

Chapter

Catalyst Development of Microbial Fuel Cells for Renewable-Energy Production

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Abstract

In this chapter, we focus on microbial fuel cells (MFCs) that convert the energy from organic matters into electrical energy using microorganisms. MFCs are greatly expected to be used as a relatively low-cost and safe device for generating renewable energy using waste biomass as a raw material. At present, however, it has not reached the desired practical application because of the low-power generation; hence, improvements on fuel cell efficiency, such as electrode materials, are still being examined. Here, we focus on the microorganisms that can be used as catalysts and play a central role in improving the efficiency of the fuel cells. Several kinds of microbial catalysts are used in MFCs. For example, *Shewanella oneidensis* has been well studied, and as known, since *S. oneidensis* transports the electrons generated within the cell to the surface layer, it does not require a mediator to pass the electrons from the cells to the electrode. Furthermore, *Escherichia coli* and *Saccharomyces cerevisiae*, a model organism for MFCs, are also used. The improvements of such microbial catalysts have also been proceeding actively. Here, we elaborated on the principle of MFCs as well as the current situation and latest research on the catalyst development.

Keywords: microbial fuel cell, MFC, catalyst, renewable energy, bioelectrochemical device, microorganism, biomass

1. Introduction

The fossil fuel depletion and inevitable global warming have become worldwide problems; thus, significant efforts have been made to generate and utilize renewable energy to alleviate these crises. Methods for obtaining energy compounds from biomass, such as ethanol, methane, and hydrogen, have been developed using environment-friendly technology, and some of these technologies have been put to practical use. It is important to establish the technologies that are able to obtain energy in various forms according to the environments and circumstances of each region. Apart from the above technologies, biofuel cells utilizing microorganisms and enzymes, which can generate renewable electrical energy from organic matters contained in biomass, begin to attract attention as a means to obtain sustainable energy. It has not been put into practical use yet, but without the problem of by-products, electricity can be directly obtained from the devices, whereby multiple operations, such as product distillation (e.g., ethanol), are not necessary. Moreover,

if biomass waste is used as the fuel, no food competition will occur. Therefore, using this method, energy can be obtained sustainably (**Figure 1**).

There are various types of biomass, e.g., sustainably harvested wood, waste paper, food waste, sewage sludge, and various wastewaters. Taking wood-based biomass as a fuel example, when everything is burnt using available technology for thermal power generation, there will be nothing left, and we will lose some other useful compounds contained in it. On the other hand, in biofuel cells, although electricity is generated from the sugar obtained from the biomass, other components in the wood, such as lignin, can be used for purposes other than power generation. Generally, the energy density of the biomass used as a fuel for MFCs is high. For example, glucose and xylose, found in various plant biomass, can produce up to 20 or 24 electrons per molecule, provided that they are completely oxidized to carbon dioxide. It is possible to generate 4430 Wh power per kg of glucose according to the calculation described later. For reference, a typical lithium-ion battery has a weight energy density of about 200 Wh per kg. This comparison means that glucose and xylose are two biofuel sources of interest, especially as electron donors. Therefore, MFCs using glucose and/or xylose as their fuel have great potential as a means of obtaining high energy.

In biofuel cells, biological reactions are used for the oxidation reaction of biomass, and they are divided into two based on the type of catalyst used: (1) enzymes and (2) microorganisms. When enzymes are used, the most widely studied mechanism is the two-electron oxidation system by glucose oxidase (GOx) or glucose dehydrogenase (GDH) [1]. Since purified enzymes are generally used, the reaction rate is faster than using microorganisms. However, the number of electrons obtained by one enzymatic reaction is smaller than that of a microorganism. For example, when GDH is used as the catalyst, glucose is oxidized to gluconic acid, and at most, only two electrons are obtained from one glucose molecule. Therefore, if only one enzyme is used, high-energy production per glucose consumed cannot be much expected. Further, the addition of cofactors, such as nicotinamide adenine dinucleotide and pyrroloquinoline quinone (PQQ), is necessary for the enzymatic reaction to enhance the energy production. Furthermore, the cost of enzyme purification is also high; hence, enzymes are better utilized in sensor applications than energy production. By contrast, as mentioned above, one completely oxidized glucose molecule gives 24 electrons when using microorganisms as the catalyst. It

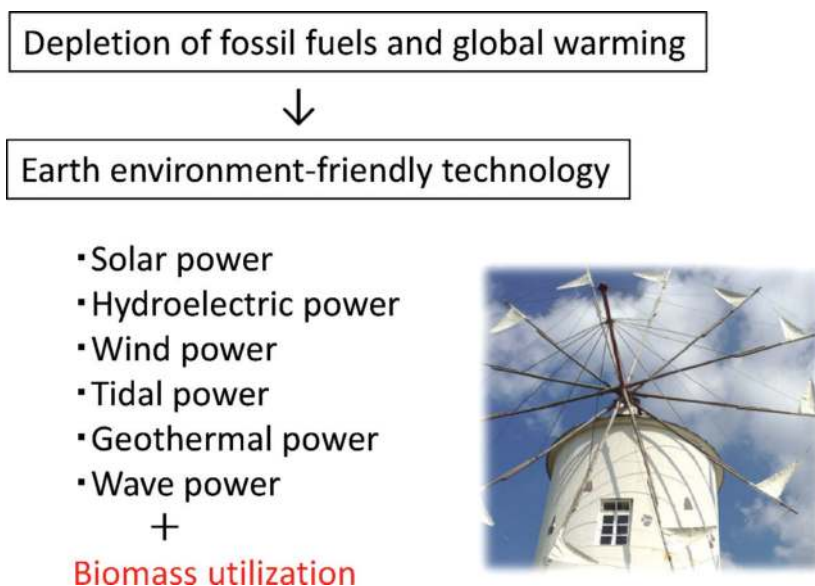


Figure 1.
Environmentally friendly energy.

shows a possibility of obtaining more electrons per glucose consumed. Moreover, the addition of cofactors is not necessary, unlike when enzymes are used. These are some substantial advantages of MFCs. However, the production of low power in MFCs is still a problem because of typical processes in living organisms, such as the uptake of glucose into cells, metabolism repression, and extraction of electrons from the inside of cells (**Figure 2**). Many researchers are working to solve such problems, and those results are summarized in recent review articles [2, 3].

Looking back at the historical background, research on the MFCs has been conducted for a long time, whereby the first idea of using microorganisms to produce electricity was conceived and reported by Potter in 1911 [4]. *Escherichia coli* and *Saccharomyces cerevisiae* were used as the catalyst, and platinum was used as the electrode. Further, in 1931, Cohen showed that a number of small fuel cells connected in series produced 2 mA of electricity at over 35 V [5]. Early MFCs used an artificial mediator, e.g., thionine, methyl viologen, and humic acid, to carry electrons from the microbial cells to the electrodes [6, 7]. The oxidized mediators came into contact with the microbial cells, and were reduced by accepting electrons, and they were then separated from the microbial cells. They diffused and came into contact with the surface of the electrode to release the electrons and were reoxidized thereafter. Overall, the addition of artificial mediators promotes the flow of electrons. Next, in the 1990s, several bacteria were found capable of acquiring electrons from the electrode via a self-mediator without the addition of an artificial mediator. Moreover, they used electrons for their growth; for example, a ferric-iron-reducing bacterium *Shewanella putrefaciens* grew on lactate by obtaining electrons from the electrode [8], and similar reports were found on *Shewanella oneidensis* and *Geobacter sulfurreducens* [9, 10]. Although the flow direction of electrons between cells and electrodes is opposite from the MFC explanation just before, these findings led to the development of mediator-free MFCs [11–13]. In the 2000s, the mechanism at the cell surface whereby bacteria directly came into contact with the electrodes and carried the electrons was reported [14, 15]. Since then, MFC research, including the analysis of the electron transport mechanism at the cell surface, has been actively conducted. In fact, the number of publications related to the MFCs grew significantly since 2010 and reached nearly 1000 in 2016 and 2017 according to ISI WEB OF SCIENCE [2]. In particular, with the discovery of new fuel cell components other than the microbial catalysts, the performance of MFCs could be increased further.

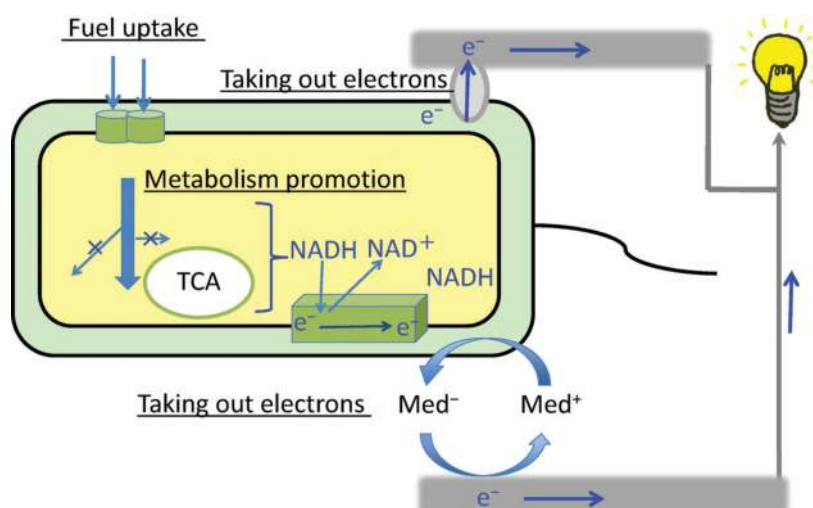


Figure 2.
Electron generation and extraction in the microbial fuel cell system. Med: mediator.

The performance of MFCs is evaluated based on some indicators. The electrical energy (Wh) used to express the capacity of dry batteries is also an important indicator, but only a few papers have reported it so far. In most cases, the performance is expressed as the maximum power per anode electrode area (power density per area) or the maximum power per cell volume (power density per volume). The latter is a straightforward index and important for practical use. For example, a relatively high-performance small-scale fuel cell (2.5 mL) using a complex (mixed) microorganism system was reported in 2007 with a power density of 1550 W/m^3 [16]. Other fuel cells performing beyond 500 W/m^3 were also reported [17–22], but many of them are still at a microliter or milliliter scale. Owing to the low proton diffusion speed and high internal resistance, the maximum power per volume tends to be small for a large-scale fuel cell. MFCs with a volume more than 1 L were also being studied in the laboratory, but the maximum power per volume was still low at the level of several W/m^3 to tens of W/m^3 [23, 24]. Scaling-up is also another issue of MFCs, and further improvements are still being conducted.

Practical applications of MFCs are still problematic because of the high cost and low-power generation. Despite this situation, research on the implementation of MFCs has been carried out. For example, an artificial stomach called Gastrobot (aka Chew-Chew train) using *E. coli* and sugar as the catalyst and fuel, respectively, EcoBot-III (a self-sustainable robot with its own circulatory system, such as ingestion, digestion, and excretion), and several environmental sensors using the MFCs for powering [2]. Such implementation studies are also important to understand the desired performance level for MFCs. Meanwhile, in order to bring MFCs closer to practical use, a combination of power generation and other effects is one of the promising methods. For example, MFCs that are installed at a wastewater treatment facility are expected not only to reduce the generated sludge amount from the treatment but also to cover a part of the electricity load used by the plant. Recently, the minimum performance of MFCs required for reaching energy neutrality in a wastewater treatment facility has been calculated [25], and the realization has been highly expected. Such research on MFCs installed for wastewater treatment has been actively carried out so far, and the experiments were examined at a pilot-scale plant of more than 10 L [26–28]. Accordingly, the practical use of MFCs is expected soon. Besides this, although it deviates from energy production, the use of MFCs as a sensor has also been extensively studied. In order to perform on-site real-time monitoring, it is important to recognize the toxic compounds rapidly. Several analytical techniques based on electrochemistry have been developed for this purpose, but in many cases, they lack practicality for environmental measurements. The MFC-based biosensor is one promising candidate, and it has already been shown that not only toxic heavy metals but also toxic organic compounds can be detected [29]. The sensitivity adjustment suitable for the detection of specific contaminants is left as a challenge, but there is still a great expectation for its application.

Here, we will explain the mechanism of electron generation in microorganisms, introduce the principle of MFCs, describe the microbial catalysts used for various MFCs mentioned above, and discuss the recent topics on microbial catalysts.

2. General principles of MFCs and various microbial catalysts

2.1 Mechanism of electron generation in microbial cells

MFCs utilize the decomposition energy of organic matters by the organisms to produce ATP, known as the energy currency, based on the energy obtained from

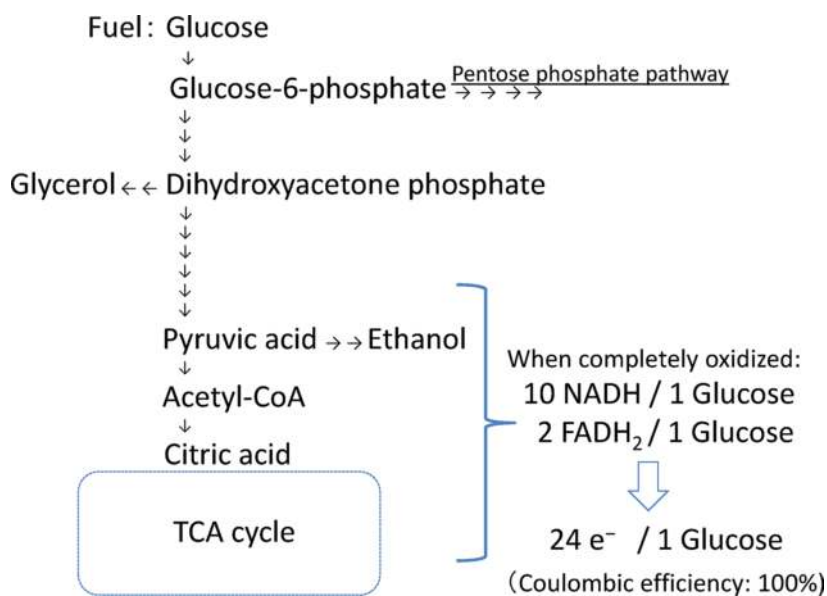
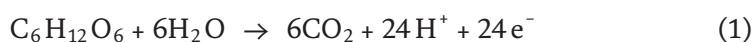


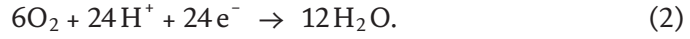
Figure 3.
 Glucose metabolism (*Saccharomyces cerevisiae*).

this process. Taking glucose decomposition in *Saccharomyces cerevisiae* as an example (**Figure 3**), glucose taken into the microbial cells by cell membrane enzymes is oxidized and decomposed to pyruvic acid by various enzymes via an intracellular glycolysis system. Next, pyruvic acid becomes carbon dioxide and water when it is completely oxidized via the TCA cycle. The electrons generated are then collected in the mitochondrial inner membrane in eukaryotes, and in prokaryotes, they were accumulated in the cell membrane via NADH and FADH₂ (both of which provide two electrons to the membrane electron flow; **Figure 3**). In addition, the flow of electrons in these membranes is maintained through multiple protein complexes localized in the membrane. Quinone compounds and cytochrome proteins are also included in the complex. ATP is synthesized by the membrane enzyme, ATP synthase, using the proton concentration gradient, which is generated inside and outside the membrane because of the flow of electrons. In MFCs, it is thought that it takes the electrons directly from NADH or the flow of electrons generated in the membrane by the decomposition of organic substances. A part of the electrons generated within the microbial cell is carried to an electrode outside the cell via an electron carrier called a mediator. When one molecule of glucose is completely oxidized into carbon dioxide and water in the cell, 10 molecules of NADH and 2 molecules of FADH₂ are also generated. In total, 24 electrons are obtained from 1 glucose molecule. If this principle were to apply to fuel cells, the Coulomb efficiency, which is an index of energy efficiency, would become 100%. Therefore, in order to obtain electrons more efficiently from the cells via such metabolism, it is important to adjust the redox balance within the microbial cells in the MFCs.

2.2 Calculation of the energy obtained from glucose

In the case of using glucose as the fuel source, the reaction occurring in the anode tank is represented by Eq. (1), and the reduction reaction occurring in the cathode tank is represented by Eq. (2).





The oxidation-reduction potential of Eq. (1) is -0.42 V , whereas the oxidation-reduction potential of Eq. (2) is 0.82 V . Therefore, the total potential difference of the MFC reaction (Eq. (1) + Eq. (2)) as represented by Eq. (3) is 1.24 V . Theoretically, the voltage exceeds 1 V , but in most cases, it has never reached that value.



Assuming that 24 electrons are obtained from 1 glucose molecule and that they can be recovered in 1 h, the quantity of electricity (Ah) obtained from the glucose (1 kg) can be calculated using the Faraday constant ($96,485\text{ C/mol}$) as shown in Eq. 4. As a result, the electrical energy of 4430 Wh can be achieved if the potential is 1.24 V ; accordingly, this value is the same as the value mentioned in the Introduction section.

$$(24 \times 96,485 \times 1000) / (3600 \times 180) = 3574\text{ Ah}. \quad (4)$$

2.3 Basic components of dual-chambered MFCs using a mediator

A dual-chambered fuel cell consisting of an anode tank and a cathode tank is the simplest and has been used for a long time for MFCs. In many cases, they are separated by a cation exchange membrane (CEM) to create a potential difference between the two tanks (**Figure 4**). CEM prevents mixing of each content and allows the protons generated in the anode to migrate to the cathode. In addition, CEM selection, especially based on its proton transfer efficiency, is important

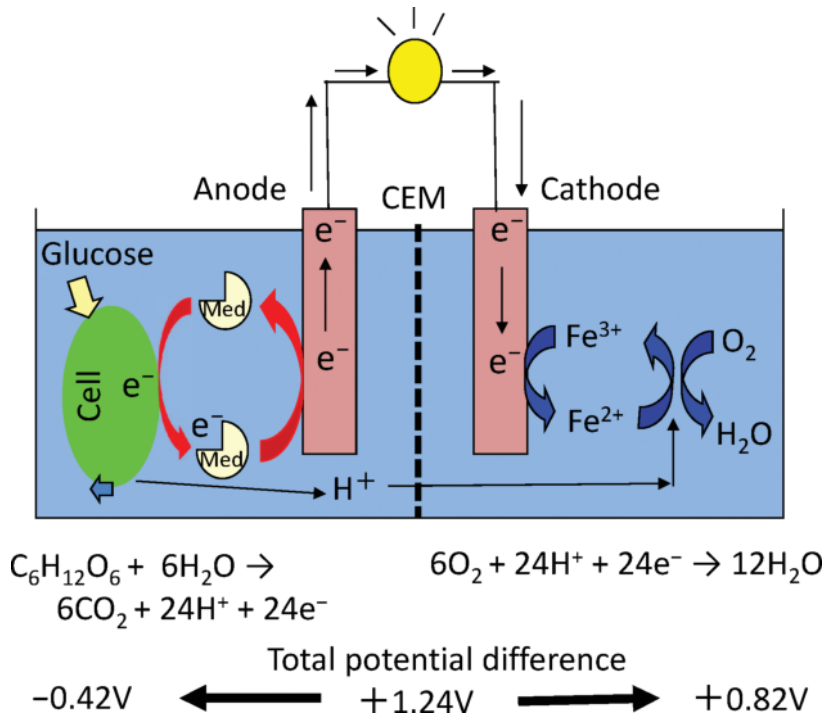


Figure 4. General dual-type MFC. Med: mediator, CEM: cation exchange membrane.

because it significantly regulates the movement of the protons responsible for the pH reduction at the anode affecting the activity of microorganisms and the delivery of electrons to the oxygen at the cathode. Also, some factors to consider, such as durability and cost, are important for selecting CEM. At present, Nafion is popular for many MFCs [30, 31].

Numerous research studies are being conducted to evaluate the influence of the electrode materials on the performance and cost of the MFCs. Carbon materials, which are noncorrosive, have been widely used because of their high electrical conductivity and chemical stability, e.g., carbon rod, carbon fiber, carbon felt, and carbon cloth [3]. Biocompatibility, specific surface area, electrical conductivity, and cost are important factors for its selection. Since its discovery in 2004, graphene has been attracting much attention for its use as an electrode because of its high specific surface area, electrical conductivity, and biocompatibility [32]. In fact, graphene has been already used in lithium-ion batteries, and the development of graphene-modified materials to increase the power density has progressed actively [33, 34]. Moreover, since biofilm formation by microorganisms on the electrodes affects the performance of MFCs, the preference of electrode materials tends to shift from two-dimensional to three-dimensional surfaces, where a larger surface area is obtained; thus, the contact with microorganisms increases. Furthermore, metals are also used as the electrodes. The conductivities are higher than those of carbon materials, but they are prone to corrosion in the anode solution. Therefore, metals are problematic to use, except for stainless and titanium. To improve such problem, materials in which metal is incorporated into graphite have been made [3].

A phosphate buffer or bicarbonate buffer solution is often used for the anode electrode solution to achieve high performance [16, 35]. The pH of the solution affects not only the activity of microorganisms but also the transfer of hydrogen ions used from the anode to the cathode when the electrons are transferred to oxygen at the cathode. The solutions contain microorganisms as the catalyst, organic matter as the fuel, and mediator as the electron carrier. In addition, there are reports that the performance of MFCs was improved by adding NaCl to increase the ionic strength [36].

Regarding the fuel, many substrates have already been studied [37]. For example, acetic acid, lactic acid, glycerol, glucose, xylose, sucrose, starch, yeast extract, malt extract, various real wastewaters, and synthetic wastewater were used depending on the purpose of each research. Generally, the fermentable substrate of microorganisms is used to generate electricity more efficiently. There is a trend where glucose is used when using *S. cerevisiae*, lactic acid when using *S. oneidensis*, and acetate when using *G. sulfurreducens* in the experiments. On the basis of a calculation, when lactic acid, acetic acid, and glycerol are completely oxidized, there are 14, 8, and 14 electrons obtained, respectively. The number of electrons obtained from each substrate depends on the metabolic pathway.

Regarding the mediator, although some microorganisms can carry electrons directly to the electrode as described later, in many cases, the electrons cannot be carried, or the performance is low even if carried, so an artificial mediator that can pass through the cell membrane is added to the anode solution. The typical compounds for artificial mediators are methylene blue, neutral red, 2-hydroxy-1,4-naphthoquinone (HNQ), thionine, benzyl viologen, 2,6-dichlorophenolindophenol, and various phenazines [38]. It was reported that the hydrogenase donates electrons to the neutral red [39], but the process was not yet clearly proven as to how these mediators deprive electrons of the cell. It is thought that, depending on the type of mediator, the electrons may be taken directly from NADH or obtained from the electron transfer system of the cell membrane. On the other hand, there is also a difficult aspect of using a mediator. In order to increase the electron transfer

efficiency by the mediator, it requires a high concentration, but because of its high toxicity, it has a strong influence on the cells; therefore, the level of use is necessary to be controlled.

Finally, the cathode solution is explained as follows. The electrons generated at the anode are carried to the cathode, where the reduction reaction takes place. When oxygen, the most common electron acceptor, is used as an oxidizing agent, aeration is necessary because oxygen has low solubility (about 8 mg/L DO). There are cases where oxygen generation by the photosynthesis of algae is used for oxygen supply [40, 41]. In the reaction at the cathode, H_2O is produced by oxygen, whereby the electrons were carried from the anode via an external circuit and protons were carried from the anode solution via CEM. There is also a report that the addition of hydrogen peroxide leads to an improvement in power generation [42]. Besides oxygen, there are various electron acceptors [43]; for example, an oxidizing agent such as iron ferricyanide is also used for the cathode. In many cases, the ferricyanide has a high mass transfer efficiency and a high cathode potential so that a high output can be obtained. The combination of carbon electrodes and ferricyanides to achieve power 50–80% higher than the combination of Pt/carbon electrodes and oxygen was reported [44]. In the case of using ferricyanide, once the trivalent iron ion receives the electrons, it becomes divalent, and when it delivers the electrons to oxygen, it reverts to the trivalent state. However, the latter reaction is less likely to occur owing to the low solubility of oxygen. Ferricyanide is an excellent electron acceptor, but owing to its toxicity, its use is generally limited to the laboratory. Other than oxygen and ferricyanide, there are also many candidates, for example, nitrate, persulfate, permanganate, and manganese dioxide. It is also possible to use the nitrate contained in the wastewater because its redox potential is close to that of oxygen, and then, the nitrate is reduced to nitrogen gas at the cathode [43].

2.4 Other types of MFCs

MFCs are typically divided into a dual-chambered cell described above and a single-chambered cell (Figure 5). In the latter, a membrane-type positive electrode with oxygen permeability called an air cathode is used [45]. The electrode is coated with the platinum catalyst, and H_2O is produced from the oxygen permeated from the atmosphere, the proton in solution, and the electron from the anode. However,

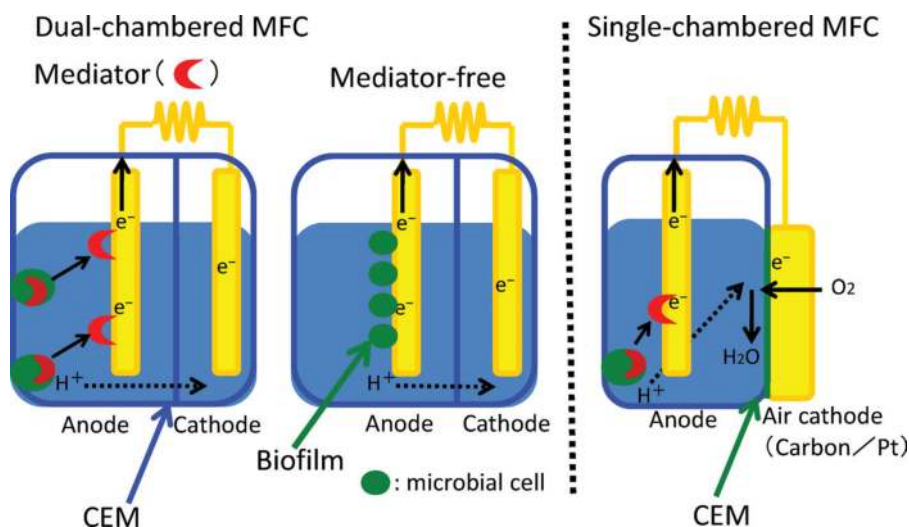


Figure 5. Other types of MFCs. Med: mediator, CEM: cation exchange membrane.

if the permeated excess oxygen diffuses and is delivered to the microorganisms at the anode, the electrons generated in the microorganisms are then transferred to the oxygen, and the energy recovery rate decreases. Therefore, a CEM is used between the anode solution and the cathode in order to prevent a decrease in energy recovery. This single-chambered type has already been widely used currently.

Moreover, there is a mediator-free type that does not require an artificial electron compound [46]. The microbial strains used here can synthesize mediators themselves and/or have an electron transfer function on the cell surface. In the former, the self-synthesized mediators are flavin compounds, hydroquinone, and phenazine that are able to transfer the electrons to the electrode. In the latter, membrane-bound proteins such as pili, c-type cytochromes, and filaments are known as cell surface structures that can directly transmit electrons. The biofilm formation on the electrodes, namely, biocompatibility of the electrodes, is also important for power generation via such direct electron transfer. Therefore, research on electrodes promoting the formation of the biofilm is actively being conducted.

In addition, with the use of MFCs in wastewater treatment, contamination on the CEM results in reduced power generation; hence, membrane-free MFCs have also been studied [46].

2.5 Various microbial catalysts

Various microorganisms have been studied for a long time since the first experiments on *S. cerevisiae* and *E. coli* [4]. The classification of these catalysts is largely based on the purity and complexity of the cultured microbial systems. Many different microorganisms are used in the pure system [37, 38, 47, 48]. *S. cerevisiae* is a safe microorganism used in foods and can grow even in the presence of a high concentration of sugar, sulfate, and ammonium nitrogen. MFCs show high performance when using *S. cerevisiae* and glucose as a catalyst and a fuel, respectively [43]. *E. coli* can also ferment sugar well and is used for the study of MFCs using glucose as a fuel. Although it can generate electricity without a mediator, in the present situation, the power generated is low, so an artificial mediator is added in order to achieve better performance. Besides the two examples, there are also *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Rhodospirillum rubrum*, *Geothrix fermentans*, *Shewanella* species, *Geobacter* species, *Clostridium* species, and sulfate-reducing bacteria. The possibility of utilizing extremophilic microorganisms is also being studied [49], and to add a new perspective to power generation by MFCs, the utilization of photosynthetic bacteria at the anode is also examined [40]. One of the advantages of these MFCs is the elimination of carbon dioxide released into the atmosphere. Meanwhile, in complex systems, the use of various wastewater and waste sludge has been reported [25, 37, 46, 50]. Many studies on bacterial communities under the control of MFCs have been conducted using those aforementioned resources. It is thought that the bacteria belonging to the phylum Proteobacteria were involved in power generation [51, 52]. However, owing to the complexity of bacterial interactions, their contribution to power generation within these communities is not well understood yet.

In such a research situation, there are relatively many examples of research on *S. oneidensis* and *G. sulfurreducens*, and the details of their power generation mechanisms are being clarified. *S. oneidensis* can produce self-synthesized mediators, like flavin compounds. The strain has not only such a mediator but also an extracellular electron transport system involved in power generation. This system, present from the inner membrane to the outer membrane, plays a role in carrying the electrons to the extracellular receptors (i.e., the electrodes in this case) by contacting them directly. In particular, cell-surface-localized cytochromes (MtrC and OmcA) are

important components for the electron transfer [53]. On the other hand, *G. sulfurreducens* has electrically conductive pili, called nanowires, which can transfer electrons to extracellular electron acceptors on the cell surface [54]. *S. oneidensis* also has an electrically conductive structure similar to the pili, but its structure is different, whereby the membrane structure containing the cytochrome protein described above was raised [54]. In any case, it has been confirmed that electrons can be delivered via such protrusions. It is expected that new developments will be made in the future, such as introducing the genes related to these mechanisms into other species, especially model organisms, such as *E. coli* and *S. cerevisiae*.

3. Recent topics of microbial catalyst and future directions

3.1 Modification of microorganisms

The utilization of chemical and biotechnological techniques is important to modify the microbial cells as biocatalysts in the MFC system. Molecular biology approaches are effective tools to improve the performance of the biocatalysts for the desired system. In this section, recent topics about the approaches for microbial catalyst development are discussed.

3.1.1 Chemical treatment

Mediators and macromolecular catabolic enzymes, which are used for electron transfer and other metabolic activities, are abundant in the cytoplasm of the microbial cells used in the MFCs. However, it is not easy to transport the mediator molecules to the bacterial outer membrane so as to reach the electrode. The lipopolysaccharide (LPS) layer on the Gram-negative outer membrane is compact and nonconductive; thus, most microbial cells are nonconductive [55]. It was found that chemically perforated pores and channels on the cell membranes accelerated electron transfer, leading to an improved power output for an MFC using *P. aeruginosa* [56]. In their study, polyethyleneimine (PEI)-treated biofilm achieved a doubled power density (41 mW/m^2) compared to the control biofilms. The large pores and channels on the cell membrane created by PEI treatment promoted the diffusion of the self-produced mediators (pyocyanin and pyorubin) of *P. aeruginosa*. The modified cell membrane surface also promoted the adherence of microbial cells to the electrode, which further improved the electron transfer. This method was also applied to *E. coli* [57]. Recently, it has been reported that lysozyme treatment increased 1.75-fold of the MFC performance with *K. rhizophila* P2-A-5 [58]. Thus, chemical treatment is one of the important approaches to modify the microbial cells for the improvement of the MFC performance.

3.1.2 Biosurfactant production by gene modification

To increase the cell permeability of biocatalysts in the MFCs, Zheng et al. proposed a new approach by inducing the biosurfactant production based on a genetic modification [59]. It is true that the efficiency of membrane permeability can be improved with a biosurfactant, which ultimately increases the transport across the membrane. In addition, overexpression of the *rhlA* gene, which is responsible for rhamnolipid (a biosurfactant) production, was also conducted [60]. The biosurfactant directly influenced the overproduction of rhamnolipids from the electrical bacteria, such as *P. aeruginosa*. The electron transport across the membrane was greatly increased as the membrane permeability increased. The power output of the

MFC-catalyzed process by the biosurfactant-producing bacterium was enhanced up to about 2.5 times compared with the wild type.

3.1.3 Introducing the extracellular electron transfer pathway

The sparse availability of genetic tools in manipulating electricity-generating bacteria and the multiple overlapping pathways for extracellular electron transfer make it challenging to modulate electron transfer and/or introduce other functions of interest. In response to this challenge, several studies have taken the complementary approach of engineering portions of the extracellular electron transfer pathways into the well-studied industrial microbe *E. coli* [61]. In these studies, MtrCAB of *S. oneidensis* was successfully expressed in the *E. coli* cells, and the activity of these proteins was confirmed by the metal reduction. Although the introduction of MtrCAB permits extracellular electron transfer in *E. coli*, the low electron flux and the absence of growth in these cells limit their practical application. Recently, in addition to surface-localized cytochromes, it has been further confirmed that CymA, the inner membrane component of *S. oneidensis*, significantly improved the extracellular electron transfer rate or cell viability. This recombinant *E. coli* achieved current generation in an MFC system without the addition of mediators [62, 63]. Our research group is trying to develop an excellent *E. coli* biocatalyst for the anode in an MFC system based on the combination of engineering of central metabolism and introduction of extracellular electron transfer in the presence of an HNQ mediator.

3.2 Extremophilic microorganisms

An extremophilic microorganism thrives in physically or geochemically extreme conditions that are detrimental to most life on Earth. They thrive in extreme hot niches, ice, and salt solutions, as well as acid and alkaline conditions; some may grow in toxic waste, organic solvents, heavy metals, or several other habitats that were previously considered inhospitable for life. Extremophiles can be used to oxidize sulfur compounds in acidic pH to remediate wastewaters and generate electrical energy from marine sediment microbial fuel cells at low temperatures. The MFC performance of these extremophilic microorganisms has been well summarized in several review papers [49, 64]. In this section, the recent advances of MFCs using extremophilic microorganisms as catalysts are briefly introduced and discussed.

3.2.1 Acidophiles and alkaliphiles

An increase of cell voltage is seen at increasing anode pH because of the additional pH gradient representing a source of energy. The practical implication of an elevated cell voltage is that more energy can be gained from MFC systems at higher pH values. By contrast, operating the anode of MFCs at an acidic condition has an advantage that the protons will not cause diffusion limitations in the cathode compartment for the reduction of oxygen, and therefore, it will not limit the current production [65]. However, under a low-pH condition, the microbial cells have to maintain a near-neutral cytoplasm [66] which consumes a portion of the energy derived from the electron transport for other processes, such as proton export, that increases the anode overpotential, leading to decreasing power generation. At pH 2.5, *Acidiphilium* sp. isolated from the environment mediates a direct electron transfer from the glucose metabolism to the anode at a rate of 3 A/m² even in the presence of air [67]. This interesting strain produces extracellular polymeric substances and forms a biofilm between the carbon microfibers and in pores on the graphite rod surface [68].

Meanwhile, at high pH, the lower anode potential results in an increased cell voltage. In general, the anode becomes acidified during the MFC operation, and the cathode becomes more alkaline, followed by a reduced cell voltage and power output. *Pseudomonas alcaliphila* is capable of electricity production at high pH, excreting phenazine-1-carboxylic acid that acts as an electron shuttle during the oxidation of citrate [69]. An MFC has also been developed to treat food wastes that comprise 30–55% of all refuse in urban societies. The waste was first treated by anaerobic digestion, the resulting food waste leachate was amended with 100 mM NaCl, and then, electricity was generated in a pH 9 MFC that had a maximum of 63% Coulombic efficiency [70]. Recently, a tubular upflow MFC utilizing seafood processing wastewater has achieved a maximum power density of 105 mW/m² at pH 9 [71].

3.2.2 Psychrophiles and thermophiles

Temperature also has a major impact on the output of MFCs. It influences the activity of microorganisms, the electrochemical reactions, and the Gibbs free energy change of the reactions. There exists an optimum temperature for enzymes in the MFCs, and the electrochemical reaction rate increases with increased temperatures. A lower operating temperature adversely influences the output, start-up time, and substrate oxidation rate in the MFC system. This negatively impacts the MFCs for processes such as wastewater treatment because the streams are generally at low temperatures. However, the advantage of low temperature for the MFCs is that they typically produce higher Coulombic efficiencies [72, 73]. The microbial community was enriched from the anaerobic sludge at the anode of an acetate-fed MFC operated at 15°C with psychrophiles *Simplicispira* sp. and *Geobacter* sp. [74]. Another study at 5–10°C enriched the low-temperature microorganisms from the genera *Arcobacter*, *Pseudomonas*, and *Geobacter* [75]. One promising application of the low-temperature MFCs is that for low-power-consuming devices like sensors that are intended to last for an extended period of time.

On the other hand, the advantages of operating at high temperatures are higher microbial activity, better substrate solubility, high mass transfer rate, and lower contamination risk. An example of an improved current generation at a high temperature (60°C) is a marine sediment MFC that generated 209–254 mA/m² compared with 10–22 mA/m² at 22°C [76]. Recently, an MFC with a higher operating temperature (70°C) has generated 6800 mA/m² [77]. Furthermore, the hyperthermophilic MFCs were operated at above 80°C [78]. However, a negative point of thermophilic MFCs is higher rates of evaporation than the MFC system itself. Therefore, a continuous mode of MFCs was proposed to allow a replacement of the anolyte and catholyte [79].

In this section, recent topics of microbial catalysts for MFCs were introduced. There are two approaches in developing the microbial catalysts. One is the modification of existing microorganisms using chemical treatments or biotechnological techniques, including gene editing. The other is exploring new microorganisms from the environment, including extreme conditions. Although new findings and knowledge were obtained from both approaches, a drastic improvement on the MFC performance to achieve a paradigm shift has not appeared yet. In parallel with the improvement of microbial catalysts, the development of the fuel cell system, including the electrodes, was intensively studied to increase the output of MFCs. In particular, the application of graphene-modified electrodes [33] and the investigation of electron acceptors [43] have shown remarkable progress in the past decade. In order to actualize the practical use of MFCs, a synergistic impact from the combination of microbial catalyst and fuel cell system is essential.

4. Conclusion

This chapter focuses on the significance of MFC development, the historical background and fundamental principles of MFCs, and their recent developments, especially on microbial catalysts. MFCs have not reached the desirable level of power generation that supports daily life because of the problems such as scaling-up. On the other hand, developments of technology combining wastewater treatment and power generation, and application for environmental sensors are progressing to a stage close to practical use. If these popularizations continue, it will further develop its application in broader fields. Owing to their limitations, it may be difficult to force MFCs to become the main power supply in our daily life in the future, but it seems possible to use them as an auxiliary power supply. In addition, MFCs may become useful as a power supply in areas where the infrastructure is not well developed, for example, a portable power supply generating electricity if water is added. Regarding microbial catalysts, it is also known that various microorganisms can generate electricity, and if this superior power-generating function of these microorganisms can be integrated into a microbial cell using the synthetic biological method developed recently, the ability of the microbial catalyst will dramatically increase. Soon, its power generation ability could be greatly improved in combination with the progress of other constituents.

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Conflict of interest


The authors declare no conflicts of interest associated with this text.

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References

- [1] Ivanov I, Vidaković-Koch T, Sundmacher K. Recent advances in enzymatic fuel cells: Experiments and modeling. *Energies*. 2010;**3**(4):803-846. DOI: 10.3390/en3040803
- [2] Santoro C, Arbizzani C, Erable B, Ieropoulos I. Microbial fuel cells: From fundamentals to applications: A review. *Journal of Power Sources*. 2017;**356**:225-244. DOI: 10.1016/j.jpowsour.2017.03.109
- [3] Choudhury P, Prasad Uday US, Bandyopadhyay TK, Ray RN, Bhunia B. Performance improvement of microbial fuel cell (MFC) using suitable electrode and bioengineered organisms: A review. *Bioengineered*. 2017;**8**(5):471-487. DOI: 10.1080/21655979.2016.1267883
- [4] Potter MC. Electrical effects accompanying the decomposition of organic compounds. *Proceedings of the Royal Society B: Biological Sciences*. 1911;**84**(571):260-276
- [5] Cohen B. The bacterial culture as an electrical half-cell. *Journal of Bacteriology*. 1931;**21**:18-19
- [6] Bennetto HP, Delaney GR, Rason JR, Roller SD, Stirling JL, Thurston CF. The sucrose fuel cell: Efficient biomass conversion using a microbial catalyst. *Biotechnology Letters*. 1985;**7**(10):699-704. DOI: 10.1007/BF01032279
- [7] Delaney GM, Bennetto HP, Mason JR, Roller SD, Stirling JL, Thurston CF. Electron-transfer coupling in microbial fuel cells. II. Performance of fuel cells containing selected microorganism-mediator combinations. *Journal of Chemical Technology and Biotechnology*. 1984;**34**(1):13-27. DOI: 10.1002/jctb.280340104
- [8] Kim BH, Kim HJ, Hyun MS, Park DH. Direct electrode reaction of Fe(III)-reducing bacterium, *Shewanella putrefaciens*. *Journal of Microbiology and Biotechnology*. 1999;**9**(2):127-131. DOI: 10.1002/(SICI)1099-1514(199905/06)20:3<127::AID-OCA650>3.0.CO;2-I
- [9] Logan BE, Regan JM. Electricity-producing bacterial communities in microbial fuel cells. *Trends in Microbiology*. 2006;**14**(12):512-518. DOI: 10.1016/j.tim.2006.10.003
- [10] Lovley DR. Microbial fuel cells: Novel microbial physiologies and engineering approaches. *Current Opinion in Biotechnology*. 2006;**17**(3):327-332. DOI: 10.1016/j.copbio.2006.04.006
- [11] Kim HJ, Park HS, Hyun MS, Changa IS, Kim M. A mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*. *Enzyme and Microbial Technology*. 2002;**30**(2):145-152. DOI: 10.1016/S0141-0229(01)00478-1
- [12] Reimers CE, Tender LM, Fertig S, Wang W. Harvesting energy from the marine sediment-water interface. *Environmental Science & Technology*. 2001;**35**(1):192-195
- [13] Bond DR, Holmes DE, Tender LM, Lovley DR. Electrode-reducing microorganisms that harvest energy from marine sediments. *Science*. 2002;**295**(5554):483-485. DOI: 10.1126/science.1066771
- [14] Fredrickson JK, Romine MF, Beliaev AS, Auchtung JM, Driscoll ME, Gardner TS, et al. Towards environmental systems biology of *Shewanella*. *Nature Reviews Microbiology*. 2008;**6**(8):592-603. DOI: 10.1038/nrmicro1947
- [15] Shi L, Squier TC, Zachara JM, Fredrickson JK. Respiration of metal (hydr)oxides by *Shewanella* and

- Geobacter: A key role for multihaem c-type cytochromes. *Molecular Microbiology*. 2007;**65**(1):12-20. DOI: 10.1111/j.1365-2958.2007.05783.x
- [16] Fan Y, Hu H, Liu H. Sustainable power generation in microbial fuel cells using bicarbonate buffer and proton transfer mechanisms. *Environmental Science & Technology*. 2007;**41**:8154-8158. DOI: 10.1021/es071739c
- [17] Fan Y, Hu H, Liu H. Enhanced Coulombic efficiency and power density of air-cathode microbial fuel cells with an improved cell configuration. *Journal of Power Sources*. 2007;**17**:348-354. DOI: 10.1016/j.jpowsour.2007.06.220
- [18] Ringeisen BR, Henderson E, Wu PK, Pietron J, Ray R, Little B, et al. High power density from a miniature microbial fuel cell using *Shewanella oneidensis* DSP10. *Environmental Science & Technology*. 2006;**40**:2629-2634. DOI: 10.1021/es052254w
- [19] Kaneshiro H, Takano K, Takada Y, Wakisaka T, Tachibana T, Azuma M. A milliliter-scale yeast-based fuel cell with high performance. *Biochemical Engineering Journal*. 2014;**83**:90-96. DOI: 10.1016/j.bej.2013.12.011
- [20] Wang H, Wang G, Ling Y, Qian F, Song Y, Lu X, et al. High power density microbial fuel cell with flexible 3D graphene-nickel foam as anode. *Nanoscale*. 2013;**5**(21):10283-10290. DOI: 10.1039/c3nr03487a
- [21] Nevin KP, Richter H, Covalla SF, Johnson JP, Woodard TL, Orloff AL, et al. Power output and Coulombic efficiencies from biofilms of *Geobacter sulfurreducens* comparable to mixed community microbial fuel cells. *Environmental Microbiology*. 2008;**10**:2505-2514. DOI: 10.1111/j.1462-2920.2008.01675.x
- [22] Choi S, Lee HS, Yang Y, Parameswaran P, Torres CI, Rittmann BE, et al. A μ L-scale micromachined microbial fuel cell having high power density. *Lab on a Chip*. 2011;**11**(6):1110-1117. DOI: 10.1039/c0lc00494d
- [23] Liu H, Cheng H, Huang L, Logan BE. Scale-up of membrane-free single-chamber microbial fuel cells. *Journal of Power Sources*. 2008;**179**:274-279. DOI: 10.1016/j.jpowsour.2007.12.120
- [24] Walter XA, Gajda I, Forbes S, Winfield J, Greenman J, Ieropoulos I. Scaling-up of a novel, simplified MFC stack based on a self-stratifying urine column. *Biotechnology for Biofuels*. 2016;**9**:93. DOI: 10.1186/s13068-016-0504-3
- [25] Stoll ZA, Dolfing J, Xu P. Minimum performance requirements for microbial fuel cells to achieve energy-neutral wastewater treatment. *Water*. 2018;**10**(3):243. DOI: 10.3390/w10030243
- [26] Dong Y, Qu Y, He W, Du Y, Liu J, Han X, et al. A 90-liter stackable baffled microbial fuel cell for brewery wastewater treatment based on energy self-sufficient mode. *Bioresource Technology*. 2015;**195**:66-72. DOI: 10.1016/j.biortech.2015.06.026
- [27] Ge Z, He Z. Long-term performance of a 200-liter modularized microbial fuel cell system treating municipal wastewater: Treatment, energy, and cost. *Environmental Science: Water Research & Technology*. 2016;**2**:274-281. DOI: 10.1039/C6EW00020G
- [28] Zhuang L, Yuan Y, Wang Y, Zhou S. Long-term evaluation of a 10-liter serpentine-type microbial fuel cell stack treating brewery wastewater. *Bioresource Technology*. 2012;**123**:406-412. DOI: 10.1016/j.biortech.2012.07.038
- [29] Zhou T, Han H, Liu P, Xiong J, Tian F, Li X. Microbial fuels cell-based biosensor for toxicity detection: A

- review. *Sensors*. 2017;**17**(10):2230. DOI: 10.3390/s17102230
- [30] Chae KJ, Choi M, Ajayi FF, Park W, Chang IS, Kim IS. Mass transport through a proton exchange membrane (Nafion) in microbial fuel cells. *Energy & Fuels*. 2008;**22**:169-176
- [31] Ghassemi Z, Slaughter G. Biological fuel cells and membranes. *Membranes (Basel)*. 2017;**7**(1):3. DOI: 10.3390/membranes7010003
- [32] Novoselov KS, Geim AK, Morozov SV, Jiang D, Zhang Y, Dubonos SV, et al. Electric field effect in atomically thin carbon films. *Science*. 2004;**306**:666-669
- [33] Yu F, Wang C, Ma J. Applications of graphene-modified electrodes in microbial fuel cells. *Materials*. 2016;**9**:807. DOI: 10.3390/ma9100807
- [34] Yang Y, Liu T, Zhu X, Zhang F, Ye D, Liao Q, et al. Boosting power density of microbial fuel cells with 3D nitrogen-doped graphene aerogel electrode. *Advanced Science (Weinh)*. 2016;**3**(8):1600097. DOI: 10.1002/advs.201600097
- [35] Nam JY, Kim HW, Lim KH, Shin HS, Logan BE. Variation of power generation at different buffer types and conductivities in single chamber microbial fuel cells. *Biosensors & Bioelectronics*. 2010;**25**(5):1155-1159. DOI: 10.1016/j.bios.2009.10.005
- [36] Jang JK, Pham TH, Chang IS, Kang KH, Moon H, Cho KS, et al. Construction and operation of a novel mediator- and membrane-less microbial fuel cell. *Process Biochemistry*. 2004;**39**:1007-1012. DOI: 10.1016/S0032-9592(03)00203-6
- [37] Garba NA, Sa'adu L, Balarabe MD. An overview of the substrates used in microbial fuel cells. *Greener Journal of Biochemistry and Biotechnology*. 2017;**4**(2):007-026. DOI: 10.15580/GJBB.2017.2.051517061.
- [38] Reddy LV, Kumar SP, Wee YJ. Microbial fuel cells (MFCs)-a novel source of energy for new millennium. *Applied Microbiology and Microbial Biotechnology*. 2010;**25**:956-964
- [39] McKinlay JB, Zeikus JG. Extracellular iron reduction is mediated in part by neutral red and hydrogenase in *Escherichia coli*. *Applied and Environmental Microbiology*. 2004;**70**(6):3467-3474. DOI: 10.1128/AEM.70.6.3467-3474.2004
- [40] Rosenbaum M, He Z, Angenent LT. Light energy to bioelectricity: Photosynthetic microbial fuel cells. *Current Opinion in Biotechnology*. 2010;**21**(3):259-264. DOI: 10.1016/j.copbio.2010.03.010
- [41] Fischer F. Photoelectrode, photovoltaic and photosynthetic microbial fuel cells. *Renewable and Sustainable Energy Reviews*. 2018;**90**:16-27. DOI: 10.1016/j.rser.2018.03.053
- [42] Tartakovsky B, Guiot SR. A comparison of air and hydrogen peroxide oxygenated microbial fuel cell reactors. *Biotechnology Progress*. 2006;**22**:241-246. DOI: 10.1021/bp050225j
- [43] Ucar D, Zhang Y, Angelidaki I. An overview of electron acceptors in microbial fuel cells. *Frontiers in Microbiology*. 2017;**8**:643. DOI: 10.3389/fmicb.2017.00643
- [44] Oh S, Min B, Logan BE. Cathode performance as a factor in electricity generation in microbial fuel cells. *Environmental Science & Technology*. 2004;**38**(18):4900-4904. DOI: 10.1016/j.electacta.2015.12.105
- [45] Watanabe K. Recent developments in microbial fuel cell technologies

for sustainable bioenergy. *Journal of Bioscience and Bioengineering*. 2008;**106**(6):528-536. DOI: 10.1263/jbb.106.528

[46] Tharali AD, Sain N, Osborne WJ. Microbial fuel cells in bioelectricity production. *Frontiers in Life Science*. 2016;**9**(4):252-266. DOI: 10.1080/21553769.2016.1230787

[47] Schaetzle O, Barrière F, Baronian K. Bacteria and yeasts as catalysts in microbial fuel cells: Electron transfer from micro-organisms to electrodes for green electricity. *Energy & Environmental Science*. 2008;**1**:607-620. DOI: 10.1039/b810642h

[48] Fan L, Xue S. Overview on electricigens for microbial fuel cell. *The Open Biotechnology Journal*. 2016;**10**:399. DOI: 10.2174/1874070701610010398

[49] Dopson M, Ni G. Possibilities for extremophilic microorganisms in microbial electrochemical systems. *FEMS Microbiology Reviews*. 2016;**40**(2):164-181. DOI: 10.1093/femsre/fuv044

[50] Khater D, El-khatib KM, Hazaa M, Hassan RYA. Activated sludge-based microbial fuel cell for bio-electricity generation. *Journal of Basic and Environmental Sciences*. 2015;**2**:63-73

[51] Clauwaert P, Aelterman P, Pham TH, De Schampelaire L, Carballa M, Rabaey K, et al. Minimizing losses in bio-electrochemical systems: The road to applications. *Applied Microbiology and Biotechnology*. 2008;**79**(6):901-913. DOI: 10.1007/s00253-008-1522-2

[52] Kim GT, Webster G, Wimpenny JW, Kim BH, Kim HJ, Weightman AJ. Bacterial community structure, compartmentalization and activity in a microbial fuel cell. *Journal of Applied Microbiology*. 2006;**101**(3):698-710

[53] Kouzuma A, Kasai T, Hirose A, Watanabe K. Catabolic and regulatory systems in *Shewanella oneidensis* MR-1 involved in electricity generation in microbial fuel cells. *Frontiers in Microbiology*. 2015;**6**:609. DOI: 10.3389/fmicb.2015.00609

[54] Sure SK, Ackland LM, Torriero AA, Adholeya A, Kochar M. Microbial nanowires: An electrifying tale. *Microbiology*. 2016;**162**:2017-2028. DOI: 10.1099/mic.0.000382

[55] Vaara M. Agents that increase the permeability of the outer membrane. *Microbiological Reviews*. 1992;**56**:395-411

[56] Liu J, Qiao Y, Lu ZS, Song H, Li CM. Enhance electron transfer and performance of microbial fuel cells by perforating the cell membrane. *Electrochemistry Communications*. 2012;**15**(1):50-53. DOI: 10.1016/j.elecom.2011.11.018

[57] Qiao Y, Li CM, Bao SJ, Lu ZS, Hong YH. Direct electrochemistry and electrocatalytic mechanism of evolved *Escherichia coli* cells in microbial fuel cells. *Chemical Communications*. 2008;**11**:1290-1292. DOI: 10.1039/b719955d

[58] Luo JM, Li M, Zhou MH, Hu YS. Characterization of a novel strain phylogenetically related to *Kocuria rhizophila* and its chemical modification to improve performance of microbial fuel cells. *Biosensors & Bioelectronics*. 2015;**69**:113-120. DOI: 10.1016/j.bios.2015.02.025

[59] Zheng T, Xu YS, Yong XY, Li B, Yin D, Cheng QW, et al. Endogenously enhanced biosurfactant production promotes electricity generation from microbial fuel cells. *Bioresource Technology*. 2015;**197**:416-421. DOI: 10.1016/j.biortech.2015.08.136

- [60] Van Gennip M, Christensen LD, Alhede M, Phipps R, Jensen PØ, Christophersen L, et al. Inactivation of the *rhLA* gene in *Pseudomonas aeruginosa* prevents rhamnolipid production, disabling the protection against polymorphonuclear leukocytes. *APMIS*. 2009;**117**:537-546. DOI: 10.1111/j.1600-0463.2009.02466.x
- [61] Heather MJ, Aaron EA, Konstantin RM, Yuri YL, Bruce EC, Brett AH, et al. Engineering of a synthetic electron conduit in living cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;**107**(45):19213-19218. DOI: 10.1073/pnas.1009645107
- [62] Sturm-Richter K, Golitsch F, Sturm G, Kipf E, Dittrich A, Beblawy S, et al. Unbalanced fermentation of glycerol in *Escherichia coli* via heterologous production of an electron transport chain and electrode interaction in microbial electrochemical cells. *Bioresource Technology*. 2015;**186**:89-96. DOI: 10.1016/j.biortech.2015.02.116
- [63] Jensen HM, TerAvest MA, Kokish MG, Ajo-Franklin CM. CymA and exogenous flavins improve extracellular electron transfer and couple it to cell growth in Mtr-expressing *Escherichia coli*. *ACS Synthetic Biology*. 2016;**5**(7):679-688. DOI: 10.1021/acssynbio.5b00279
- [64] Shrestha N, Chilkoor G, Vemuri B, Rathinam N, Sani RK, Gadhamshetty V. Extremophiles for microbial-electrochemistry applications: A critical review. *Bioresource Technology*. 2018;**255**:318-330. DOI: 10.1016/j.biortech.2018.01.151
- [65] Erable B, Etcheverry L, Bergel A. Increased power from a two-chamber microbial fuel cell with a low-pH air-cathode compartment. *Electrochemistry Communications*. 2009;**11**(3):619-622. DOI: 10.1016/j.elecom.2008.12.058
- [66] Slonczewski JL, Fujisawa M, Dopson M, Krulwich TA. Cytoplasmic pH measurement and homeostasis in bacteria and archaea. *Advances in Microbial Physiology*. 2009;**55**:1-79. DOI: 10.1016/S0065-2911(09)05501-5
- [67] Malki M, De Lacey AL, Rodriguez N, Amils R, Fernandez VM. Preferential use of an anode as an electron acceptor by an acidophilic bacterium in the presence of oxygen. *Applied and Environmental Microbiology*. 2008;**74**:4472-4476. DOI: 10.1128/AEM.00209-08
- [68] Tapia JM, Munoz JA, Gonzalez F, Blazquez ML, Ballester A. Interrelation between cells and extracellular polymeric substances (EPS) from *Acidiphilium 3.2Sup(5)* on carbon surfaces. *Advanced Materials Research*. 2009;**71-73**:287-290. DOI: 10.4028/www.scientific.net/AMR.71-73.287
- [69] Zhang T, Zhang L, Su W, Gao P, Li D, He X, et al. The direct electrocatalysis of phenazine-1-carboxylic acid excreted by *Pseudomonas alcaliphila* under alkaline condition in microbial fuel cells. *Bioresource Technology*. 2011;**102**:7099-7102. DOI: 10.1016/j.biortech.2011.04.093
- [70] Li XM, Cheng KY, Wong JW. Bioelectricity production from food waste leachate using microbial fuel cells: Effect of NaCl and pH. *Bioresource Technology*. 2013;**149**:452-458. DOI: 10.1016/j.biortech.2013.09.037
- [71] Jayashree C, Tamilarasan K, Rajkumar M, Arulazhagan P, Yogalakshmi KN, Srikanth M, et al. Treatment of seafood processing wastewater using upflow microbial fuel cell for power generation and identification of bacterial community in anodic biofilm. *Journal of Environmental Management*. 2016;**180**:351-358. DOI: 10.1016/j.jenvman.2016.05.050

- [72] Jadhav GS, Ghangrekar MM. Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration. *Bioresource Technology*. 2009;**100**:717-723. DOI: 10.1016/j.biortech.2008.07.041
- [73] Catal T, Kavanagh P, O'Flaherty V, Leech D. Generation of electricity in microbial fuel cells at sub-ambient temperatures. *Journal of Power Sources*. 2011;**196**:2676-2681. DOI: 10.1016/j.jpowsour.2010.11.031
- [74] Liu L, Tsyganova O, Lee DJ, Su A, Chang JS, Wang A, et al. Anodic biofilm in single chamber microbial fuel cells cultivated under different temperatures. *International Journal of Hydrogen Energy*. 2012;**37**:15792-15800. DOI: 10.1016/j.ijhydene.2012.03.084
- [75] Zhang L, Shen J, Wang L, Ding L, Xu K, Ren H. Stable operation of microbial fuel cells at low temperatures (5-10°C) with light exposure and its anodic microbial analysis. *Bioprocess and Biosystems Engineering*. 2014;**37**:819-827. DOI: 10.1007/s00449-013-1054-8
- [76] Mathis BJ, Marshall CW, Milliken CE, Makkar RS, Creager SE, May HD. Electricity generation by thermophilic microorganisms from marine sediment. *Applied Microbiology and Biotechnology*. 2008;**78**(1):147-155. DOI: 10.1007/s00253-007-1266-4
- [77] Shehab NA, Ortiz-Medin JF, Katuri KP, Hari AR, Amy G, Logan BE, et al. Enrichment of extremophilic exoelectrogens in microbial electrolysis cells using Red Sea brine pools as inocula. *Bioresource Technology*. 2017;**239**:82-86. DOI: 10.1016/j.biortech.2017.04.122
- [78] Fu Q, Fukushima N, Maeda H, Sato K, Kobayashi H. Bioelectrochemical analysis of a hyperthermophilic microbial fuel cell generating electricity at temperatures above 80°C. *Bioscience, Biotechnology, and Biochemistry*. 2015;**79**(7):1200-1206. DOI: 10.1080/09168451.2015.1015952
- [79] Carver SM, Vuoriranta P, Tuovinen OH. A thermophilic microbial fuel cell design. *Journal of Power Sources*. 2011;**196**:3757-3760. DOI: 10.1016/j.jpowsour.2010.12.088