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Electric Power from Rice Paddy Fields

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1. Introduction

One of the largest innovations in the twentieth century is the use of petroleum (and other fossil fuels, such as, coal, and natural gases) for the energy source that supports the human society. Petroleum is an easy-to-use, energy-intensive fuel as well as a material for a variety of chemical products. It is produced by pumping-up from natural underground reservoirs. In 2006, petroleum and other fossil fuels support 86% of the total energy supply for the human society (Energy Information Administration 2007), indicating that they are currently indispensable for us.

Recently, however, humans face problems associated with the use of fossil fuels. One of such problems is the limitation in fossil fuels stored in our planet. It is known that fossil fuels (as the name indicates) had been mostly generated by long-term geochemical reactions with ancient plants and algae as original resources and accumulate in underground reservoirs. Such reactions may also occur currently, while the recent consumption of fossil fuels is much faster than that, resulting in the rapid decrease in the amounts of fossil fuels in underground reservoirs. In addition, a large fraction of petroleum stored in easily accessible reservoirs is considered to have already been consumed, and engineers predict that the cost for producing petroleum will dramatically increase in the 21th century. Another problem results from the combustion of fossil fuels; this process generates carbon dioxide that is released and accumulates in the air, resulting in the green-house effect and global warming. According to an assessment report in 2007 by the Intergovernmental Panel on Climate Change (IPCC), global surface temperature increased 0.74 ± 0.18°C during the 20th century (IPCC 2007). This increase corresponds to an increase in the carbon dioxide concentration in the atmosphere (from 0.03% to 0.037%) in the 20th century (Global Warming Art 2007). We therefore consider that the use of fossil fuels is such a process that converts underground carbons into carbon dioxides and release them into the atmosphere.

Under such circumstances, energy sources alternative to fossil fuels are strongly desired for supporting human activities in the 21th century, particularly those that are renewable and not associated with the global warming. The primary important is the solar energy. It has been estimated that the amount of solar energy that strikes the Earth every hour (~4.3×10^{20} J) is approximately equal to the total amount of energy consumed by human society every year (Donohue & Cogdell 2006). Hence, global energy needs can be substantially satisfied even with a small fraction of the available solar energy, and the use of photovoltaic solar cells (Fahrénbruch & Bube 1983) is currently expanding. We also consider that biomass is another important renewable energy source. Biomass includes all biologically synthesized

chemicals, primary by photosynthetic organisms using sun light as the energy source and carbon dioxide as the raw material (i.e., the biological primary production). It is also known that the geochemical energy is also available for the synthesis; this process involves bacteria, called chemolithotrophs, that use the chemical energy conserved in reduced inorganic chemicals to synthesize organic compounds from carbon dioxide. Biomass is the most abundant carbonaceous material at the surface of our planet, and the total amount has been estimated to be approximately 75 billion tons. Although the combustion of biomass also generates carbon dioxide, this is considered to be a part of carbon cycling on our planet along with the biological primary production. In this sense, the use of biomass as an energy source is regarded as the "carbon neutral process". The history of using biomass as our energy source is long, starting from the finding of fire on woods that ancient people used for heating and cooking, while recent technical achievements include the conversion of raw biomass into convenient forms of energy (e.g., liquid fuels, and electricity), and the use of biomass that is difficult to use (e.g., those that contain a large amount of water). Accordingly, scientists and engineers are recently keen in developing technologies for utilizing energies conserved in biomass; these include the production of bio-ethanol (Gray et al. 2006), bio-hydrogen (Levin et al. 2004), and bio-electricity (Logan et al. 2006; Watanabe 2008).

As described, we expect that the solar light and biomass will become important energy sources in the 21th century, and biological energy conversion (BEC) processes will be widely used in association with this energy shifts. In such processes, organisms (in particular, microbes) are catalysts that convert energy forms (e.g., the solar energy into the chemical energy) and chemical species (e.g., biomass into ethanol). A representative example is a photosynthetic organism that conserves the light energy as organic compounds (rich in the chemical energy) (Gust et al. 1993). In another case, bacteria ferment biomass chemicals, such as starch, to produce bio-ethanol (Gray et al. 2006). Besides, recent studies have found that some bacteria convert chemical energies in organic compounds into electricity (McConnell et al. 2010). Accordingly, we can say that we have a large repertoire of energy conversion processes, if biological systems are used. There are advantages of BEC processes over chemical processes; these include (i) they are environmentally friendly, (ii) the diversity of catabolic capacities expands a range of chemicals that can serve as fuels, and (iii) they are self-sustaining. Based on these advantages, BES processes are also able to use waste biomass as energy sources to generate fuel gases (hydrogen, and methane) and electricity. Such a process must be impossible, when we use chemical catalysts.

This chapter introduces technologies for generating bio-electricity from the sun light, and/or biomass, particularly those that use in rice paddy fields (RPFs). Since electricity generation in RPFs is a process that is a combination of microbial solar cells (MSCs) and microbial fuel cells (MFCs), these BEC processes are explained before describing RPF electricity generation.

2. Microbial solar cells (MSCs) and Microbial fuel cells (MFCs)

2.1 MFCs

MFCs are devices that exploit microbial catabolic activities to generate electricity from a variety of materials, including complex organic waste and renewable biomass. In MFCs, microbes utilize organic compounds as energy and carbon sources. In order to generate energy for growth, organics are decomposed, and chemical energy is released (i.e.,

fermentation). In addition, high-energy electrons released from organics are transferred to oxidized chemicals (i.e., electron acceptors, such as molecular oxygen) to conserve electrochemical energy (i.e., respiration). In microbial cells, electrons released from organics are initially accepted by intercellular electron-shuttling compounds (e.g., nicotinamide adenine dinucleotide [NAD]), and subsequently transferred to electron acceptors via respiratory electron-transport chains. If a mechanism is present by which electrons released from organics can be transferred from any step in the intercellular electron-transfer pathway to an extracellular electrode (i.e., anode), then microbial oxidation of organics can be coupled to electricity generation (i.e., an MFC).

Fig.1 illustrates typical structures of MFCs. Fig. 1A shows a membrane-type oxygen-diffusion cathode that is frequently used in recent studies (Watanabe 2008). This type of reactor is called "a single-chamber MFC reactor", while two-chamber MFCs are also used, which are comprised of anode and cathode chambers separated by proton-exchange membranes (Ishii et al. 2008a). As shown in this figure, electrons released by microbes are captured by an anode and transferred to a cathode according to the potential gradient between them. The electrons are used in cathode chemical reactions, in which protons released at the anodes and diffusively transferred to the cathodes are also utilized, thereby completing an electric circuit. Cathode reactions are dependent on the species of electron acceptor and catalyst (see below). Ambient oxygen is the most commonly used electron acceptor, while other oxidants, such as ferricyanide, can also be used (Logan et al. 2006). As a cathode catalyst, platinum is widely used for oxygen reduction.

It has been suggested that MFCs have many possible future applications; these include water treatments coupled to energy recoveries, portable fuel cells, biosensors, and in-situ energy sources. Among them, the first one, waste treatments, is considered to be the most promising, and MFCs may be able to work as energy- and cost-saving options for waste treatments (Watanabe 2008). The MFC technology has however not yet been applied to practical waste treatments. This is primarily because it is an emerging technology and more time is required for technical maturation. Another reason is that its process performance is considered low when compared to its competitors (e.g., methanogenic anaerobic digesters). On average, modern methanogenic anaerobic digesters treat organic wastes at efficiencies of ~8–20 kg chemical oxygen demand (COD) units per m^3 of reactor volume per day (Lettinga 1995), equivalent to ~1,200–3,000 watts per m^3. Given that waste-to-gas, and gas-to-electricity conversion efficiencies are typically 80%, and 40%, respectively, these methanogenic digesters generate electricity at ~380–960 watts per m^3. On the other hand, a recent MFC study reported a power density of 130 watts per m^3 with a model organic waste (comprised of starch, peptone, and fish extract) as the fuel (Shimoyama et al. 2008), suggesting that ~7-fold increases in power output may be necessary for MFCs to exceed performances of anaerobic digesters. Approaches to how power outputs of MFCs can be increased have been summarized in a literature (Watanabe 2008).

Recent studies have identified that there exist bacteria that can use self-sustaining extracellular electron transfer mechanisms to respire MFC anodes (Lovley 2008; Watanabe et al. 2009). Some bacteria excrete water-soluble electron-shuttling compounds that are reduced by bacterial cells and oxidized by transferring electrons to MFC anodes (Watanabe et al. 2009). Other bacteria use secreted and/or cell-surface electron-transporting proteins (e.g., cytochromes) for the electron transfer toward MFC anodes (Lovley 2008). An important point is that they have self-sustaining anode-respiring mechanisms without the need of artificial assistance, e.g., the supplementation of MFC with artificial electron
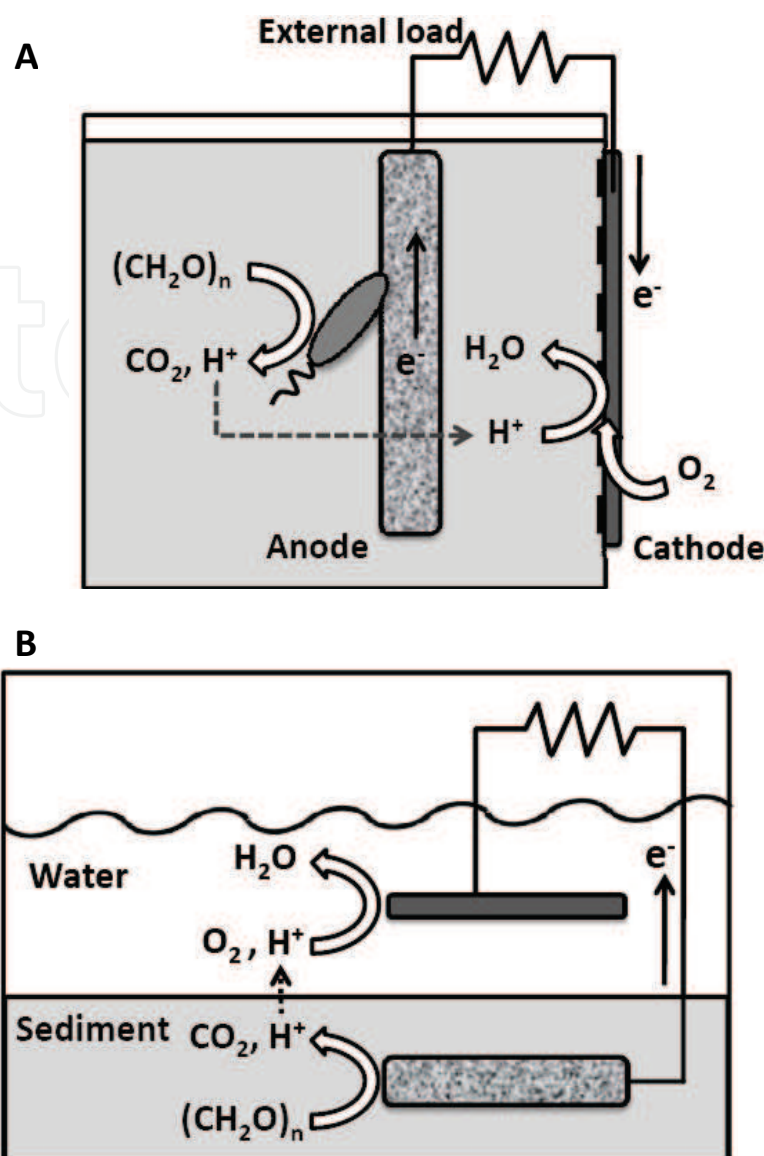
Fig. 1. Schematic diagrams for a single-chamber MFC (A) and sediment MFC (B).

shuttles. This finding is really important, when one consider the application of MFC to waste treatment. Furthermore, with such bacterial self-sustaining electron-transfer mechanisms, sediment MFC (this includes RPF electricity generation) can also be constructed (Fig. 1B). We consider that deeper understanding of bacterial self-sustaining electron-transfer mechanisms will facilitate more efficient MFCs.

Here we also explain sediment MFCs (sMFCs) as the final part in the MFC section (Fig. 1B). In sMFCs (Reimers et al. 2001), anodes are submerged in freshwater and marine sediments, while cathodes are placed in water phases above the sediments. Since sediment and water are anaerobic and aerobic, respectively, potential gradients are generated between them. It is therefore possible to generate electricity between them, if anode microbes have self-sustained extracellular electron transfer mechanisms (Lovley 2006). So far, sMFCs have mostly been set in shallow marine coasts, since the high ionic strength in the sea water is beneficial to the electron transfer. Researchers aim at applying sediment MFCs to on-site power sources for environmental monitoring systems (Donovan et al. 2008).

2.2 MSCs

As the name of MSC represents its function, MSC is a type of solar cells used for the light/electricity conversion with living phototrophic microbes serving as catalysts for the conversion (Rosenbaum et al. 2010). In one work, a phrase, photosynthetic MFCs (photoMFCs), is used for biological light/electricity conversion processes (Rosenbaum et al. 2010). In MSCs, photosynthetic microbes, such as microalgae and cyanobacteria, use the light energy to liberate high energy-level electrons from water molecules, and these electrons are transferred through photosynthetic electron-transport chains and finally used to fix carbon dioxides and synthesize organic molecules (Gust et al. 1993). Fig. 2 shows two representative types of MSCs, namely, mediator-type MSCs (Fig. 2A), and syntrophic MSCs.
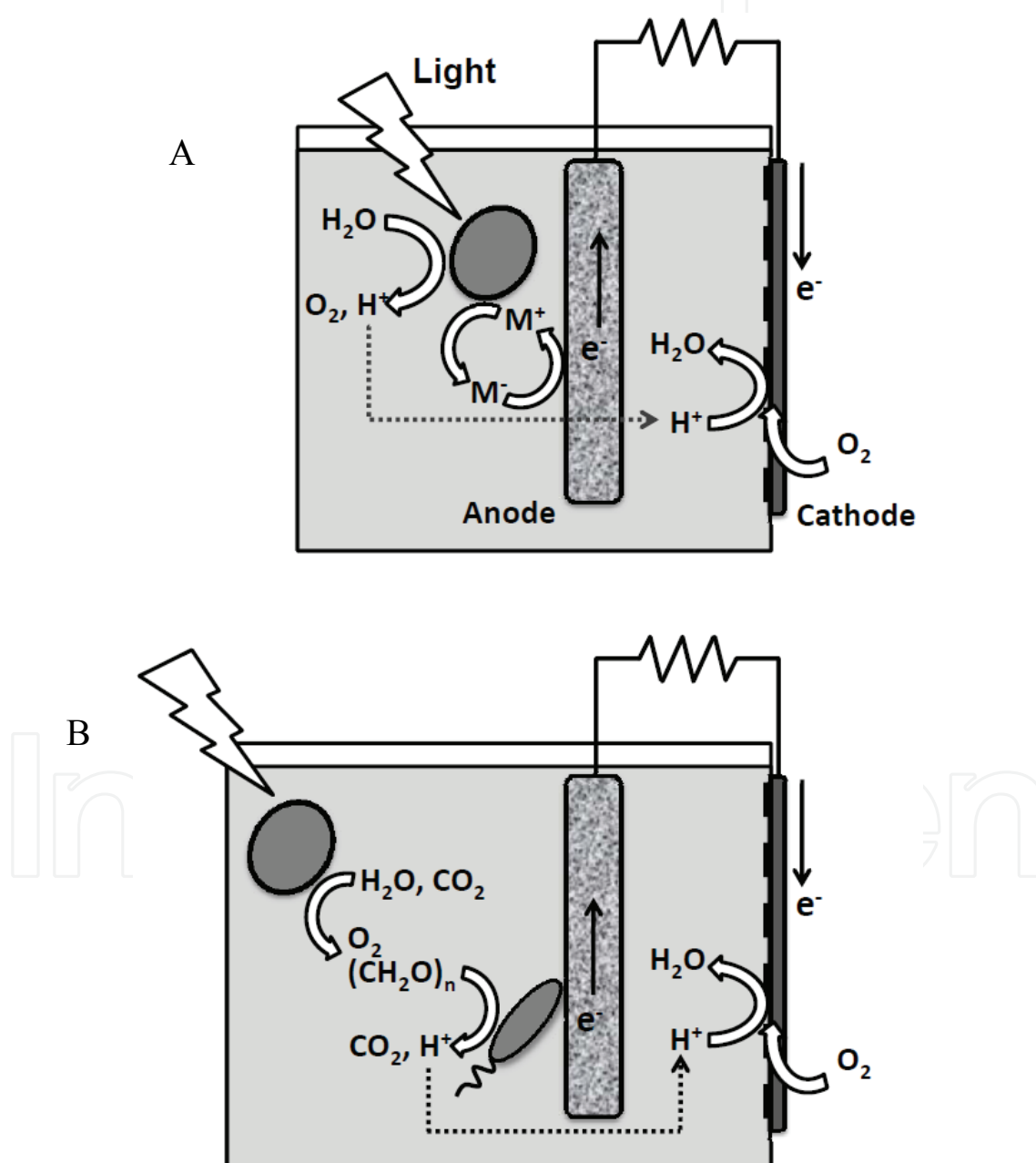

Fig. 2. Systematic diagrtams for a mediator-type MSC (A) and syntrophic MSC (B).

In mediator-type MSCs (Fig. 2A), electron-shuttling compounds (also called as electron mediators; "M" in Fig. 2A) are supplemented. These compounds are reduced (accept electrons) at anywhere in electron-transport chains in photosynthetic microbes and are oxidized (release electrons) at the surface of MSC anodes. Subsequently, electrons are transferred to cathodes according to the potential gradient between them. The electrons are used in cathode chemical reactions, in which protons released at the anodes and diffusively transferred to the cathodes are also utilized, thereby completing an electric circuit. This cathode reaction is the same with MFCs. Mediator-type MSCs are possible only by supplementing with artificial mediators, since there has been no known photosynthetic microbe that is able to produce mediator compounds; in this sense, mediator-type MSCs are not self-sustaining processes. This type of MSCs was actively investigated ~10 years ago (Rosenbaum et al. 2010). In that period, cyanobacteria (e.g., *Anabaena* and *Synechocystis*) were mainly used as biocatalysts with 2-hydroxy-1,4-naphtoquinone (HNQ) as an artificial mediator (Tanaka et al. 1985; Yagishita et al. 1997; Tanaka et al. 1988; Yagishita et al. 1999). Most of these studies however observed the increases in power outputs in association with the dark reaction, where intracellular carbon storages were oxidized, and electrons were liberated. On the other hand, oxygen production limited power outputs in the light (Tanaka et al. 1985; Yagisgita et al. 1998). Such researches are not actively performed in recent years, since it has been realized that artificial mediators are toxic and costly, and mediator-type MSCs are thereby not sustainable and practical.

The idea of syntrophic MSCs (Fig. 2B) has recently been proposed (Nishio et al. 2010). Since the addition of artificial mediator compounds is not sustainable, researchers have been looking for ways that make MSCs sustainable. In the research performed by Nishio et al. (2010), single-chamber MFC reactors were inoculated with natural microbial communities (those in fresh water samples and hot-spring green mats), irradiated, and investigated for their electric outputs. They found that, with some natural inoculants, light-dependent electric outputs were observed when anodes were covered with green biofilms. Analyses of anode biofilms established from a hot-spring green mat revealed that they included microalgae and hetertrophic bacteria closely related to those found in MFCs (Nishio et al. 2010). It was therefore suggested that these two types of microbes had syntrophic relationships, in which microalgae produced organic compounds by photosynthesis, and bacteria used them to generate electricity. Although a power conversion efficiency (PCE; conversion of light into electricity) was low (approximately 0.03%), the concept of syntrophic MSCs is considered to be widely applicable, and further researches are awaited to find strategies to increase PCEs.

A self-sustained biological process for the light/electricity conversion has also been reported recently (He et al. 2009). In that report, when a sediment MFC was irradiated, photosynthetic microbes grew in the water phase and subsequently deposited on the sediment, serving as substrates for heterotrophic bacteria in the sediment to generate electricity (He et al. 2009). They suggested that self-sustained phototrophic microbial fuel cells could be constructed based on the synergistic cooperation between photosynthetic microorganisms and heterotrophic bacteria. A difference between these two studies was that electricity was generated only under dark conditions in the latter study. Although authors of that study speculated that oxygen molecules evolved by photosynthesis in the light may have been inhibitory to electricity generation, clear mechanisms are still unclear, partially because microbes involved in the light/electricity conversion have not yet been analyzed. Despite such a difference in the condition for electricity generation, these two studies are

outstanding examples for self-sustained MSCs with a common feature that microbial communities were used rather than pure cultures of photosynthetic microbes. This feature is important, since it suggests a possibility for constructing syntrophic MSCs in natural environments. On the other hand, complex community structures could cause difficulties in analyzing conversion mechanisms and improving PCEs. We therefore consider that it will also be necessary to construct simple models of syntrophic MSCs using defined mix cultures of photosynthetic microbes and electricity-generating bacteria and to examine syntrophic relationships between these organisms.

3. Plant-assisted sMFCs

In 2008, three reports have been published, which describe sMFCs (Fig. 1B) in the presence of plants (Kaku et al. 2008; Strik et al. 2008; De Schamphelaire et al. 2008). Among them, two studies used rice plants (Kaku et al. 2008; De Schamphelaire et al. 2008), while the other used reed mannagrass (Strik et al. 2008). Two studies used laboratory pot-culture systems (Strik et al. 2008; De Schamphelaire et al. 2008), while the other used a real rice paddy field (RPF; Kaku et al. 2008). This section initially explains a concept of these plant-assisted sMFCs, followed by detailed explanations for two pot-culture sMFCs.

Fig. 3 illustrates conceptual models for plant-associated sMFCs. A RPF sMFC system is shown in Fig. 3A, while a pot-culture sMFC is illustrated in Fig. 3B. An original idea for RPF sMFC was that rice paddy soil was rich in organics, including residues of past rice plant bodies, and self-sustained electricity-generating bacteria in soil use these organics for generating power using sMFC systems. However, Kaku et al. (2008) and other groups have found that organics excreted from rice roots (i.e., root exudates) largely contributed to electricity generation. Since these organics are photosynthesized by plants using the solar energy, these systems can be called "plant-assisted sMFCs". Plant-assisted sMFCs can also be considered as solar cells in which plants and microbes establish syntrophic relationships. In this sense, a RPF sMFC should be included in syntrophic MSCs, in which rice plants are used rather than microalgae. In RPF sMFC, cathodes are placed in flooded water above the anode-submerged soil, and oxygen dissolved in the water is used for the cathode reaction. In a pot-culture sMFC (Fig. 3B), the anode system is the same with a RPF sMFC, while the two studies (Strik et al. 2008; De Schamphelaire et al. 2008) prepared additional cathode chambers that were connected to the anode pots via proton-exchange membranes. In one system reported by Strik et al. (2008), oxygen was used for the cathode reaction, while ferricyanide was also used in the other study (De Schamphelaire et al. 2008). The use of ferricyanide could improve power outputs, although the system was no longer sustainable.

Among the three studies, I first introduce that done by Strik et al. (2008). In that study, Reed mannagrass (*Glyceria maxima*), also named Reed sweetgrass, was used, because it is an abundant grass species in America, Europe, and Asia, and it is one of the few local species in the Nederland that can grow in anaerobic freshwater sediments. These anaerobic conditions were considered necessary for an anode compartment to function. To start a pot-culture sMFC, an anode chamber was filled with graphite granules that contained microorganisms pre-grown on acetate under electricity-generating conditions and inoculated with Reed mannagrass. Graphite granules were used to fill the anode pot, because they were conductive and could serve as the plant support as well as electrode. The pot-culture sMFCs were operated for 118 days, and substantial increases in cell voltages (over 100 mV) were observed after day 50 only in MFCs inoculated with plants. This level of cell voltage was
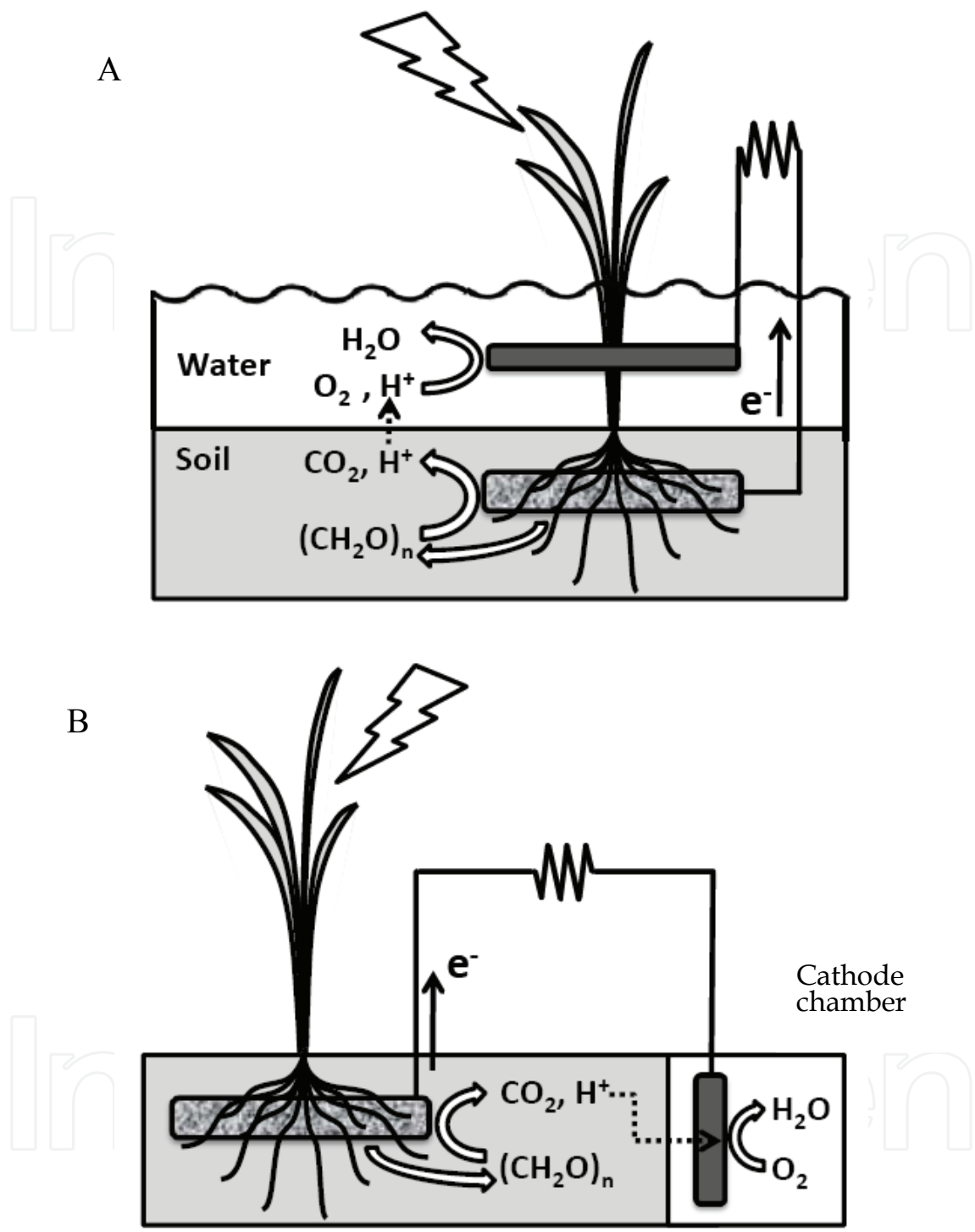
Fig. 3. Systematic diagrams for a rice paddy-field MFC (A) and plant pot-culture MFC (B).

continued for 50 days afterward, followed by an unexpected drop down to below 50 mV. Polarization-curve analyses (refer to Watanabe 2008 for methods) on day 90 estimated a maximum power density (per anode surface) of 67 mW per m^2. They deduced that the voltage drop resulted from the decline of plant vitality. They also reported that the cell voltage showed oscillatory behavior which was most pronounced between days 50 and 60 and was probably related to the daily light cycle. The photosynthates produced during the

day time may have led to increased exudate production and substrate availability in the MFC thereby increasing the cell voltage. The simultaneous daily temperature fluctuations of 4°C probably affected the microbial activity as well, and thus the cell voltage. From day 60 on, the electricity production continued at night, which means that the plant-MFC has the equivalent of a battery built into its system.

In their subsequent study (Timmers et al. 2010), the plant used was changed to a salt marsh species, *Spartina anglica*, and it was reported that electricity was generated for 119 days or more. A maximum power density was increased up to 100 mW per m^2 (geometric anode area); they described that it was the highest reported power output for a plant-assisted sMFC. Electrochemical analyses revealed that cathode overpotential was the main potential loss in the period of oxygen reduction due to slow oxygen reduction kinetics at the cathode. Ferricyanide reduction improved the kinetics at the cathode and improved electricity generation with a maximum of 254%. In the period of ferricyanide reduction, the main potential loss was transport loss of hydrogen between the anode and cathode. It is likely that a salt-rich condition for the growth of *Spartina anglica* was favorable for electron transfer in the MFC system, resulting in the increased electric output. It is therefore reasonable to deduce that marine sediment MFCs can be coupled to plants for getting more powers.

A study done by De Schamphelaire et al. (2008) used pot cultures of rice plants (*Oryza sativa* ssp. *indica*). In their pot-culture sMFCs, natural soils, vermiculites, or graphite granules were used as plant supports, graphite mats were used as anodes, and cathodes were set either as indicated in Fig. 3A (graphite rods floating in water above the plant support) or Fig. 3B (graphite granules set in cathode chambers filled with 100 mM ferricyanide). The primary aim of these experiments was to identify experimental settings that generate more electricity than others. They initiated pot-culture sMFCs experiments by inoculating the MFCs with microbes obtained from an acetate-fed MFC and those from a methanogenic tank. Besides, a cathode culture in pre-operated sediment MFC was also inoculated to the floating graphite-rod cathode. These MFCs were operated over 100 days, and they confirmed that plant inoculation substantially (typically 7 folds) enhanced electricity generation, when the plants were actively grew (in summer). Concerning plant support media, soil was the best among them in term of electric output. In contrast, there were no substantial differences in MFC performances between these two cathode settings, while, in some pots, the leakage of ferricyanide solutions into anode pots resulted in decreases in the plant vitality and electric output. Consequently, the highest power output achieved in that study was reported to be 33 mW per m^2 geometric anode area, equivalent to 330 W per ha, while it should be noted that this is not a value based on land area. They also reported that start-up periods of 50 to 100 days were needed to produce electric powers with the aid of living plants. They assumed that the time delay could be due to one or some of following reasons, such as the life cycle dependency of the exudate release, both qualitatively and quantitatively (Grayston et al. 1997), the omission of nutrients (which can induce exudation) (Marschener 1998), the release of oxygen, conducted through the aerenchyma (Neue et al. 1996), scavenging the electrons otherwise collected at the anode, and the lack of an adapted anodic microbial consortium. Another important point shown in that study was that there was a certain level of positive relationship between the concentration of organics in vermiculite support media and the electric output, suggesting that rhizodeposits (organics excreted from roots) were fuels used to generate electricity in the pot-culture MFCs.

In their subsequent study (De Schamphelaire et al. 2010), microbial communities established on anode graphite mats in rice pot-culture sMFCs containing vermiculite or natural soils as

support media were analyzed using molecular ecological tools (including, clone-library analyses, denaturating gradient gel electrophoresis [DGGE], and terminal restriction fragment length polymorphism [T-RFLP]) for the domain *Bacteria* and *Archaea*. They focused their analyses on microbial communities on anodes, because these were considered responsible for the generation of electrical current and hence fulfilled pivotal roles in MFCs. Furthermore, they compared community structures between open-circuit and closed-circuit MFCs to more clearly identify microbial species involved in electricity generation. As a result, they found that closing the electrical circuit, allowing a capture of electrons by the anode, resulted in clear shifts in the bacterial community of soil MFCs, while shifts were not clear in vermiculite MFCs; reasons for this phenomenon is not clear. It was also found that the electrical circuit also influenced the archaeal community, despite that the *Archaea* were less influenced than the *Bacteria* were. They concluded that closed-circuit anodes in potting soil were enriched with *Desulfobulbus*-like species, members of the family *Geobacteraceae*, and as yet uncultured representatives of the domain *Archaea*, suggesting that they were involved in electricity generation. Future studies will be performed to isolated and characterize bacteria and archaea that were detected by the molecular ecological analyses.

4. Rice paddy-field sMFC

As described above, rice paddy-field (RPF) sMFC is a type of plant-assisted sMFCs, and uses a real RPF rather than a pot-culture system. RPF-sMFC experiments have been performed only by our research group (Kaku et al. 2008; Takanezawa et al. 2010), and here we explain experimental settings and results obtained in these studies.

A paddy field is a flooded parcel of arable land used for growing rice and other semiaquatic crops. In Japan, rice paddy fields cover 2.5 million ha and occupy more than 50% of the total arable land areas (Ministry of Agriculture, Forestry and Fisheries 2006). When a paddy field is flooded, the soil immediately below the surface becomes anaerobic (Takai 1969), and a community of anaerobic microbes (comprised mainly of sulfate-reducing bacteria, iron-reducing bacteria, fermenting bacteria and methanogenic archaea) is established (Grosskopf et al. 1998; Chin et al. 1999). Since a potential gradient is known to be formed between the soil and the flooded water, it was anticipated that an sMFC system could operate in a paddy field.

In order to demonstrate this idea, electrodes were set in RPF as presented in Fig. 3A concomitant with the transplantation of rice seed cultures (in May 2007) (Kaku et al. 2008). In the experiment in 2007, *Oryza sativa* L. cv. Sasanishiki was used as a rice plant, while the experimental RPF in the university farm of Yamagata University (Tsuruoka, Japan) was the experimental field. Anode and cathode electrodes were made of graphite felts without any modification. Monitoring of the cell voltage (the potential difference between the anode and cathode) was initiated immediately after the electrodes were set in the RPF, and an electric output of approximately 0.05 V was immediately detected after that, which then increased to more than 0.2 V (measured in the daytime) within 7 days. It was also found that the electric output increased in the daytime and decreased at night. These observations implied that the electric output was dependent on the sunlight. Such circadian oscillation was observed throughout the experiment. A similar trend of electric output was also observed in experiments performed in 2008 using a RPF of the Kanagawa Agricultural Technology Center in Hiratsuka, Japan (Takanezawa et al. 2010). A typical trend was presented in Fig. 4,

in which the experiment was initiated in June. In this case, the increase in electric output was somewhat slow compared to that observed in the 2007 experiment, while the clear circadian oscillation was also observed. The difference in the start-up period was likely ascribable to differences in field manipulations, nutrients in soil, and microorganisms in soils. The oscillation suggested that sunlight affected the electric output, although effects of temperature should have also been considered. This is because we also found that the electric output was influenced by whether and temperature. In the 2007 experiment, the polarization-curve analysis in June and August showed that the RPF sMFC could generate the power as much as 6 mW per m^2 anode geometric area. This value was low compared to those reported for the pot-culture sMFCs. This was in part because the anode was large in area and covered not only rice rhizospheres but also soils between rice hills.
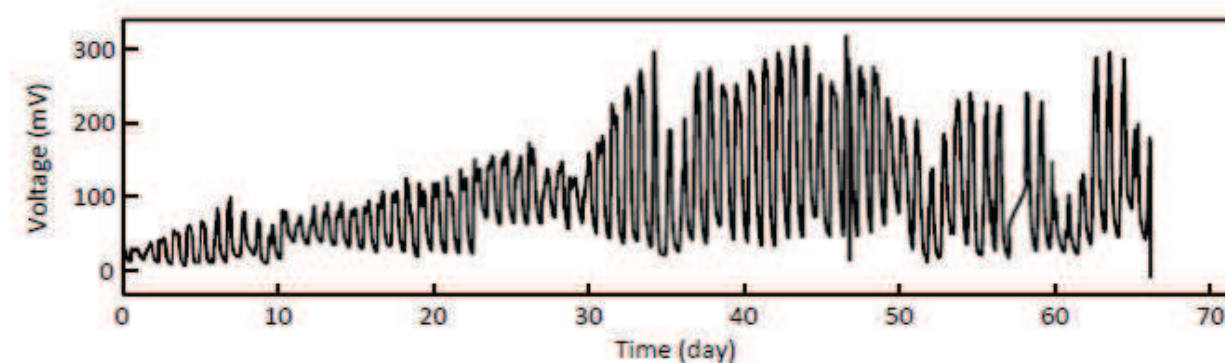

Fig. 4. A trend of electric output from an RPF sMFC set in the Hiratsuka field in 2008, showing the presence of a circadian oscillation in the electric output.

In order to examine how sunlight influenced the electric output (in August, 2007), we shaded either rice plants or the surface of the cathode and then monitored the electric output (Kaku et al. 2008). This experiment revealed that the shading of rice plants largely reduced the output, while there was no significant effect when only the cathode surface was shaded. This result suggested that plant photosynthesis enhanced the electricity generation. Nest, we examined if sunlight could stimulate rice plants to exhaust photosynthesized organic compounds from their roots (Kaku et al. 2008). It has been reported that plants exhaust photosynthesized organic compounds from their roots (Jones 1998; Walker et al. 2003), and organic acids and sugars are the main components (Jones 1998). In the experiment, rice plants were pulled from the paddy field and incubated them under the light and dark conditions by soaking their roots in water supplemented with the antibiotics. Total organic concentration (TOC), organic acids, and sugars in the root-soaked water were analyzed 2 h after commencing the incubation and compared with values at hour 0. It was found that the TOC value increased only under sunlight, indicating that sunlight largely enhanced the amounts of organics exhausted from the roots. Among the organic compounds analyzed, acetate was the most abundant, followed by glucose, and their production was also found to be sunlight dependent. Since we also found that rice roots penetrated the anode graphite felt and occasionally entwined with the graphite fibers, it is reasonable to conclude that these organics can serve as fuels in PRF sMFCs.

In MFCs, bacteria are considered to be responsible for electricity generation (Logan and Regan 2006; Lovley 2006). In the 2007 experiment (Kaku et al. 2008), bacterial populations

attaching onto the anode and cathode of the RPF sMFCs were analyzed by DGGE of PCR-amplified 16S rRNA gene fragments (refer to Watanabe et al. 2001 for methods). The DGGE profiles were compared with those of bacteria associated with the graphite felts in the control system (electricity not generated) and also those in soil, allowing us to identify bacteria that specifically occurred in response to electricity generation. This electrophoresis analysis detected several intense bands that specifically occurred on the anode or cathode of the power-generation system; sequence analyses revealed that bands from the anode were related to *Natronocella acetinitrilica* (Sorokin et al. 2007) and *Rhizobiales bacterium* A48 (Satoh et al., 2002), while that from the cathode was related to *Rhodobacter gluconicum* (Do et al. 2003). Among them, A48 was isolated from a rice root surface (Satoh et al., 2002), and its taxonomy has recently been described as the genus *Rhizomicrobium* gen nov. (Ueki et al. 2010). In addition, closely related bacteria were detected in laboratory MFCs operated with cellulose as the sole fuel (Ishii et al. 2008a; Ishii et al. 2008b). It is therefore considered that bacteria related *Rhizomicrobium* was involved in the electricity generation. Information concerning roles of other bacteria detected by the DGGE analysis is to date not sufficient.

All together, these results suggest the RPF electricity-generation system was an ecological solar cell in which the plant photosynthesis was coupled to the microbial conversion of organics to electricity. In our next study (Takanezawa et al. 2010), we identified factors affecting electric outputs from RPF sMFCs. According to that study, in the next section, we discuss factors that can affect electric outputs from RPF sMFCs.

5. Factors affecting electric outputs from rice paddy-field sMFCs

5.1 System configurations

In the study done by Takanezawa et al. (2010), sMFC systems were set under different experimental conditions (Table 1) based on our knowledge on laboratory MFC reactors, and power outputs from these RPF sMFCs were evaluated by the polarization-curve analyses. Among these conditions (Table 1a), a number of anode felt was changed (one versus five [vertically aligned]; compare conditions 1 and 2), since it had been considered that more organic exudates from roots could be utilized for microbial anode respiration, if the roots were contacted with more anode felts. The anode position (a depth of the anode) corresponded to a distance between the anode and cathode that was placed at the surface of the soil (conditions 1 and 3); an anode/cathode distance is known to influence MFC performances, since it affects the proton diffusion from the anode to cathode (Cheng et al. 2006). We also examined several different external loads (conditions 1, 5, and 6), since it has been reported that external loads influenced the electric output from laboratory MFC reactors (Jadhav, G.S. & Ghangrekar 2009). In these experiments, it was found that the anode depth and external load largely affected the electric output, while the anode number did not (Table 1). Among them, the result for the anode/cathode distance was unexpected, since it suggests that the proton-transfer efficiency (from the anode to cathode) did not limit the electric output. Two possible reasons are conceivable for the high performance with the anode at 5 cm in depth: (i) a zone at the depth of 2 cm was not sufficiently anaerobic, resulting in the presence of oxygen that served as an alternative electron sink; (ii) more rice-plant roots could access to the anode at 5 cm in depth than that at 2 cm, resulting in the larger amounts of organics supplied for the anode at 5 cm. We consider that the second possible reason is more likely, since oxidation/reduction potentials (vs. a standard

hydrogen electrode) for zones at 2 cm and 5 cm in depth were not largely different (-158 mV and -165 mV, respectively).

Condition	Anode GF-felt number	Anode depth below the soil surface (cm)	External resister (Ω)	Maximum power (mW per m^2)
1	5	2	100	3.52
2	1	2	100	3.96
3	5	5	100	9.82
4	5	2	100	2.32
5	5	2	10	2.16
6	5	2	1000	14.44

Table 1. Experimental conditions of RPF sMFCs to identify factors affecting electric outputs.

5.2 Electrode materials

Studies on MFC reactors have indicated that electrode modification, both for anode and cathode, substantially improves MFC performances (Watanabe 2008). It is therefore reasonable to deduce that electrode modification is also beneficial for RPF sMFCs. In our study (Takanezawa et al. 2010), it was found that the modification of cathode with platinum catalysts (originally developed for hydrogen fuel cells) largely improved the electric output. This is in accordance with previous studies showing that the catalyst can largely accelerate oxygen reduction (Lefebvre et al. 2008).

A substantial amount of studies have been done to modify MFC anodes (Watanabe 2008), and these studies have suggested that the surface area and electrochemical properties are important factors for efficiently capturing electrons released by microorganisms. To cite an instance, Zhao et al. reported that the modification of graphite anodes with nano-porous conductive polymers substantially (~10 folds) improved the electric outputs from laboratory single-chamber MFCs (Zhao et al. 2010). In another example, carbon electrodes were coated with a composite of conductive polymers and carbon nanotubes and successfully used for MFC anodes (Qiao et al. 2007). It should however be noted that it is difficult to sustain improved anode performances for a long time, since these materials are not sufficiently resistant to microbial attacks (Niessen et al. 2004). In some cases, such as organic polymers, microorganisms use these materials as substrates for their growth. This would be particularly true in RPF sMFCs, since the soil contains very diverse microorganisms with a wide variety of catabolic abilities. It is desired to develop materials that are electrochemically active and persistent in rice paddy-field soil.

5.3 Root exudates

On average, 30-60% of photosynthesized organics are allocated by plants to roots, and a substantial portion of these organics are released from or secreted by roots into the rizosphere (Marschner 1995). The RPF sMFC experiments indicates that the electricity generation was dependent on root exudates (Kaku et al, 2008), suggesting that better understanding of root exudates will allow RPF sMFCs to function more efficiently. In

addition, it is also necessary to understand effects of allelopathic compounds produced by rice roots (Olofsdotter et al. 2002) to electricity generation by rhizosphere bacteria.

5.4 Microbes in rhizosphere

A large amount of studies has been carried out concerning microorganisms inhabiting RPF soil (Liesack et al. 2000), particularly those associated with rice rhizosphere. There may be two reasons for the active research on RPF microorganisms. First, they are considered to influence (positively and negatively) the rice production. Second, these microorganisms are responsible for the methane emission from RPFs; methane is known to exert strong green house effects (Prinn 1994). Since these studies have revealed that RPF soils harbor surprisingly diverse microbial species, we deduce that a variety of bacteria capable of transfer electrons to anodes may also be present there. Actually, MFCs were successfully developed using RPF soils as inocula (Ishii et al. 2008a; Ishii et al. 2008b). It is also important to note that the electric output was relatively rapidly (within 7 days) increased in our 2007 experiment (Kaku et al. 2007), while the pot-culture sMFC experiments needed substantially longer times (~50 days) despite that these sMFCs were inoculated with microbial communities sufficiently acclimatized in MFCs (Strik et al. 2008; De Schamphelaire et al. 2008). This fact supports the idea that PRF soils harbor substantial amounts of electricity-generating bacteria that adapt to the soil environment. A bacterial strain was isolated from RPF soil which is capable of electricity generation in pure-culture MFCs (Kodama & Watanabe 2008), and a novel species (*Rhizomicrobium electricum*) has been proposed for this bacterium (Kodama & Watanabe 2010). This organism is the first isolate of electricity-generating bacteria inhabiting RPFs, and further studies on this organism will provide valuable information concerning the physiology and genetics of microbial electricity generation in the rhizosphere.

6. Conclusions

The RPF sMFC is a representative of BEC processes that clearly demonstrates the concept of self-sustainable biological systems. The fact that rice is the world's most important agronomic plant with 143 million ha under cultivation globally suggests a great possibility for the future contribution of the RPF sMFC to global energy issues. In addition, similar systems may also be applicable to wetland ecosystems. Besides, it is also important to suggest that the sMFC systems may possibly reduce methane emission from rice paddy fields and natural wetlands. This is currently under investigation in our laboratory.

RPF sMFCs are also interesting from the scientific viewpoint, since the electric output enables the real-time monitoring of ecosystem functions in the rhizosphere; for instance, the circadian oscillation of root deposition was for the first time observed using the RPF sMFC system (Fig. 4). This would leads to deeper understanding of ecological interactions between plants and rhizoshere microorganisms.

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Paths to Sustainable Energy

Edited by Dr Artie Ng

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