rice is one of the major agricultural crops in the world, in particular, in Asian countries, including Japan, and paddy fields reach over 2 million hectares in Japan, sharing over 50% of the total cultivated field (Ministry of Agriculture, Forestry and Fisheries 2018). It is therefore suggested that RP-MFC has a large potential, while further research is necessary for practical application.

In this chapter, we describe fundamentals and recent progresses for RP-MFCs and other S-MFCs with focuses on structures, performances, and microbes involved in power generation. Based on current knowledge, we discuss future directions of the research on RP-MFCs.

13.2 Structures and Performances of RP-MFCs and Other Plant MFCs

13.2.1 RP-MFCs

The use of MFC systems for electricity generation in rice paddy fields was first reported by Kaku et al. (2008), and, since then, such systems are termed RP-MFCs. A similar idea of MFC was also examined in pot cultures of rice plants

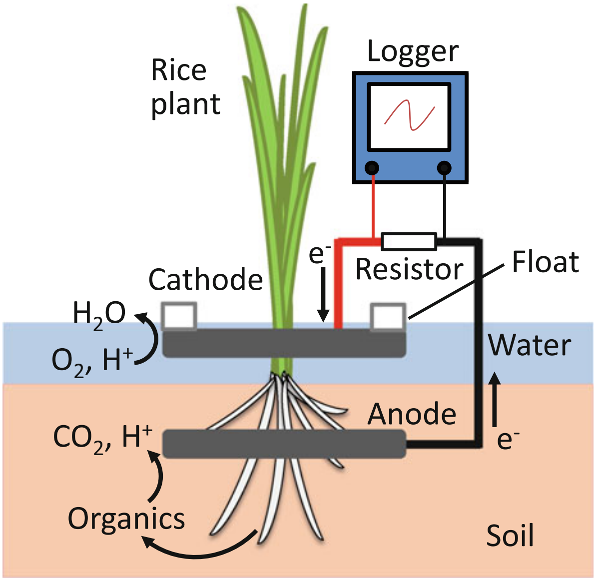

Fig. 13.1 Schematic diagram for RP-MFC

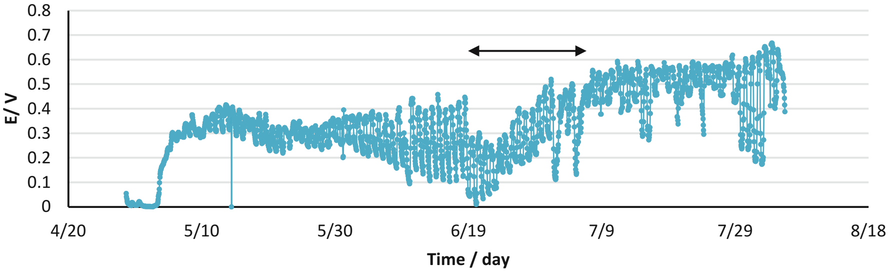

Fig. 13.2 Time course of cell voltage of RP-MFC. The operation was started at the end of April and terminated in August. An arrow indicates a rainy period

(de Schamphelaire et al. 2008). In RP-MFCs, anodes are set in rhizospheres of rice plants, while cathodes are placed in flooded water (Fig. 13.1). These electrodes were made of graphite, a material most widely used for electrodes in MFCs. In the rhizosphere, rice plants excrete photosynthesized organics from roots, and these organics serve as substrates (electron donors) for exoelectrogenic bacteria and other microbes. Electrons released from organics by oxidative catabolic reactions of exoelectrogens are captured by anodes, transferred to cathodes via external circuits, and used for the reduction of oxygens at the cathode surface (Fig. 13.1). According to the electromotive force between organics oxidation and oxygen reduction, electrons are transferred from the anode to cathode, resulting in the power generation at the external circuit. A characteristic feature of RP-MFC is the circadian oscillation of the electric output that increases in the day time and drops at night (Fig. 13.2). In order to investigate mechanisms behind the oscillated electric output, Kaku et al. (2008) conducted two experiments, namely, measurement of electric outputs after rice plants were shaded with black clothes, and detection of root exudates in the light and dark. They have found that the shading of rice plants substantially decreases the electric output, and rice roots excrete organics, e.g., organic acids, only in the light. From these results, they suggest that the oscillated electric output from RP-MFC is attributable to the daily oscillation of sun irradiation and resultant oscillated organics excretion from rice roots. It is therefore considered that the oscillated electric output is an evidence for the plant/microbe cooperation for the conversion of light energy (sun irradiation) into electric energy (i.e., microbial solar cell).

The above work has provided the concept of RP-MFC, while the maximum power density reported in that work was 6 mW m^{-2} (normalized to the projection area of the anode) at the maximum (Kaku et al. 2008). Subsequent studies have been conducted for examining technical breakthroughs to improve power outputs from RP-MFCs. To cite an instance, Takanezawa et al. (2010) examined several factors affecting electric outputs from RP-MFCs, including anode thickness, anode depth, modification of cathodes with oxygen-reduction catalysts, and external resister used during the start up. The study has shown that anode depth, oxygen-reduction catalysts, and external resister largely affect the resultant power output. Another study has optimized sizes of anodes and cathodes and reported the maximum power density of 140 mW m^{-2} (based on the projected area of the anode) using relatively small anodes (Ueoka et al. 2016). It should also be noted that outputs from RP-MFCs are largely influenced by weather (temperature and sun irradiation); for instance, Japan has a rainy season in June and July, during which the output from RP-MFC drops substantially (Fig. 13.2).

13.2.2 Other Plant MFCs

In addition to RP-MFC, S-MFCs are also operated in association with other plants, such as *Typha latifolia*, a perennial herbaceous plant, and *Spartina anglica*, species of cordgrass (Strik et al. 2008). These MFCs are termed plant MFCs (P-MFCs), and Table 13.1 summarizes representative studies on P-MFCs. In P-MFCs, as has been reported for RP-MFCs, plants provide rhizosphere exoelectrogenic microbes with organic substrates (Strik et al. 2008). Liu et al. (2013) examined P-MFCs planted with *Ipomoea aquatica* (water spinach), showing that their power densities were twice as high as those of unplanted MFCs. In addition, the study has also shown that nitrogen-removal efficiencies of the planted MFCs are much better than those of unplanted MFCs (e.g., 90.8% vs. 54.4%), and it has been deduced that untreated nitrate in unplanted MFCs lowered electricity generation (Liu et al. 2013).

Saz et al. (2018) operated P-MFCs using four different plants, *Typha latifolia*, *Typha angustifolia*, *Juncus gerardii*, and *Carex divisa*, for examining wastewater treatment in experimental wetlands. It has been shown that P-MFCs with *T. angustifolia* exhibit the best performances in terms of ammonia removal and

Table 13.1 Representative P-MFC studies

Study	Support medium	Anode material	Cathode material	Plant	P_{max} (mW m^{-2})^a
Helder et al. (2010)	Graphite granule	Graphite rod	Graphite felt	*Spartina anglica*	222^b
				Arundinella anomala	22^b
Liu et al. (2013)	Gravel	Granular achieved carbon	Stainless steel mesh	*Ipomoea aquatica*	12
Villaseñor et al. (2013)	Gravel	Graphite	Graphite	*Phragmites australis*	43
Oon et al. (2015)	Gravel	Carbon felt	Carbon felt	*Typha latifolia*	6
Lu et al. (2015)	Gravel	Graphite disk	Carbon cloth	*Canna indica*	18^c
Liu et al. (2017)	Gravel	Granular activated carbon	Granular activated carbon	*Spartina alterniflora*	60
Saz et al. (2018)	Gravel	Graphite	Magnesium	*Typha latifolia*	13
				Typha. angustifolia	18
				Juncus gerardii	8
				Carex divisa	9
Rathour et al. (2019)	Gravel	Stainless steel	Stainless steel	*Fimbristylis dichotoma*	199
Xu et al. (2019)	Ceramics/sand	Titanium cylinder	Titanium mesh	*Phragmites australis*	16

[a] Normalized to the anode projected area unless otherwise stated
[b] Normalized to the planting surface area
[c] Normalized to the cathode projected area

power output among the examined P-MFCs, and the authors have considered that *T. angustifolia* is able to provide rhizosphere microbes with appropriate environments for organics degradation and electricity generation. From these results, the authors recommend *T. angustifolia* for fueling P-MFCs that are installed in wetlands (Saz et al. 2018). In addition to plant species, plant growth phases are also considered to affect MFC performances (Moqsud et al. 2015); in that study, authors have revealed that electricity generation of P-MFCs is high during the vegetative growth phase compared to that during the reproductive phase.

Another study has shown that salinity affects the performance of P-MFCs (Xu et al. 2019). In that study, authors constructed wetlands for treating wastewater, in which P-MFCs were operated. As a result, P-MFCs treating saline wastewater exhibited high power densities compared to those treating non-saline wastewater (16.4 mW m^{-2} vs. 3.9 mW m^{-2}, for example). Microbiome analyses have shown that putative exoelectrogens were more abundantly detected at anodes in the presence of saline wastewater than those in non-saline wastewater, and it has been suggested a relatively high ionic strength of the electrolyte may be necessary for exoelectrogens to actively grow in P-MFCs (Xu et al. 2019). It is therefore likely that P-MFCs exhibit good performances in coastal areas and salt-damaged areas.

In summary, P-MFCs, including RP-MFCs, have been operated in rice paddy fields, wetlands, and experimental pod cultures. While comparisons of these MFCs in terms of power output suggest that rice is one of the best plants used for P-MFCs, plants that naturally occur in wetlands, such as *T. angustifolia*, are also useful for efficient power generation.

13.3 Microbes at Work in RP-MFCs and Other P-MFCs

13.3.1 Anode-Associated Microbes

In order to characterize microbiomes that occur around anodes of P-MFCs, including RP-MFCs, studies have used metabarcoding of 16S rRNA gene amplicons and shotgun metagenomics (Kouzuma et al. 2014). It is well known that microbiomes occurring in the rhizosphere are highly diverse (Berg 2009). Similar trends have also been observed in anode-associated microbiomes in RP-MFCs, in which bacterial species related to exoelectrogens, such as members of the genera *Geobacter*, *Clostridium*, *Bacillus*, and *Desulfobulbus*, have frequently been detected (Kouzuma et al. 2013; Wang et al. 2015; Lu et al. 2015; Abbas et al. 2019; Gustave et al. 2019). Among them, members of the genus *Geobacter* have been considered to be the major exoelectrogens in many natural and engineered ecosystems, owing to the widespread distribution in anaerobic environments and the high capability of extracellular electron transfer to electrodes via outer membrane-localized cytochromes (Kouzuma et al. 2018; Logan et al. 2019). On the other hand, Wang et al. (2019) have suggested that physicochemical properties of soil, including, water content, electrical conductivity, total sulfur, and iron content, significantly affect the abundance and species of exoelectrogens. The authors have reported that among the genera related to *Desulfobulbus* and *Bacillus* are the most abundant in coastal and arid-land soils, respectively, while *Geobacter*, *Clostridium*, and *Anaeromyxobacter* are abundant in paddy and lakeshore soils. Other studies have however reported that members of *Desulfobulbus* and *Bacillus* occur in anode-associated microbiomes in RP-MFCs (Wang et al. 2015; Lu et al. 2015). These differences may be ascribable to salinity; a study on MFCs using brackish sediments as inocula has shown that *Geobacter* occurs only when NaCl concentrations in electrolytes are lower than 0.1 M, while *Desulfuromonas* occurs at higher concentrations (Miyahara et al. 2016). It is hence concluded that dominant exoelectrogens can vary depending on environmental conditions.

In the rhizosphere, plant-root exudates, such as carbohydrates, fatty acids, and amino acids, are utilized as carbon and energy sources of microbial residents, thereby significantly affecting the structure of rhizosphere microbiomes (Smalla et al. 2001; Berg and Smalla 2009). Plant species are therefore considered important factors that determine the structure of exoelectrogenic microbiomes in P-MFCs. Timmers et al. (2012) characterized anode-associated microbiomes in P-MFCs planted with reed mannagrass (*Glyceria maxima*), in which members of *Geobacter* were abundantly detected along with anaerobic cellulolytic bacteria affiliated with the families *Clostridiaceae* and *Ruminococcaceae*. From these observations, it has been suggested that electricity generation in *G. maxima*-planted P-MFCs is based on syntrophic interactions among exoelectrogenic and fermentative bacteria.

In RP-MFCs, members of *Anaeromyxobacter*, as well as those of *Geobacter*, have been abundantly detected in association with anodes (Kouzuma et al. 2013; Cabezas et al. 2015; Lu et al. 2019). *Anaeromyxobacter* is regarded as a genus that includes exoelectrogens (Wang et al. 2019), and an isolate of *Anaeromyxobacter dehalogenans* has been reported to be capable of dissimilatory metal reduction (Marshall et al. 2009). These studies suggest that members of *Anaeromyxobacter* play a particularly important role in electricity generation in RP-MFCs. Studies have also reported that members of the class *Anaerolineae*, which includes a fermentative isolate of *Anaerolinea thermolimosa* (Yamada et al. 2006), are relatively abundant in anode-associated microbiomes in RP-MFCs (Kouzuma et al. 2013; Cabezas et al. 2015). It is likely that members of this class contribute to the fermentative degradation of rice root exudates and supply electron donors to exoelectrogens, such as members of *Geobacter* and *Anaeromyxobacter*.

The studies introduced herein suggest that ecological interactions between fermentative and exoelectrogenic microbes work at anodes of P-MFCs, thereby facilitating the current generation with the expense of root exudates. Further studies will be conducted to address how such ecological interactions can be managed to improve electric outputs from P-MFCs.

13.3.2 Cathode-Associated Microbes

In MFCs, including RP-MFCs, cathodes are made of graphite/carbon, and, in many cases, doped with oxygen-reduction catalysts (Takanezawa et al. 2010). This is because the oxygen-reduction activity at the graphite/carbon surface is generally low, and it is necessary to enhance the activity to circumvent cathode-limited electric outputs from MFCs. Most of these catalysts, e.g., platinum catalysts, however, are expensive, and studies have been conducted to develop cheap and sustainable alternatives and/or technologies to reduce amounts of catalysts used at the cathode.

One approach to tackle this requirement would be the use of microbes present at cathode surfaces. It has been known that a variety of microbes occur at the cathode, and some of them contribute to the cathodic reaction in MFCs (Rabaey and Rozendal 2010; Kracke et al. 2015). Cathodes using microbes as catalysts are termed biocathodes, which have been examined in various bioelectrochemical systems (Marshall et al. 2012). A distinctive feature of cathodes in S-MFCs, including RP-MFCs, would be that these are exposed to ever-changing and, in some cases,

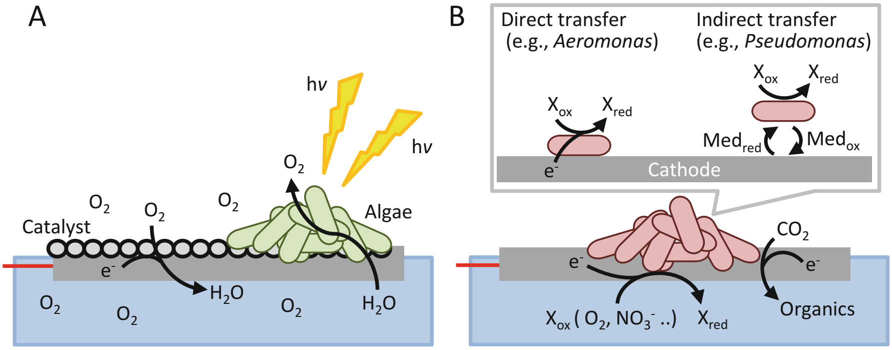

Fig. 13.3 Schematics for microbe-stimulated cathode reactions in RP-MFCs. (**a**) Oxygenic photosynthesis-assisted cathode reaction. (**b**) Biocathode reaction. X_{ox}, oxidized form; X_{red}, reduced form. *Med* electron mediator

harsh environments, e.g., the direct exposure to air, which may cause the development of distinctive microbiomes at cathodes of S-MFCs and RP-MFCs.

Studies have suggested two possible mechanisms underlying microbe-stimulated cathode reactions in S-MFCs. First, several studies have shown that microalgae are present in biofilms formed on cathodes of RP-MFCs, and they increase dissolved oxygen concentrations around cathodes due to oxygenic photosynthesis, resulting in improved cathode activities (Fig. 13.3a) (Chen et al. 2012; Srivastava et al. 2019). In another study, S-MFCs were subjected to light/dark cycles, also showing that light irradiation promotes oxygen production by phototrophs and lowers mass transport limitations for the oxygen-reduction reaction at cathodes (Bardarov et al. 2018).

On the other hand, it has been reported that there exist microbes in the natural environment that can uptake electrons from cathodes and utilize them for their intracellular metabolism, such as, oxygen respiration and CO_2 fixation (Fig. 13.3b) (Huang et al. 2011). Microbes exhibiting such activities are the focus of recent studies on microbial electrosynthesis (Claassens et al. 2016), in which anaerobes, such as those affiliated with the genera *Sporomusa*, *Clostridium*, *Methanosarcina*, have been reported to use electrons from cathodes for fixing carbon dioxide to produce acetate or methane (Rabaey and Rozendal 2010; Karthikeyan et al. 2019). However, microbes that grow at the cathode surface of RP-MFCs may be different from these anaerobes, since oxygen concentrations around cathodes are substantially high (Rago et al. 2017).

Biofilm microbiomes formed on cathodes of S-MFCs have been analyzed (Reimers et al. 2006; de Schamphelaire et al. 2010). These studies have shown that gammaproteobacteria, such as members of the genera *Pseudomonas* and *Aeromonas*, are abundantly present. These genera include facultative aerobes that exhibit electrochemical activities (Logan et al. 2019); in particular, *Aeromonas hydrophila*, a close relative of the genus *Shewanella*, is known to possess an extracellular electron transport pathway (Conley et al. 2018), thereby performing direct electron transfer for taking electrons from cathodes (Fig. 13.3b). On the other

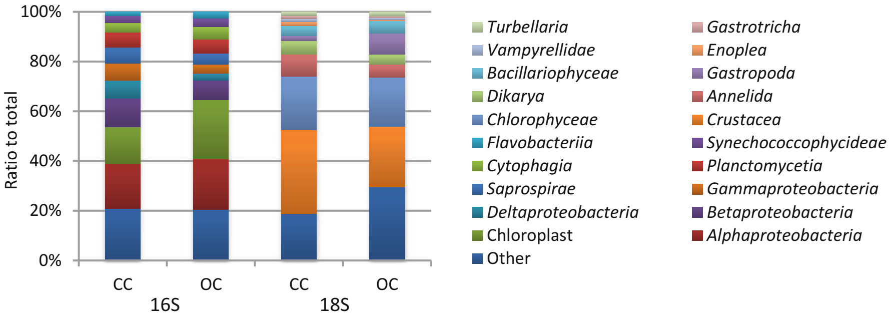

Fig. 13.4 Microbiome structures in cathode biofilms in RP-MFCs as assessed by metabarcoding of rRNA gene amplicons. Analyses were conducted as described by Vamshi Krishna and Venkata Mohan (2016). Bacterial (16S) and eukaryotic (18S) communities established under open-circuit (OC) and closed-circuit (CC) conditions were compared, indicating that some bacteria, including those affiliated with *Gammaproteobacteria* and *Deltaproteobacteria* were more abundantly detected under the CC condition than those under the OC condition

hand, *Pseudomonas aeruginosa* is known to secret electron mediators and perform indirect electron transfer (Wang et al. 2010) (Fig. 13.3b).

Our recent study has found that *Gammaproteobacteria* (e.g., members of the family *Sinobacteraceae*) and *Deltaproteobacteria* (e.g., members of the family *Geobacteraceae*) are more abundantly detected at the surface of cathodes in RP-MFCs under closed-circuit conditions than those under open-circuit conditions (Fig. 13.4), suggesting that these bacteria would grow by using electrons taken from cathodes (Hirose et al. unpublished results). The occurrence of *Geobacter* relatives in cathode biofilms is unexpected since oxygen is abundantly present around cathodes. A possible explanation would be that thick biofilms are formed on cathodes, in which anaerobic regions are formed at the bottom (immediately above the cathode surface). Among eukaryotes, members of *Chlorophyceae* (algae) and *Crustacea* (arthropods) were abundantly detected by metabarcoding of 18S rRNA gene amplicons from RP-MFC cathodes (Fig. 13.4). Further studies would be necessary for identifying the functions of these microbes.

13.4 Future Perspectives

It has been shown that RP-MFCs are devices that convert light energy into electricity under the cooperation between rice plants and microbes. As described above, studies have shown that microbes present not only around anodes but also around cathodes play important roles for electricity generation. It has therefore been considered that a deeper understanding of these microbes is necessary for further improving RP-MFCs. In particular, although two possible roles of cathode microbes in RP-MFCs have been proposed, information is limited as to how much these microbes contribute to electricity generation relative to the activity of chemical catalysts. In addition, studies may also be necessary for enhancing the performance of anode microbes for transferring electrons to electrodes. We expect that achievements to be obtained in these studies will provide us with reliable on-site power sources that are applicable to wireless sensors useful for smart agriculture.

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