Cellulose-fueled Single-chamber Microbial Fuel Cells Using *Cellulomonas fimi*

2019

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Contents

Chapter 1
General Introduction

1.1 Cellulose

Cellulose was first found in 1838 by French chemist Anselme Payen, while studying different types of wood. He named this new substance “cellulose” because he had obtained it from the cell walls of plants (1). The structure of cellulose was then elucidated in 1920 by Hermann Staudinger. He found that cellulose was composed of D-glucose units, linked to each other to form long chains. The structure of cellulose was made up of many glucose moieties linked in the form of β-D-anhydroglucopyranose units (AGUs). The AGUs are linked to each other through glycosidic bridges on the C1 and C4 carbon atoms as shown in Fig. 1.1. The number of repeating AGUs is defined as the degree of polymerization (DP) of cellulose. The common range of DP for wood pulp is about 300–1700 and is 800–10000 for cotton or other plant fibers (2).

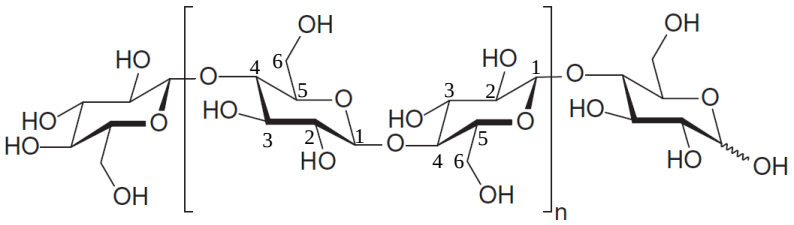

Fig. 1.1 Chemical structure of the cellulose chain, where n = 2000 to 10000 (3)

Cellulose is the most abundant naturally occurring biopolymer (1), the most abundant organic compound on the earth, the major structural component of the cell wall of higher plants, and is found in raw materials such as forestry, agricultural residues and various kinds of waste (4). The consequence of the supra-molecular structure of cellulose is its insolubility in water, as well as in common organic liquids. Poor solubility in common solvents is one of the reasons why cellulose is converted to its cellulose esters (3).

1.2 *Cellulomonas fimi*

Cellulomonas fimi is a soil-dwelling, gram-positive, rod-shaped, mesophilic, facultative anaerobe as shown in Fig. 1.2. It grows optimally at 30°C, under aerobic and anaerobic conditions, and can utilize cellulose as carbon source, with growth demonstrated on wood, agricultural wastes, and crystalline cellulose (5). *C. fimi* has been studied for degrading cellulosic material. Prior to the release of genome by the United States Department of Energy (US DOE), there were 14 cellulolytic enzymes known in *C. fimi* as shown in Table 1.1 (6).

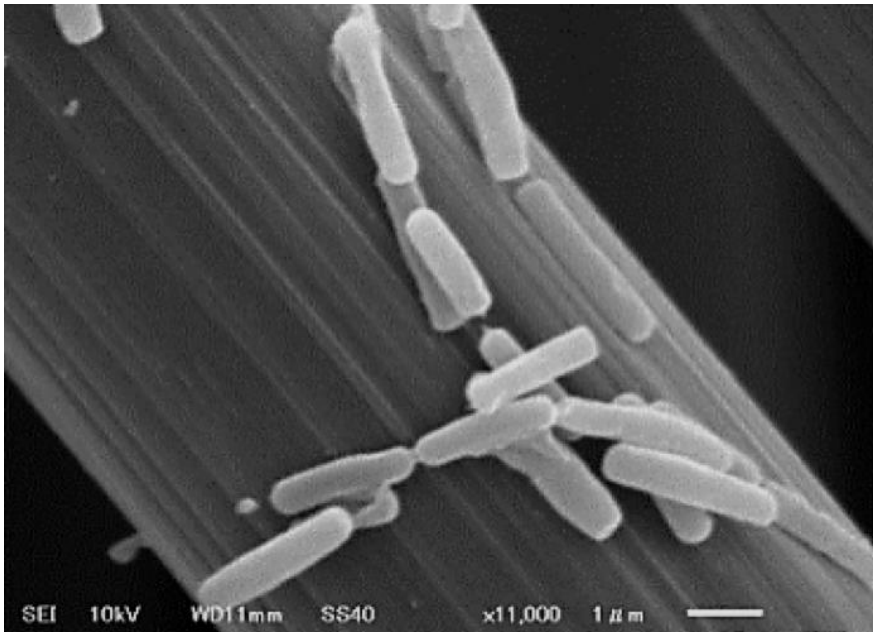

Fig. 1.2 Scanning electron microscope image of *Cellulomonas fimi* NBRC 15513 on non-woven carbon fiber observed by scanning electron microscope (at 10 kV)

Furthermore, it has been shown almost 40 years ago to produce extracellular enzymes That could efficiently degrade pretreated sugarcane bagasse. As interest in enzymatic saccharification growth for production of fuel ethanol from biomass. Much research also reported that *C. fimi* secreted cellulases and hemicellulases (7).

Table 1.1 A selection of putative functions and known gene products required for cellulose degradation found in the *C. fimi* genome (6).

Putative Enzyme Function	GH Families	Already Known in *C. fimi*
Endo 1,4-β-glucanase	5, 6, 9, 16, 64, 81	CenA, CenB, CenC, CenD
1,4-β cellobiohydrolase	6, 48	CbhA, CbhB
Endo 1,4-β xylanase	10, 11, 43	XynB, XynC, XynD, XynE
Xylan 1,4-β xylosidase	39, 43	
α-L-arabinofuranosidase	43, 51, 62	AbfCelF(AfsA)
arabinanase	43	
β-mannanase/mannosidase	None, 2, 26	ManA, ManD
α-amylase	None, 13	
Pectate Lyase	None	
Chitinase	None, 3, 18, 20	NagA, NagB
Acetyl Xylan Esterase	None	

The cellulases of *C. fimi* are well studied, and the genome has been sequenced. Two of the best-studied cellulases of *C. fimi* are the endoglucanase CenA and the bifunctional exoglucanase/xylanase Cex. The N-terminal family-2 CBM of CenA, unlike those of other endoglucanases, has been shown to disrupt the structure of cellulose fibers, resulting in the release of fine cellulose particles (8). Warren et al. has reported that the enzymes were shown to be both free in the supernatant and the cellulose bound. The composition of the cellulases produced was shown to be a complex mixture, including some proteins which were clearly proteolytic breakdown products. The advent of molecular cloning soon revealed that indeed there were many genes for cellulases and xylanases in the type strain *C. fimi* ATCC 484 (7).

Summary of the observed CAZyme content. In order to facilitate visual comparison of the supernatant CAZymes, enzyme family was summarized in a pie chart (Fig. 1.3).

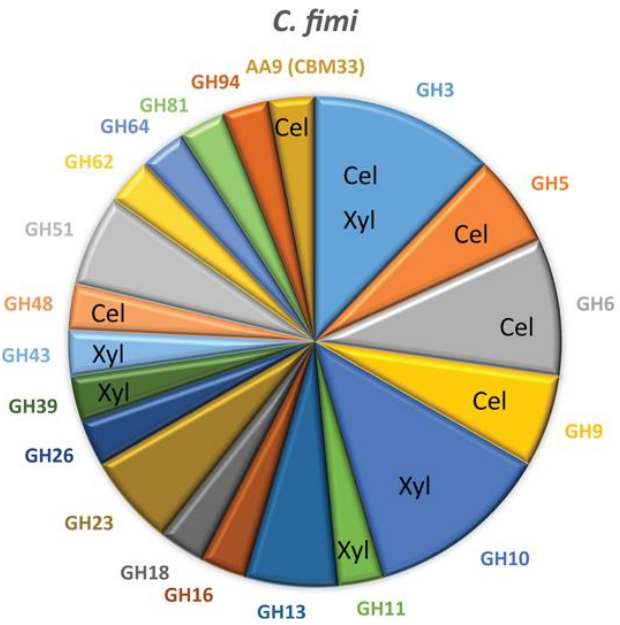

Fig. 1.3 Observed CAZyme content in different supernatant inoculated with *C. fimi* (7)

Those families that have known activities toward cellulose or xylan degradation are labeled with “Cel” or “Xyl”. The sections are proportional to the number of family members that were identified in each supernatant (7). In addition to the type species, In 1980 the Approved Lists of Bacterial Names already listed six species: *C. flavigena*, *C. biazotea*, *C.-gelida*, *C. uda*, *C. fimi*, and *C. cellasea.* Currently, 17 species belonging to the genus *Cellulomonas* are noted in the actual version of the “List of Procaryotic names with Standing in Nomenclature” (9). Due to the cellulolytic activity of these organisms, their preferred habitats are cellulose enriched environments such as soil, bark, wood, and sugar fields, but they were also successfully isolated from rumen and from activated sludge.

1.3 Cellulose degradation by microbes

Cellulose, the major component of plant cell walls, is the most abundant biopolymer on the earth, and is an attractive source for biofuel production for many reasons, besides it contains the most abundant terrestrial source of carbon (5). Its structure is made of glucose molecules

linked by β-1,4-glycosidic bonds forming long fibers that interact through hydrogen bonds and van der Waal forces to produce microfibrils of crystalline and semicrystalline cellulose (6). Cellulose can be degraded naturally and used as a source of energy by various bacteria and fungi. Such organisms produce endo-β-1,4-glucanases, exo-β-1,4-glucanases or cellobiohydrolases and β-glucosidases. The combined action of these enzymes results in the saccharification of cellulose. Endoglucanases and cellobiohydrolases break the long cellulose into short-chain units which are hydrolyzed by β-glucosidases into glucose. The endoglucanases and cellobiohydrolases usually consist of two functional units, the catalytic domain which hydrolyzes the β-1,4-glycosidic bonds in cellulose and a non-catalytic CBM which binds to cellulose (8). Cellulose degradation by microbes can be divided into two distinct strategies. These include the "secreted enzyme" strategies, where cellulases are released into the extracellular environment away from the cell, and the "surface enzyme" strategies, where an organism uses surface-associated cellulases to degrade fiber near the cell surface. The secreted enzyme approach appears to be employed by a few bacterial phyla and, is typically associated with aerobic organisms (5).

1.4 Microbial fuel cells

Microbial fuel cells (MFCs) are a device that directly converts the metabolic activity and chemical energy of the microorganism adhering on an anode under anaerobic conditions to generate electric power from variety of organic matters, including, agricultural waste, and renewable biomass, as fuels, and are a prospective technology that purifies wastewater which gained a lot of attention in recent years as a future alternative energy for human being. MFCs are classified into two types depending on how electrons are transferred from the bacteria to the electrode. The general principle of microbial fuel cells relies on the oxidation of organic matter by the microbes that are living in an anaerobic medium. In the absence of dioxygen, or

other natural electron acceptors, some microbial species have the ability to transfer electrons to the MFC anode. The electric current/ energy is then used through a load in the external circuit, and eventually reduces at the cathode an electron acceptor such as dioxygen (10,15). Mediator acts as electron shuttle between cells and electrode, and many microorganisms require it for transferring electron for generating electricity of MFC. For mediator MFC system, exogenous electron shuttles or mediators are added in order to improve the electron transfer efficiency (Fig. 1.4a). The currently reported electron mediators in MFCs are mainly compounds containing quinone functional group such as neutral red, anthraquinone-2,6-disulfonate. While, some microorganisms, for example *Shewanella* sp. secretes quinone and flavin derivatives employing mediators (16) and has metal-reducing extracellular electron transfer pathway of transfer electron from surface of the cell to electrode (17). Such microorganisms can realize mediator- less MFC without the addition or self-secretion of external electron shuttles shown in Fig. 1.4b. Although, the remarkable improvement in power density, the large- scale application of MFCs has yet to be implemented due to low yields of power generation and high costs. The performance of any fuel cell strongly depends on the efficiency of the electrode. Therefore, electrode design is a key factor for efficient and inexpensive MFC fabrication. The materials used in these devices are still not economic and researchers use different materials as an anode in MFC (10–12).

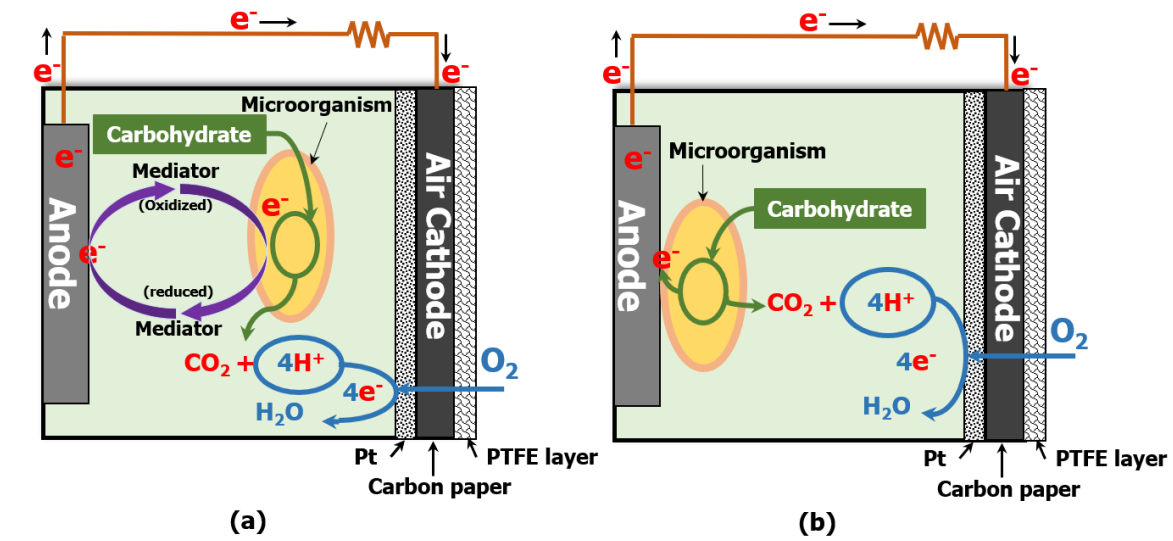

Fig. 1.4 The air cathode-microbial fuel cells: Microbial fuel cell with addition of mediator (a) and mediator-less microbial fuel cell (b)

1.5 Palm kernel shell

Palm kernel shell is a waste material obtained during the crushing of palm nuts in the palm oil mills for palm oil extraction as shown in Fig. 1.5. The shell part that remained after the nut has been removed is obtained as residual waste after separating the palm seed during the extraction process (18,19). Besides, the crushed palm kernel shell is an economically and environmentally sustainable raw material for renewable energy industry. Oil palm nativity had been associated with the tropical rainforest of West Africa but has spread to most of the equatorial tropics of South-East Asia and America. It forms part of foreign income earner for most of Asian countries such as Malaysia, Indonesia and Thailand. Palm kernel shell as one of the “wastes” which can be suitably converted to renewable energy by applying suitable thermochemical process. Gasification, torrefaction and fast pyrolysis are the three common thermochemical processes recently applied to biomass conversion to obtain higher energy density fuels (20). Nearly 2.4 million tons of palm shell wastes, equivalent to 45.84 PJ (Peta

joule) of energy, is produced annually in Malaysia alone. The waste is not currently utilized fully, and some is burnt without energy recovery. Efficient management of the waste, such as performing thermochemical conversion to valuable products, is required to improve the process efficiency and economics of the overall oil production (22). Oil palm demand is projected to increase from 51 million tonnes today to 75 million tonnes by 2050. Due to its high caloric value, palm kernel shell potentially was used as fuel to replace conventional fuel from fossil energy (23).

Fig. 1.5 Palm kernel shells after oil extraction (approximately 30 g)

1.6 Purpose of this study

This study is mainly the development of a single-chamber microbial fuel cell utilizing cellulose as fuel for *Cellulomonas* spp. with/without the addition of electron mediator. The thesis reveals the advantages of MFC compared with thermal power generation of the Carnot cycle which has a limitation, while MFC has no such limitation for converting chemical bond energy of organic substrate to electric energy. Herein, the direct electron transfer ability of *Cellulomonas fimi* is also studied whether it is self-electron transferable microorganism or not. Additionally, a suitable cellulose fuel solution from commercial and agricultural sources for the MFCs is studied. The purpose of each study is divided as follows;

Firstly, *Cellulomonas* spp. is a well-known microorganism that utilizes cellulose as a carbon source. Furthermore, its strains are not pathogenic. There is a good review of microbes used in MFCs (5). However, there is no information as which species is suitable for the medium condition of MFC. Therefore, in this study, three types of cellulose-degrading bacteria, namely, *Cellulomonas fimi*, *Cellulomonas biazotea*, and *Cellulomonas flavigena*, were selected and compared in terms of their respective microbial degradation of cellulose and the performance of the MFCs with different kinds of fuel solution and mediator was evaluated to study the suitable condition for current generation.

Secondly, to the best of our knowledge, no study concerning MFCs using cellulose as fuel without addition of mediator. The author investigates the mediator-less MFC fed with cellulose in water, and clarify the bacterial electron transfer mechanism from *C. fimi* to the electrode for the electric generation of the MFCs, using antimycin A as an inhibitor of electron transfer activity of *C. fimi*.

Finally, there is no report to date employing palm kernel shell as fuel for MFCs. The problem of applying these cellulosic materials to MFCs should be solved while considering *C. fimi*, because the morphologies of cellulosic materials would affect its utilization by the microorganism. In this study, three types of cellulosic fuel, kernel shell cellulose, kernel shell nanocellulose, and kernel shell nanocellulose residual are prepared, along with commercially available cellulose powder. The performance of the MFCs is evaluated using the synthesized cellulose from palm kernel shell compared with the commercially available cellulose powder.

References

1. **Devabaktuni, L., Kulkarni, P. K., Mudit, D., Prudhvi, K. R., and Krishna, L. N. V.:** Sources of cellulose and their applications-A review, IJDFR, **2**, (2015).
2. **Yao, B. H. and Yao, F. T.:** Hydrolysis of cellulose to glucose by solid acid catalysts, Green Chemistry, **15**, 1095–1111 (2013).
3. **Rudnik, E.:** Cellulose, pp.201 in: Ebnesajjad, S. (Ed.), 10 Compostable polymer materials: Definitions, structures, and methods of preparation. Handbook of biopolymers and biodegradable plastics: Properties, processing and applications, Elsevier, (2013).
4. **Peciulyte, A., Karlström, K., Larsson, P. T., and Olsson, L.:** Impact of the supramolecular structure of cellulose on the efficiency of enzymatic hydrolysis, Biotechnol. Biofuels, **8**, 1–13 (2015).
5. **Melissa, R. C., Garret, S., Shanti, B., Kelsea, A. J., Frank, O. A., David, M., and Phillip, J. B.:** The Genome sequences of *Cellulomonas fimi* and '*Cellvibrio gilvus*' reveal the cellulolytic strategies of two facultative anaerobes, transfer of '*Cellvibrio gilvus*' to the genus *Cellulomonas*, and proposal of *Cellulomonas gilvus* sp. nov., PLoS One, **8**, e53954 (2013).
6. **Kane, S. D. and French, C. E.:** Characterisation of novel biomass degradation enzymes from the genome of *Cellulomonas fimi*, Enzyme Microb. Technol., 113, 9–17 (2018).
7. **Warren, W. W., Denis, B., Simon, F., Anna, R., Hirak, S., Tamara, and E., John, K.:** Proteomic analysis of the secretome of *Cellulomonas fimi* ATCC 484 and *Cellulomonas flavigena* ATCC 482, PLoS One, **11**, 1–13 (2016).
8. **Duedu, K. O. and French, C. E.:** Characterization of a *Cellulomonas fimi* exoglucanase/xylanase-endoglucanase gene fusion which improves microbial degradation of cellulosic biomass, Enzyme and Microbial. Technology, Elsevier Inc., 93–94 (2016).

9. **Birte, Abt., Brian, F., Alla, L., Alicia, C., Hui, S., Rüdiger, P., Susan, L., Tijana, G. D. R., Matt, N., Hope, T., and more 23 atuhers:** Complete genome sequence of *Cellulomonas flavigena* type strain, Standards in Genomic Sciences, **3**, 15–25 (2010).

10. **Liping, H. and Irini, A.:** Effect of humic acids on electricity generation integrated with xylose degradation in microbial fuel cells, Biotechnol. Bioeng., **100**, 413–422 (2008).

11. **Jincheng, W., Peng, L., and Xia H.:** Recent progress in electrodes for microbial fuel cells, Bioresource Technology, **102**, 9335–9344 (2011).

12. **Deepak, P., Gilbert, V. B., Ludo, and Diels, K. V.:** Anode and cathode materials characterization for a microbial fuel cell in half cell configuration, Water Science & Technology, **63**, 2457–61 (2011).

13. **Santimoy, K., Soumya, P., Jhansi, L. V., Debabrata, D., and Debabrata, P.:** Bifunctional manganese ferrite/ polyaniline hybrid as electrode material for enhanced energy recovery in microbial fuel cell, Appl. Mater. Interfaces, **7**, 20657–20666 (2015).

14. **Yumiko, K. and Kazuya, W.:** An electricity-generating prosthecate bacterium strain Mfc52 isolated from a microbial fuel cell, Microbiol. Lett., **288**, 55–61 (2008).

15. **Olivier, S., Frédéric, B., and Keith, B.:** Bacteria and yeasts as catalysts in microbial fuel cells: electron transfer from micro-organisms to electrodes for green electricity, Energy Environ. Sci., **1**, 607–620 (2008).

16. **Von Canstein, H., Ogawa, J., Shimizu, S., and Lloyd, J. R.:** Secretion of flavins by *Shewanella* species and their role in extracellular electron transfer, Appl. Environ. Microbiol.**, 74**, 615–623 (2008).

17. **Phansroy, N., Aso, Y., Sasaki, S., Aoki, T., and Ohara, H.:** Immobilization of the iron on the surface of non-woven carbon fiber for use in a microbial fuel cell, Mater. Renew. Sustain. Energy, **5**, 1–7 (2016).

18. **Ikumapayi, O. M. and Akinlabi, E. T.:** Review article composition, characteristics and socioeconomic benefits of palm kernel shell exploitation-an overview, J. Environ. Sci. Technol., **11**(**5**), 220–232 (2018).

19. **Itam, Z., Beddu, S., Mohd Kamal, N. L., Alam, M. A., and Ayash, U. I.:** The feasibility of palm kernel shell as a replacement for coarse aggregate in lightweight concrete, IOP Conf. Ser. Earth Environ. Sci., **32**, 7–11 (2016).

20. **Okoroigwe, E. C. and Saffron, C. M.:** Determination of bio-energy potential of palm kernel shell by physicochemical characterization, Niger. J. Technol., **31**, 329–335 (2012).

21. **Abdul, K. A., Sarani, N. A., and Abd, K. S. A.:** Incorporation of Ppalm kernel shell into fired clay brick, MATEC Web Conf., **103**, 01017 (2017).

22. **Kim, S. W., Koo, B. S., and Lee, D. H.:** Catalytic pyrolysis of palm kernel shell waste in a fluidized bed, Bioresour. Technol., **167**, 425–432 (2014).

23. **Febriansyah, H., Setiawan, A. A., Suryopratomo, K., and Setiawan, A.:** Gama stove: Biomass stove for palm kernel shells in Indonesia, Energy Procedia, **47**, 123–132 (2014).

Chapter 2

Microbial fuel cells using *Cellulomonas* spp. with cellulose as fuel

2.1 INTRODUCTION

Cellulose is the main component of cell walls and fibers of plant cells. It is the most abundant carbohydrate source and comprises glucose polymers with a Gibbs energy of approximately 2890 kJ/mol. Electrical energy is the foundation of our everyday lives, and thus, methods involving the efficient conversion of energy from various sources to electrical energy have grown in importance. For example, in the conversion of thermal energy into electrical energy, the efficiency of the conversion process is limited by the Carnot cycle and the efficiency is determined by the ratio of the temperature of burnt fuels to atmospheric temperature. Given this limitation, thermal power plants cannot convert more than 40% of chemical energy from the burnt materials to electrical energy. A few recent studies attempted to overcome the limitation of this energy conversion. Studies on fuel cells reported the production of electrical energy using enzyme reactions on cellulose (1–3). However, several processes are required for the production and purification of enzymes; moreover, using enzymes over long periods is difficult because of denaturation. Hence, microbial fuel cells (MFCs) utilize the self-propagation abilities of microorganisms instead of using enzymes (4). In an MFC, respiration activities of microorganisms release electrons and these electrons are transferred to the anode, which is used as an electron acceptor. This technique is extensively used to transfer microbial electrons to an anode for electric current production in MFCs under anaerobic conditions with the reduction of oxygen at the cathode (5). Ren et al. (6) analyzed electricity generation and the microbial ecology of cellulose-fed MFCs of a two-chamber type by using a defined co-culture of *Clostridium cellulolyticum* and *Geobacter sulfurreducens*.

However, *Clostridium* spp. is an anaerobic bacterium, and some of its strains exhibit pathogenicity. Rezaei et al. (7) reported on a sediment-based system for chitin and cellulose as substrate. The sediment used in their study was anaerobic sediment obtained from the Delaware Bay, and it contained several types of microorganisms. Rismani-Yazdi et al. (8) examined the generation of electricity with rumen microorganisms as biocatalysts and cellulose as the electron donor in two-compartment MFCs. Ishii et al. (9) inoculated with rice paddy field soil and fed cellulose as a carbon and energy source for a two-chamber type MFC. Their results indicated that sub-culturing biofilms attached on anode electrodes enriched the electricity-generating microorganisms and resulted in phylotypes frequently detected by clone library analyses as being affiliated with *Clostridiales*, *Chloroflexi*, *Rhizobiales*, and *Methanobacterium*.

Cellulomonas spp. is a well-known microorganism that utilizes cellulose as a carbon source (10–15). Furthermore, its strains are not pathogenic. There is a good review about microbes used in MFCs (16). Besides this review, to the best of author's knowledge, there are no studies on MFCs with *Cellulomonas* spp. that consume cellulose as fuel. Despite there being many studies regarding cellulose degradation with *Cellulomonas* spp., there is no information as to which species is suitable for medium condition of MFC. Therefore, in this study, three types of cellulose-degrading bacteria, namely, *Cellulomonas fimi*, *Cellulomonas biazotea*, and *Cellulomonas flavigena*, were selected and compared in terms of their respective microbial degradations of cellulose and development of MFCs.

2.2 MATERIALS AND METHODS

2.2.1 Chemicals A Cellulose powder (38 mm, Nacalai Tesque Inc., Kyoto, Japan), Yeast extract (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan), Tryptone (Nacalai Tesque), Anthraquinone-2,6-disulfonic acid disodium salt (AQDS; Combi–Blocks Inc., CA,

USA) and neutral red (Sigma–Aldrich Co., MO, USA) were used in this study.

2.2.2 Bacterial strains and medium The strains used in this study were *C. fimi* NBRC 15513, *C. biazotea* NBRC 12680, *C. flavigena* NBRC 3775, *Shewanella oneidensis* MR-1 (ATCC 700550). The cellulose-containing medium was composed of 0.5 g of cellulose powder, 0.05 g of yeast extract, and 1.0 g of NaCl, dissolved in 100 mL of 0.1 M phosphate buffer (pH 6.8), and was then autoclaved (121°C, 20 min). Luria–Bertani (LB) medium was composed of 1 g of Tryptone, 0.5 g of yeast extract, and 1 g of NaCl, dissolved in 100 mL of distilled water, and was then autoclaved (121°C, 20 min).

2.2.3 Degradation test of cellulose Three species of *Cellulomonas* spp. were precultured in an LB medium and then centrifuged (1800 ×g, 5 min). The concentration of the cellulose-containing medium was adjusted to an optical density (OD_{600}) of 2.0. This solution was added to a Sakaguchi flask and was shaken for 4 days (30°C, 120 rpm). Sampling was performed every 24 h. Then, 1 mL of this sample was added to 9 mL of distilled water. A filter paper (5A, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) was used to filter the resulting solution. The obtained residue was washed with 10 mL of distilled water and heated at 80°C for 24 h. Finally, the dry weight of the sample was measured.

2.2.4 The chamber structure of MFC The setup of the MFC used in this study was modified according to that in a previous study (17) and is shown in Fig. 2.1. The internal volume of the MFC was 100 mL, the MFC anode was composed of carbon felt (LFP-210, Osaka Gas Chemicals Co., Osaka, Japan), and the cathode was an air-cathode fabricated according to specifications used in an extant study (18). The air-cathode had three layers (Fig. 2.1), namely a catalyst layer consisting of Pt-supported carbon (IFPC40-III, Ishifuku Metal Industry Co., Tokyo, Japan) with Nafion (510211, Sigma–Aldrich, Tokyo, Japan), a carbon paper layer (TGP-120, Toray Co., Tokyo, Japan), and a polytetrafluoroethylene layer (PTFE, 60% dispersion, 31-JR, Du Pont-Mitsui Fluorochemicals Co., Tokyo, Japan).

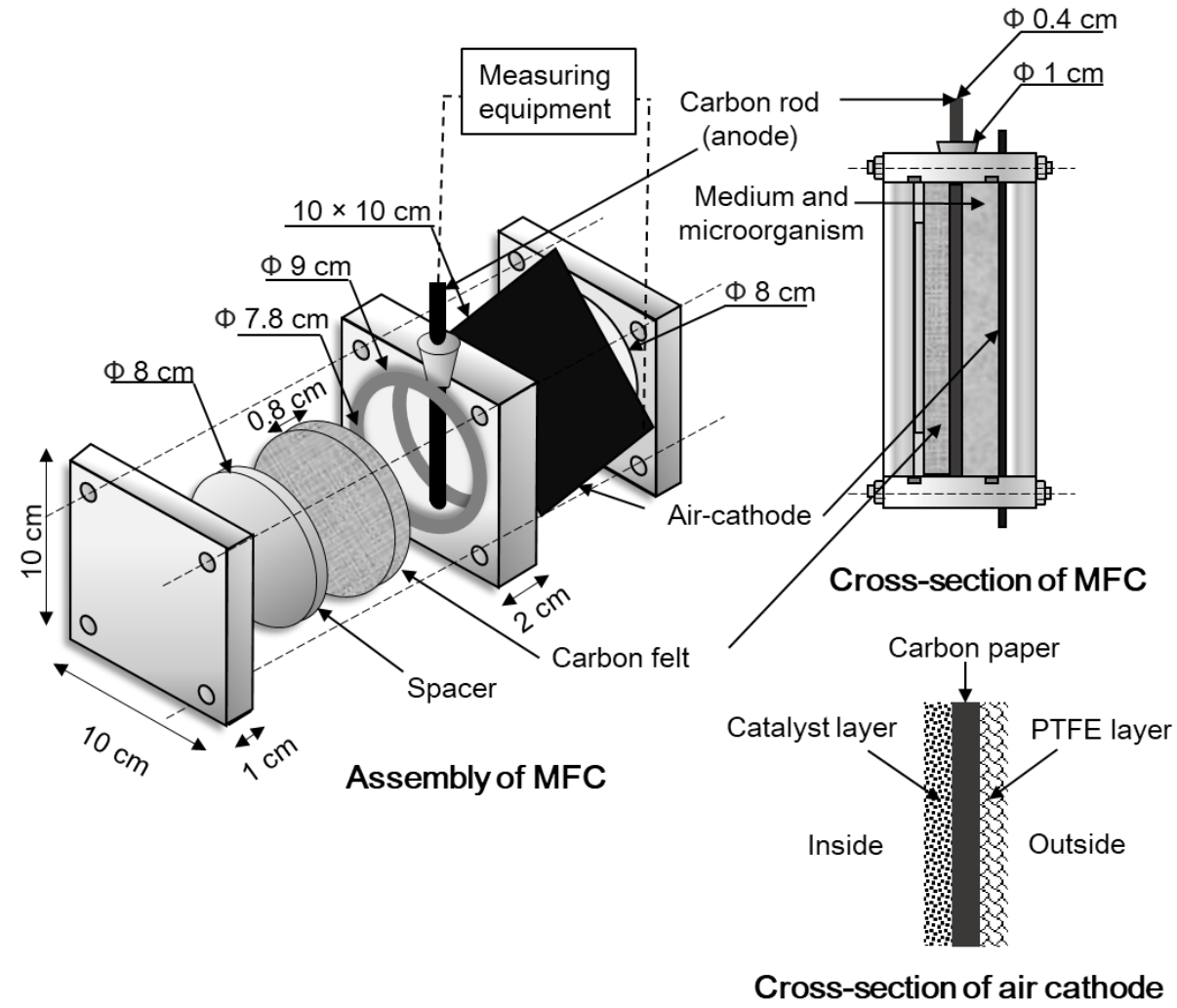

Fig. 2.1 Assembly and cross-section of MFC and the air cathode. The vessel parts are made of polycarbonate. Spacer adjusts the volume of vessel (100 mL). The carbon rod conducted to carbon felt and not the air cathode.

The voltage and electric current generated by the MFC were measured using a digital multimeter (KEW 1062, Kyoritsu Electrical Instruments, Tokyo, Japan). Internal resistance for DC voltage measurement of this meter is 100 MΩ, and the accuracy of this meter for current measurement is 0.2% of indicated values 5 least-significant digits. Therefore, in this study, internal resistance for current measurement was considered to be negligibly small, and for voltage, it was considered to be infinite.

2.2.5 Evaluation of the MFC chamber A method to evaluate the battery is estimated by using the internal resistance, since the reaction speed of the battery had limitations, and it is expressed as resistance. In the case of the MFC, the reaction speed was limited by the metabolism of the microorganism and the chamber structure consisting of components including an air-cathode and anode. Additionally, factors such as the volume and surface area of the air-cathode affect the performance of the MFC (19). The purpose of this study includes demonstrating the generation of electrical energy by *Cellulomonas* spp. using cellulose and evaluating the generated values. Therefore, in order to validate the performance of the MFC chamber used in this study, the internal resistance of the MFC was measured by using the same microorganism previously reported for the MFC, and the values of internal resistance were evaluated. That is, the performance of the MFC chamber used in this study was evaluated using *S. oneidensis* MR-1 by measuring the electric current and voltage curves.

The electric circuit was designed to estimate the internal resistance of the MFC (Fig. 2.2). In the figure, R_A, R_V, R_C, and R_{in} denote the internal resistance values of the ammeter, voltmeter, rheostat, and MFC, respectively. The I denotes the current value in the line and E denotes electromotive force. In the apparatus, R_A was considered negligibly small, and R_V was considered as infinite. This resulted in the following equation:

$$E = I R_{in} + I R_C \quad (1)$$

Conversely, the indicated value of the voltmeter (V) is given by the following equation:

$$V = I R_C \quad (2)$$

This resulted in the following equation:

$$V = E - I R_{in} \quad (3)$$

This implied that the internal resistance of the MFC could be estimated by the slope of the graph of V with respect to I. In this study, the R_C changed from 0 to 20 kΩ. E was plotted

with respect to *I*, and the internal resistance of the MFC linear portion of the graphs was estimated.

S. oneidensis MR-1 was precultured in the LB medium, and its cells were collected via centrifugation. The cell density was adjusted to an OD_{600} of 2.0 and added to the MFC inoculated at 30°C. *S. oneidensis* MR-1 secretes flavins mediating the extracellular electron transfer (20–22), and thus there was no need to add a mediator.

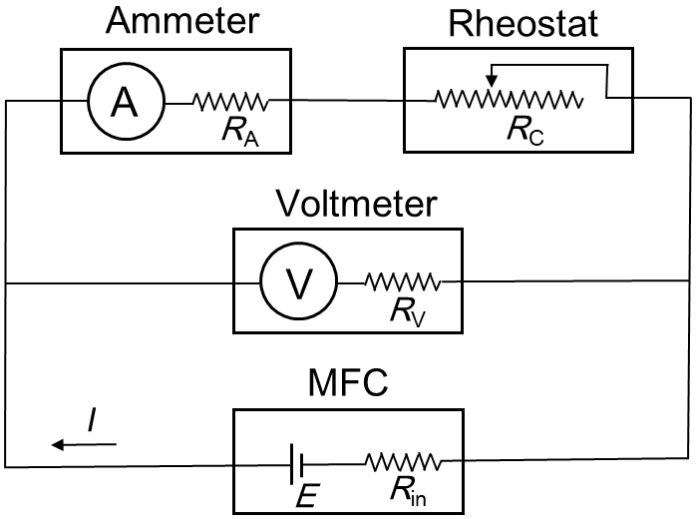

Fig. 2.2 The electric circuit to estimate an internal resistance of MFC. The R_A, R_V, R_C, and R_{in} stand for internal resistance values of ammeter, voltmeter, rheostat, and MFC, respectively. The *I* represents current value in the line and *E* electromotive force. In this apparatus, R_A is negligibly small, and R_V is considered to be infinite.

2.2.6 Comparison of mediators As far as the author searched, there is no previous study using *Cellulomonas* spp. for MFC. Therefore, the author has no information as to the amount and type of mediator required for MFC of this study. Anthraquinone-2,6-disulfonate (AQDS) (23) or neutral red (24) are often used as exogenous mediators. Neutral red and humic acid analog AQDS are commonly used as electron shuttles in studies examining extracellular electron transfer (25) due to their excellent stability, high solubility, low cost, and special electrocatalytic processes. Previous studies indicate that they are convenient redox mediators

for conducting electrochemical investigations of biological systems (26). In this study, AQDS at concentrations of 0.05, 0.4, and 2.0 mM and neutral red at a concentration of 0.4 mM were added into the LB medium containing the bacterial cells with an OD_{600} of 2.0 in the MFC inoculated at 30°C. Furthermore, the electric current generated by the inoculation of the LB medium containing 0.4 mM AQDS without bacterial cells was used as a control.

2.2.7 Current generation and determination of reducing sugar The MFC with a cellulose-containing medium was inoculated with *Cellulomonas* spp. precultured in the LB medium. The concentration was adjusted to an OD_{600} of 2.0. The MFC was inoculated in an incubator at 30°C and measured by the electric current measurements. The internal resistance of the ammeter was negligibly small, and therefore it was directly connected between an anode and a cathode of the MFC to measure the current. As an experimental control, 0.5 g of glucose instead of cellulose was added to the medium, and 1 mL of this solution was sampled every 24 h with simultaneous current measurements. In this study, cellulose and oligosaccharides were hydrolyzed and determined in terms of reducing sugars. The hydrolysis was performed: the sample and concentrated sulfuric acid were mixed in 3:1 ratio and the resultant mixture was heated at 120°C in an oil bath for 6 h. The sample changed color and a slightly brownish tinge was observed. The sample was then cooled and neutralized with NaOH (10 wt% aq) to pH 7–9 in water with ice. The reducing sugar in the samples was quantified by using the dinitrosalicylic acid (DNS) method (27). This was followed by diluting 1 mL of the sample with 10 mL of distilled water and then filtering the sample with a filter paper (5A, Toyo Roshi Kaisha, Ltd.). The residue was hydrolyzed, and the DNS method was performed to indicate the remaining cellulose. The filtered solution was centrifuged to remove microorganisms, and the DNS method was performed on a part of the supernatant fraction to indicate the reducing sugar. Another part of the supernatant fraction was hydrolyzed, and the DNS method was performed to indicate oligosaccharides.

2.3 RESULTS AND DISCUSSION

2.3.1 MFC operation Degradation test of cellulose Fig. 2.3 shows the result of the cellulose degradation by the three types of *Cellulomonas* spp. The 1.0 g of cellulose was decomposed by *C. fimi* and *C. biazotea* to 50% and 25%, respectively, in 4 days. An analysis of *C. biazotea* and *C. flavigena* indicated approximately equal amounts of cellulose degradation. In the above-mentioned cultures, the cellulose was more finely decomposed, and the solution became colloidal.

2.3.2 Evaluation of the MFC chamber After 24 h, the MFC was set in a thermostatic chamber at 30°C. This was followed by a step-by-step increase in the resistance values from 0 to 20 kΩ, and the values of electric current and voltage were measured at each step when it was stable (about 1 min). The measurement process was completed in 1 h. The cell and medium condition appeared stable such short period when compared with that over the entire measurement period of the MFC. A plot of the voltage and electrical energy with respect to the electric current was obtained by using *S. oneidensis* MR-1 in the LB medium in Fig. 2.4.

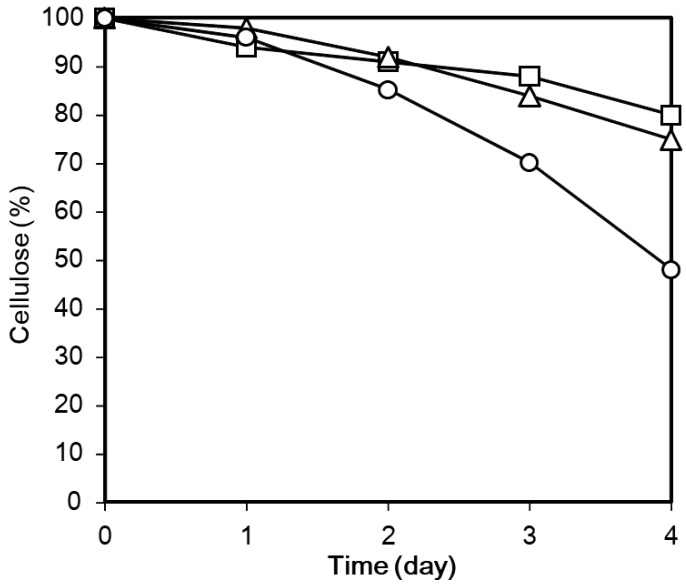

Fig. 2.3 Result of the cellulose degradation test with *C. fimi* (circles), *C. biazotea* (triangles), and *C. flavigena* (squares).

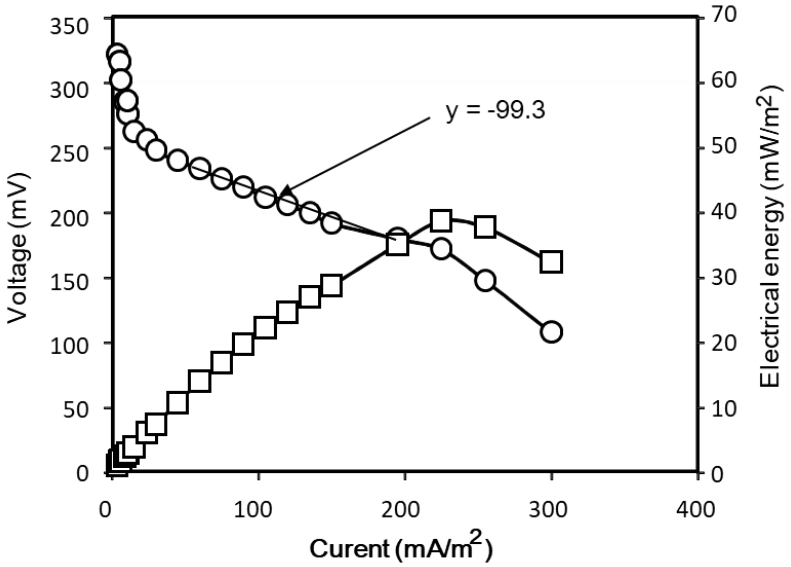

Fig. 2.4 Voltage (circles) and electrical energy (squares) against current of MFC with *S.oneidensis* MR-1 using LB medium. As calculated from the slope of the plot (y), the internal resistance is 81.3 Ω, and electromotive force from the intercept of the vertical axis is 339 mV.

As observed in the figure, a linear relationship was observed between the electric current and the voltage (from 100 to 250 mA/m^2). The internal resistance calculated from the slope of the plot was 81.3 Ω, and electromotive force calculated from the vertical axis intercept was 339 mV. Additionally, the maximum power density was 61.2 mW/m^2. Park and Zeikus (28) reported a maximum power density of 10.2 mW/m^2 in MFCs using *Shewanella putrefaciens*. Hence, this suggested that the MFC chamber used in the present study exhibited high efficiency.

2.3.3 Comparison of mediators Mediators play an important role in the electron transfer for microorganisms that transfer the electron to the anode using soluble redox mediators. A shuttle is a compound that diffuses to the surface of the electrode to transport electrons from the bacteria, and the shuttle itself is oxidized. The shuttle in its oxidation state then diffuses back to the cells and continues shuttling electrons between bacteria and the

electrode surface (29). Humic substances can serve as electron acceptors, and also as electron shuttles to ferric oxides (30–33). An alternative to humic substances involves a mobile small molecule capable of undergoing redox cycling of electron. Small quinoid compounds like AQDS have the characteristic of humic acid analog (34–37). Although *Escherichia coli* cannot reduce iron with humic acids (32), it can reduce iron with neutral red. Compounds similar in structure to neutral red such as riboflavin, pyocyanin, quinones, and humic acids occur in natural environments, and they also reduce iron when coupled with bacterial metabolism (20,25,32,38,39). Therefore, two mediators were tested in this study.

Electric current generation was not observed in the control of the LB medium containing 0.4 mM AQDS without bacterial cells. Also, electric current generation was not observed in only the LB medium. This indicated that the reduction material in the LB medium and the reducing AQDS did not affect the detection of the electric current generated from a microorganism. Fig. 2.5 shows the electric current generated by the MFC using AQDS and neutral red as mediators. Among the AQDS concentrations of 0.05, 0.4, and 2.0 mM, the AQDS at 0.4 mM showed the highest current value. This result indicated that AQDS at a concentration of 0.05 mM insufficiently performed an electron transfer between the cells and the anode. Conversely, the concentration of 2.0 mM was too high, and it had an adverse effect on cell metabolism. The most productive routes for energy generation typically involve the establishment of a proton-motive force across a membrane (40). Given that, 2 mM of AQDS transported the electron that required metabolism in the cell. It is reported that small aromatic compounds may have alternative roles, including iron reduction. For example, pyocyanin and AQDS can reduce iron but also have antibiotic activities (25).

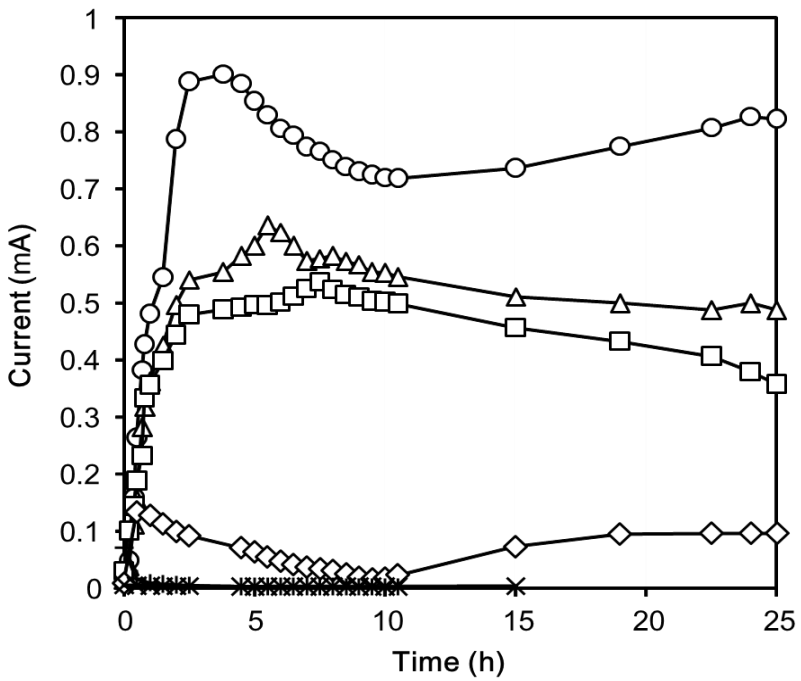

Fig. 2.5 Effect of mediator to the current of MFC using *C. fimi* and LB medium contains 0.4 mM AQDS (circles), 0.05 mM AQDS (triangles), 2.0 mM AQDS (squares), and 0.4 mM neutral red (diamonds). The LB medium containing 0.4 mM AQDS without bacteria cells (times) and only LB medium (pluses) did not generate current.

Given the same concentration of neutral red (that is, 0.4 mM), a lower value of electric current was generated when compared with that with AQDS. Therefore, the AQDS at a concentration of 0.4 mM was used as a mediator in this study. Electric current generation and determination of glucose, Fig. 2.6 shows the current generation using glucose and *C. fimi*. The electric current value reached a maximum of 1.8 mA in 12 h. On the third day, the glucose was exhausted, and the electric current was not generated.

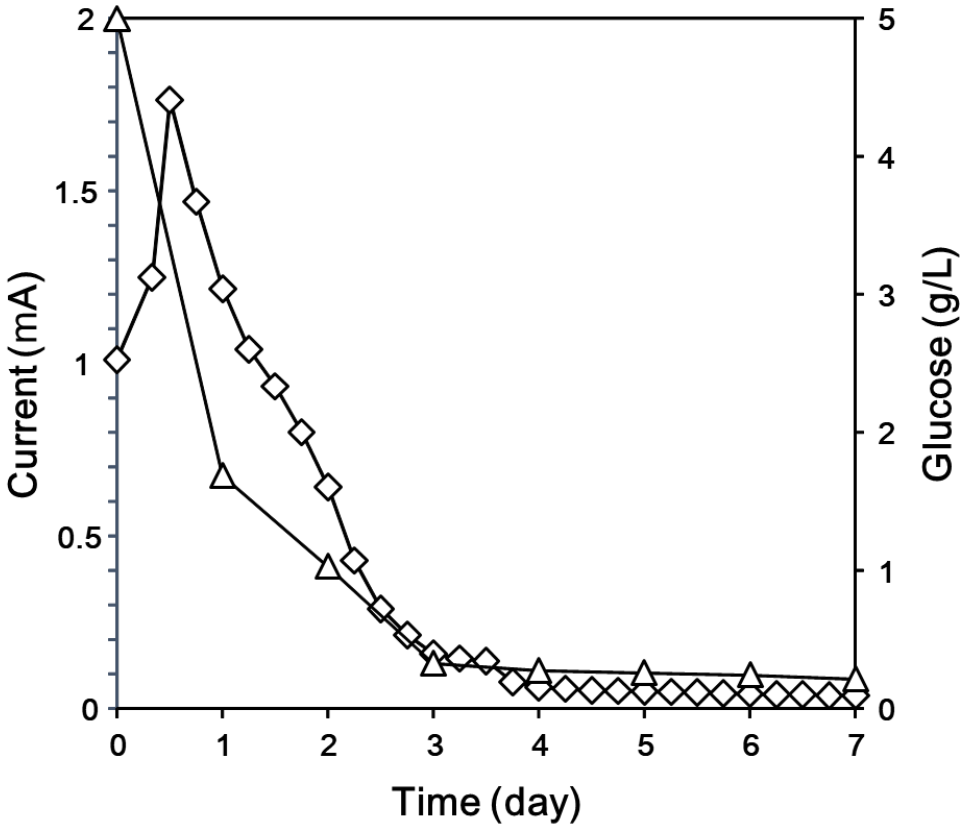

Fig. 2.6 Current generation (diamonds) and concentration of glucose (triangles) using *C. fimi*. The medium contains glucose instead of cellulose.

Fig. 2.7 shows the change in the concentration of the reducing sugar, and the electric current generated. *C. fimi* decreased the cellulose concentration by the decomposition of cellulose, whereas the concentration of the reducing sugar increased. The concentration of glucose decreased, indicating that the cells immediately consumed the glucose. The generated electric current decreased with the decrease in cellulose concentration, exhibiting a maximum value of 0.64 mA in 1.5 days. This result, when compared to that obtained for the glucose-containing medium as shown in Fig. 2.6, could be attributed to the time taken for the cellulose oligosaccharides to pass through the bacterial cells and decompose to form glucose. The concentration of cellulose on the seventh day reached 1/10 or less when compared with the original value despite the fact that the initial and final concentrations of the cell were constant at an OD_{600} of 2.0. Therefore, the decrease in the generated current value after the

seventh day was independent of the bacteriolytic process but depended on the decrease in the cellulose concentration.

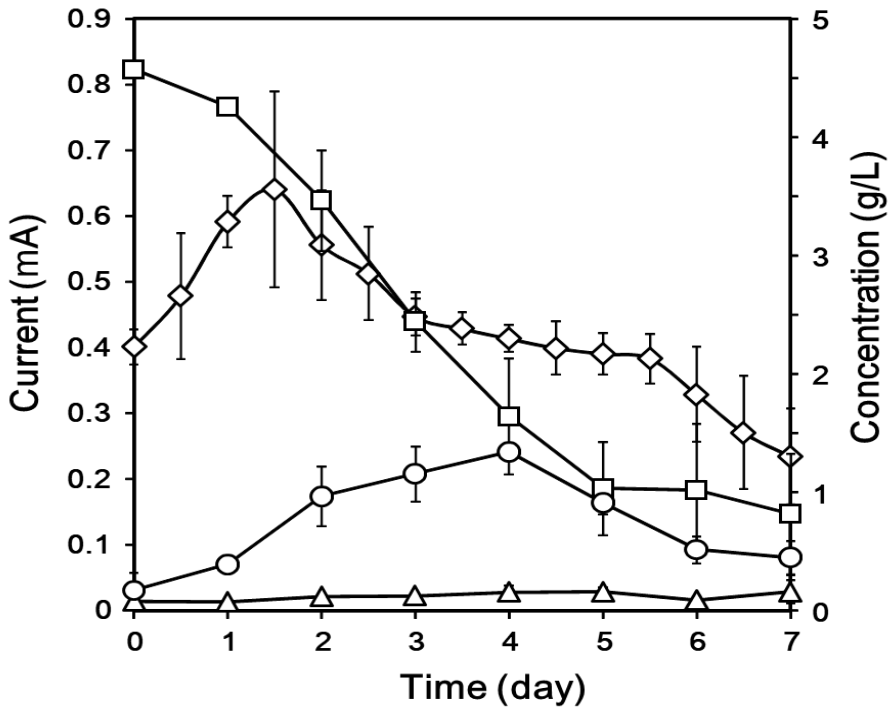

Fig. 2.7 Current generation and concentration of reducing sugar. In this study, cellulose and oligosaccharides were hydrolyzed and determined in terms of reducing sugars. Symbols: diamonds, current; circles, reducing sugar from oligosaccharide and reducing sugars; triangles, reducing sugars; squares, reducing sugar from cellulose. The error bars mean points of double experiments.

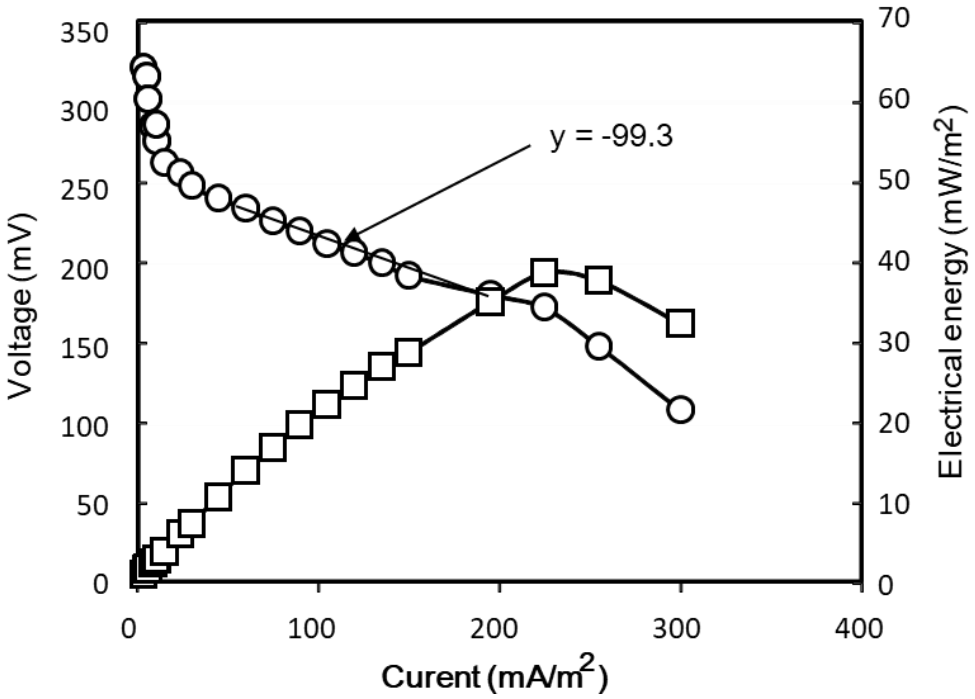

Fig. 2.8 Voltage (circles) and electrical energy (squares) against current of MFC with *C. fimi* using cellulose medium. As calculated from the slope of the plot (y), the internal resistance is 99.3 Ω, and electromotive force from the intercept of the vertical axis is 265 mV. The maximum power density was 38.7 mW/m^2.

Fig. 2.8 shows a linear relationship between the electric current and voltage between 75 and 200 mA/m^2. As calculated from the slope of the plot, the internal resistance was 99.3 Ω, and the electromotive force calculated from the vertical axis intercept was 265 mV. Additionally, the maximum value of the maximum power density was 38.7 mW/m^2. This value is 63 % of that exhibited by MFCs using *S. oneidensis* MR-1 in this study (Fig. 2.4). Previous studies examining cellulose-containing mediums obtained maximum power densities of 83 mW/m^2 using a co-culture of *C. cellulolyticum* and *G. sulfurreducens* (6), 54–112 mW/m^2 using an anaerobic sediment (7), 55 mW/m^2 using rumen microorganisms (8), and 10 mW/m^2 using a biofilm *Rhizobiales* in the main microorganism (9). The value obtained in the present study is comparable to the values obtained by these studies. However, the MFCs used in these reports used multiple or unknown microorganisms and medium components. In contrast, the

MFC in this report used a single microorganism and clear medium composition, and this could be advantageous for future biological analyses.

In the cathode, the electron flowed from the anode in the circuit and reacted with O_2 and H^+. These two reactions can be expressed by the following equations:

Anodic reaction $C_6H_{12}O_6 + 6\ H_2O \rightarrow 6\ CO_2 + 24\ H^+ + 24\ e^-$ (4)

Cathodic reaction $O_2 + 4\ e^- + 4\ H^+ \rightarrow 2\ H_2O$ (5)

Twenty-four Faradays of electrons are generated with the degradation of one mole of glucose to CO_2, and the electron is passed to the anode. While in the cathode, electron flowed from an anode in the circuit reacts with O_2 and H^+.

The microorganisms in the MFC governed the anode reaction, and the cathode reaction proceeded with Pt as catalyst. The cellulose-containing medium used for the experiment as shown in Fig. 2.7 contained 0.5 g of cellulose. The electron charge generated was estimated as 6432 C in the case of 0.5 g of cellulose degraded to CO_2 through glucose based on the above anodic reaction equation. Conversely, 545 C of electron charge was actually generated for 7 days from the cellulose given the integration of current over time. That is, the calculated Coulomb yield was 8.5%. The same calculation was applied to Fig. 2.6, wherein the calculated Coulomb yield was 9.8% in the case of when 0.5 g glucose was used. The maximum power obtained using glucose (Fig. 2.6) exceeded twice the value corresponding to that obtained using cellulose (Fig. 2.7). Nevertheless, the Coulomb yield obtained using glucose was only 1.2 times that obtained using cellulose. This suggested that the cellulose gradually degraded to reducing sugar as shown in Fig. 2.7 and was then metabolized in a manner that was almost the same as that of glucose.

The MFC showed great potential as it generated electricity along with wastewater treatment containing several types of organic compounds (41–50). In particular, the single chamber air-cathode MFC that was used in this study had advantages, including no aeration required, a simplified operation, and a smaller cell volume (43–48). However, the Coulombic efficiency of air-cathode single chamber MFCs is usually low due to oxygen crossover through the air-cathode that results in aerobic substrate degradation, which competes with anode reduction (31). Therefore, it is necessary to improve the chamber structure and air-cathode to achieve higher performance for this type of MFC (18,51). A large amount of waste paper is excreted in the environment, and wastewater contains large quantities of this waste paper. Although the use of mediators presents a barrier to the actual use of the system proposed in this study, this problem can be overcome by developing an anode that directly accepts electrons from *Cellulomonas* spp. or by the parallel fermentation of another microorganism that excretes substances that could function as mediators, for example, flavins. The findings of this study indicated the ability of *Cellulomonas* spp. to generate electricity from glucose. Future work can use the findings obtained by the MFC chamber in this study to realize more effective electricity generation.

References

1. **Barton, S. C., Gallaway, J., and Atanassov, P.:** Enzymatic biofuel cells for implantable and microscale devices, Chem. Rev., **104**, 4867–4886 (2004).
2. **Kim, J., Jia, H., and Wang, P.:** Challenges in biocatalysis for enzyme-based biofuel cells, Biotechnol. Adv., **24**, 296–308 (2006).
3. **Yan, Y., Zhen, W., Su, L., and Mao, L.:** Carbon-nanotube-based glucose/O_2 biofuel cells, Adv. Mater., **18**, 2639–2643 (2006).
4. **Xiong, Y., Shi, L., Chen, B., Mayer, M. U., Lower, B. H., Londer, Y., Bose, S., Hochella, M. F., Fredrickson, J. K., and Squier, T. C.:** High-affinity binding and direct electron transfer to solid metals by the *Shewanella oneidensis* MR-1 outer membrane c-type cytochromeOmcA, J.Am. Chem. Soc., **128**, 13978–13979 (2006).
5. **Lovley, D. R.:** Powering microbes with electricity: direct electron transfer from electrodes to microbes, Environ. Microbiol. Rep., **3**, 27–35 (2011).
6. **Ren, Z., Steinberg, L. M., and Regan, J. M.:** Electricity production and microbial biofilm characterization in cellulose-fed microbial fuel cells, Water Sci. Technol., **58**, 617–622 (2008).
7. **Rezaei, F., Richard, T. L., Brennan, R. A., and Logan, B. E.:** Substrate-enhanced microbial fuel cells for improved remote power generation from sediment-based systems, Environ. Sci. Technol., **41**, 4053–4058 (2007).
8. **Rismani-Yazdi, H., Christy, A. D., Dehority, B. A., Morrison, M., Yu, Z., and Tuovinen, O. H.:** Electricity generation from cellulose by rumen microorganisms in microbial fuel cells, Biotechnol. Bioeng., **97**, 1398–1407 (2007).
9. **Ishii, S., Shimoyama, T., Hotta, Y., and Watanabe, K.:** Characterization of a filamentous biofilm community established in a cellulose-fed microbial fuel cell, BMC Microbiol., **8**, 1–12 (2008).

10. **Stackebrandt, E. and Schumann, P.:** The family *Cellulomonadaceae*, pp. 163–184, in: Rosenberg, E., DeLong, E. F., Lory, S., Stackebrandt, E., and Thompson, F. (Eds.), The Prokaryotes: Actinobacteria. Springer Reference, Berlin (2014).
11. **Mansfield, S. D. and Meder, R.:** Cellulose hydrolysis e the role of monocomponent cellulases in crystalline cellulose degradation, Cellulose, **10**, 159–169 (2003).
12. **Dziga, D. and Jagiełło-Flasinska, D.:** Wheat straw degradation and production of alternative substrates for nitrogenase of *Rhodobacter sphaeroides*, Acta Biochim. Pol., **62**, 395–400 (2015).
13. **Bayer, E. A., Shimon, L. J. W., Shoham, Y., and Lamed, R.:** Cellulosomes structure and ultrastructure, J. Struct. Biol., **124**, 221–234 (1998).
14. **Sandercock, L. E., Meinke, A., Gilkes, N. R., Kilburn, D. G., and Warren, R. A. J.:** Degradation of cellulases in cultures of *Cellulomonas fimi*, FEMS Microbiol. Lett., **143**, 7–12 (1996).
15. **Dermoun, Z. and Belaich, J. P.:** Microcalorimetric study of cellulose degradation by *Cellulomonas uda* ATCC 21399, Biotechnol. Bioeng., **27**, 1005–1011 (1985).
16. **Reddy, V. L., Pradeep Kumar, S., and Wee, Y. J.:** Microbial fuel cells (MFCs) e a novel source of energy for new millennium, pp. 956–964, in: Méndez-Vilas, A. (Ed.), Current research, technology and education topics in applied microbiology and microbial biotechnology. Formatex, Spain (2010).
17. **Ichihashi, O., Yamamoto, N., and Hirooka, K.:** Power generation by and microbial community structure in microbial fuel cell treating animal wastewater, J. Jpn. Soc. Water Environ., **35**, 19–26 (2012).
18. **Cheng, S., Liu, H., and Logan, B. E.:** Increased performance of single-chamber microbial fuel cells using an improved cathode structure, Electrochem. Commun., **8**, 489–494 (2004).

19. **Oh, S. and Logan, B. E.:** Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies, Water Res., **39**, 4673–4682 (2005).

20. **Newman, D. K. and Kolter, R.:** A role for excreted quinones in extracellular electron transfer, Nature, **405**, 94–97 (2000).

21. **Von Canstein, H., Ogawa, J., Shimizu, S., and Lloyd, J. R.:** Secretion of flavins by *Shewanella* species and their role in extracellular electron transfer, Appl. Environ. Microbiol., **74**, 615–623 (2008).

22. **Marsili, E., Baron, D. B., Shikhare, I. D., Coursolle, D., Gralnick, J. A., and Bond, D. R.:** Shewanella secretes flavins that mediate extracellular electron transfer, Proc. Natl. Acad. Sci. USA, **105**, 3968–3973 (2008).

23. **Aulenta, F., Maio, V. D., Ferri, T., and Majone, M.:** The humic acid analogue antraquinone-2,6-disulfonate (AQDS) serves as an electron shuttle in the electricity-driven microbial dechlorination of trichloroethene to cis-dichloro-thene, Bioresour. Technol., **101**, 9728–9733 (2010).

24. **Park, D. H. and Zeikus, J. G.:** Electricity generation in microbial fuel cells using neutral red as an electronophore, Appl. Environ.Microbiol., **66**, 1292–1297 (2000).

25. **Shyu, J. B. H., Lies, D. P., and Newman, D. K.:** Protective role of tolC in efflux of the electron shuttle anthraquinone-2,6-disulfonate, J. Bacteriol., **184**, 1806–1810 (2002).

26. **Jeykumari, D. R. S., Kalaivani, R., and Narayanan, S. S.:** Nanobiocomposite electrochemical biosensor utilizing synergic action of neutral red functionalized carbon nanotubes, Nano-Micro Lett., **4**, 220–227 (2012).

27. **Sumner, J. B.:** Dinnitrosalicylic: a reagent for the estimation of sugar in normal and

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28. **Park, D. H. and Zeikus, J. G.:** Impact of electrode composition on electricity generation in single-component fuel cell using *Shewanella purrefaciens*, Appl. Microbiol. Biotechnol., **59**, 58–61 (2002).

29. **Kracke, F., Vassilev, I., and Krömer, J. O.:** Microbial electron transport and energy conservation e the foundation for optimizing bioelectrochemical systems, Front. Microbiol., **11**, 1–18 (2015).

30. **Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, E. J. P., and Woodward, J. C.:** Humic substances as electron acceptors for microbial respiration, Nature, **382**, 445–448 (1996).

31. **Coates, J. D., Ellis, D. J., Blunt-Harri, E. L., Gaw, C. V., Roden, E. E., and Lovley, D. R.:** Recovery of humic-reducing bacteria from a diversity of environments, Appl. Environ. Microbiol., **64**, 1504–1509 (1998).

32. **Benz, M., Schink, B., and Brune, A.:** Humic acid reduction by *Propionibacterium freudenreichii* and other fermenting bacteria, Appl. Environ. Microbiol., **64**, 4507–4512 (1998).

33. **Scott, D. T., McKnight, D. M., Blunt-Harris, E. L., Kolesar, S. E., and Lovley, D. R.:** Quinone moieties act as electron acceptors in the reduction of humic substances by humics-reducing microorganisms, Environ. Sci. Technol., **32**, 2984–2989 (1998).

34. **Lovley, D. R., Fraga, J. L., Blunt-Harris, E. L., Hayes, L. A., Phillips, E. J. P., and Coates, J. D.:** Humic substances as a mediator for microbially catalyzed metal reduction, Acta Hydrochim. Hydrobiol., **26**, 152–157 (1998).

35. **Hernandez, M. E. and Newman, D. K.:** Extracellular electron transfer, Cell. Mol. Life Sci., **58**, 1562–1571 (2001).

36. **Myers, C. R. and Nealson, K. H.:** Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor, Science, **240**, 1319–1320 (1988).

37. **Tratnyek, P. G. and Macalady, D. L.:** Abiotic reduction of nitro aromatic pesticides in anaerobic laboratory systems, J. Agri. Food Chem., **37**, 248–254 (1989).

38. **Cox, C. D.:** Role of pyocyanin in the acquisition of iron from transferrin, Infect. Immun., **52**, 263–270 (1986).

39. **Worst, D. J., Gerrits, M. M., Vandenbroucke-Grauls, C. M. J. E., and Kusters, J. G.:** *Helicobacter pylori ribBA*-mediated riboflavin production is involved in iron acquisition, J. Bacteriol., **180**, 1473–1479 (1998).

40. **Mitchell, P.:** Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism, Nature, **191**, 144–148 (1961).

41. **Abourached, C., English, M. J., and Liu, H.:** Wastewater treatment by microbial fuel cell (MFC) prior irrigation water reuse, J. Clean. Product., **137**, 144–149 (2016).

42. **Liu, H. and Logan, B. E.:** Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane, Environ. Sci. Technol., **38**, 4040–4046 (2004).

43. **Liu, H., Ramnarayanan, R., and Logan, B. E.:** Production of electricity during wastewater treatment using a single chamber microbial fuel cell, Environ. Sci. Technol., **38**, 2281–2285 (2004).

44. **Tender, L. M., Reimers, C. E., Stecher, H. A., III, Holmes, D. E., Bond, D. R., Lowy, D. A., Pilobello, K., Fertig, S. J., and Lovley, D. R.:** Harnessing microbially generated power on the seafloor, Nat. Biotechnol., **20**, 821–825 (2002).

45. **Kim, J. R., Min, B., and Logan, B. E.:** Evaluation of procedures to acclimate a microbial fuel cell for electricity production, Appl. Microbiol. Biotechnol., **68**, 23–30 (2005).

46. **Rabaey, K., Boon, N., Siciliano, S. D., Verhaege, M., and Verstraete, W.:** Biofuel cells select for microbial consortia that self-mediate electron transfer, Appl. Environ.

Microbiol., **70**, 5373–5382 (2004).

47. **Liu, H., Cheng, S., and Logan, B. E.:** Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell, Environ. Sci. Technol., **39**, 658–662 (2005).
48. **Park, D. H. and Zeikus, J. G.:** Improved fuel cell and electrode designs for producing electricity from microbial degradation, Biotechnol. Bioeng., **81**, 348–355 (2003).
49. **Cheng, S. A., Liu, H., and Logan, B. E.:** Power densities using different cathode catalysts (Pt and CoTMPP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells, Environ. Sci. Technol., **40**, 364–369 (2006).
50. **Gil, G. C., Chang, I. S., Kim, B. H., Kim, M., Jang, J. K., Park, H. S., and Kim, H. J.:** Operational parameters affecting the performance of a mediator-less microbial fuel cell, Biosens. Bioelectron., **18**, 327–334 (2003).
51. **Fan, Y., Hu, H., and Liu, H.:** Enhanced coulombic efficiency and power density of air-cathode microbial fuel cells with an improved cell configuration, J. Power Sour., **171**, 348–354 (2007).

Chapter 3

Direct electron transfer of *Cellulomonas fimi* and microbial fuel cell fueled by cellulose

3.1 INTRODUCTION

Nowadays mankind is facing serious energy issue, which is why technologies with high efficiency of energy conversion are required. Microbial fuel cell (MFC) is one of the important technologies for a sustainable society, and can convert the electrochemical energy from biomass to electricity by microbial metabolism of microorganism. Cellulosic biomass is one of the most abundant renewable sources of energy on the earth. A large amount of agricultural cellulosic wastes was discarded to the environment, and definitely wastewater contained a large amount of cellulose (1). If the electrochemical energy of cellulose in water can be converted to electric energy, this will help contribute to reducing the amount of petroleum usage and to realizing a sustainable society. MFCs are a possible option for evaluating the electrical performances of cellulosic materials, however, cellulose-fed MFCs particularly need to overcome the following problems: the low efficiency of electric generation, suitable microorganism for cellulose decomposition, and the addition of mediator to facilitate electron transfer, which is rather expensive and harmful to microorganisms. In chapter 2, it had mentioned that the excess addition of anthraquinone-2,6-disulfonate in the *C. fimi*-based MFCs caused the decrease of current density.

The MFCs using *Shewanella oneidensis* required no mediator because the strain secreted flavins to the outside of the cell (2), and had outer-membrane cytochromes which transfer electron from the cell to the electrode (3). This microorganism produced electrically conductive

networks of pilus-like appendages called bacterial nanowires (4). This strain cannot use cellulose, whereas mainly lactic acid as fuel for the MFCs (5).

The self-electron transferable microorganism has been extensively investigated because of no requirement for external electron shuttles which are expensive, and may affect the bacterial growth as mentioned in chapter 2. Some microorganisms also inherently transferred the electron using self-provided electron shuttles such as flavins, coenzyme A, cytochrome, and NADH (6–8). However, the cellulose-fed, mediator-less MFC with *C. fimi* has not yet been reported.

Mediator-less, double-chambered MFC using *Pseudomonas aeruginosa* had been reported (9). This microorganism is known as cytoplasmic membrane-embedded respiratory chain (10) which plays a role in the electron transfer. Similarly, mediator-less MFC with *Hansenula anomala* using glucose as fuel had been reported with the analysis of the isolated membrane containing redox enzymes such as lactate dehydrogenase, NADH-ferricyanide reductase, NADPH-ferricyanide reductase, and cytochrome *b5* (11). Ren et al. analyzed electricity generation and the microbial ecology of cellulose-fed MFCs of a two-chambered type by using the defined co-culture of *Clostridium cellulolyticum* and *Geobacter sulfurreducens* (12(. Ishii et al. inoculated the soil from rice paddy fields using cellulose as a carbon and energy source for a two-chambered MFC with *Clostridiales*, *Chloroflexi*, *Rhizobiales,* and *Methanobacterium* (13). In these two reports, the fuel solution contained vitamins, consequently it is difficult to consider whether cell directly transfers electron or secretes mediator because vitamins can perform as mediator. Rezaei et al. reported a sediment-based system of chitin and cellulose used as substrate. The sediment used in their study was the anaerobic sediment obtained from the Delaware Bay, and it contained several types of microorganism (14). Inoue et al. reported the MFC using lignocellulosic biomass in cattle manure as fuel, and explained the bacterial community of *Bacteroides* and *Clostridium*

performing as electric generators (15). In these two reports, microbial species has not been clarified, therefore there is a possibility that the fuel itself contains substances which facilitate as mediators. Rismani-Yazdi et al. examined the electricity generation using rumen microorganisms as biocatalysts and cellulose as the electron donor in two-chambered MFCs (16). While, the microorganisms in rumen had not also been clarified in this research, and the possibility that the components of rumen fluid perform as a mediator cannot be denied.

To the best of author's knowledge, no study concerning MFCs using cellulose as fuel with direct electron transfer has been examined. Chapter 2 had reported the MFC using *Cellulomonas* spp. with cellulose as fuel and demonstrated that the cellulose-fed MFC with *C. fimi* generated current approximately 0.9 mA (180 mA/m^2) in Luria–Bertani (LB) medium with the addition of 0.4 mM of anthraquinone-2,6-fisulfonic acid disodium salt (AQDS). In this present chapter, investigated the mediator-less MFC fed with cellulose in water, and clarified the bacterial electron transfer mechanism from *C. fimi* to the electrode.

3.2 MATERIALS AND METHODS

3.2.1 Bacterial strain and fuel solution *C. fimi* NBRC 15513 was used in this study. Luria–Bertani (LB) medium was prepared by 1 g of Tryptone (Nacalai Tesque Inc., Kyoto, Japan), 0.5 g of yeast extract (Nacalai Tesque), and 1 g of NaCl, dissolved in 100 mL of distilled water. The cellulose fuel solution was prepared by 0.5 g of cellulose powder (38 μm: through 400 mesh, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and 1.0 g of NaCl, dissolved in 100 mL of phosphate buffer (0.1 M, pH 7.0). A pre-culture was prepared with LB medium cultivated at 30°C for 48 h, shaken at 120 rpm. The cell was centrifuged (MX-301, Tomy digital biology Co., Tokyo, Japan) at 2,500 × *g* for 5 min, at 4°C. Then the precipitate was washed with physiological saline for 3 times to eliminate the components of LB medium.

3.2.2 The chamber configuration of MFC and MFC operation The MFC chamber used in this study was a single chamber MFC which was the same as chapter 2 (Fig. 2.1). The following is the points: The anode is a carbon felt (LF-210, Osaka Gas Chemicals Co., Osaka, Japan). The air cathode has the effective area of 50 cm^2 with three layers, namely a catalyst layer consisting of Pt-supported carbon (IFPC40-III, Ishifuku metal industry Co, Tokyo, Japan) with perfluorinated resin (Nafion 510211, Sigma–Aldrich, MO, USA), a carbon-paper layer (TGP-120, Toray Co., Tokyo, Japan), and a polytetrafluoroethylene layer (PTFE, 60% dispersion, 31-JR, Du Pont–Mitsui Fluorochemicals Co. Tokyo, Japan) and the MFC chamber volume is 100 mL. Before assembling the MFC, the chamber components were sterilized by irradiating UV light on the disassembled components in a clean bench. The MFC was filled with the 100 mL of cellulose culture containing the washed cell at the optical density (OD_{600}) of 0.2, and then Nitrogen gas (N_2) was bubbled (100 mL/min for 3 min) into the culture in the MFC. The experiment without the addition of the cell was performed as a reference to support author's idea regarding the electron transfer mechanism, the reference experiments of the *C. fimi*-free system were carried as described above. After preparation, the MFC was incubated for 5 days at 30°C. The current measurement was started at this time. The 1 mL of fuel solution was daily sampled to measure colony forming unit (CFU) and optical density (OD_{600}). After sampling, the fuel solution in the MFC was purged with N_2 again.

3.2.3 Measurement of electric generation The circuit for measuring the generated current of the MFC was the same as chapter 2 (Fig. 2.2). The following is the points: The electric current generated by the MFC was monitored using a digital multimeter (KEW 1062, Kyoritsu Electrical Instruments, Tokyo, Japan) and was automatically recorded by application software (model 8241, Kyoritsu Electrical Instruments). Polarization and power density curves were obtained using a rheostat at various external resistances (0–30 kΩ). The current had steadily risen to the maximum point, when the current and voltage of each step

were measured. The potential was recorded after it had stabilized approximately 1 min. The maximum power density and ohmic resistance of the MFC were acquired by the polarization curves.

3.2.4 Cyclic voltammetry The electron transfer of the cellulose fuel solution was confirmed whether it contains mediator-like compounds or not, the cellulose fuel solution was filtered with a polytetrafluoroethylene membrane filter (pore size = 0.22 μm, As one Co., Osaka, Japan) to remove the precipitates of cellulose, and measured by the CV measurement. The direct electron transfer from the cell surface of *C. fimi* to electrode was also studied, two samples were prepared by adding 0.5 g of the washed cell into 5.0 mL of phosphate buffer (0.1 M, pH 7.0). One sample was filtered to remove the cell before the CV measurement but another sample still containing the cell was measured without filtration. An effect on inhibition of antimycin A was carried out, more samples were prepared as above mentioned and then each sample was added antimycin A (from *Streptomyces* sp, Sigma–Aldrich) of 1 μg, 10 μg and 1 mg to 5.0 mL of phosphate buffer (0.1 M, pH 7.0) containing 0.5 g of the washed cell. All the samples were kept at 30°C for 24 h under anaerobic condition, and then the CV measurement was carried out. Each 5 ml of sample was poured into the CV test vessel, then N_2 was bubbled (100 mL/min for 3 min) in the sample not only before keeping the sample for 24 hours but also before the CV measurement. To clarify the effect of remaining oxygen in the fuel solution, the filtrated cellulose fuel solution without bubbling N_2 at the initial operation and after the 5-day operated MFC were measured as an experimental reference.

The condition of the CV measurement was as follows: A glassy carbon (diameter: 3 mm), platinum wire, and Ag/AgCl (1 M KCl), (+0.20 V vs. NHE) were used as a working electrode, a counter electrode, and a reference electrode respectively. The glassy carbon electrode was polished using 0.5 μm alumina powder (Wako Pure Chemical Industries, Osaka, Japan), and then sonicated and washed to remove the remaining alumina powder with distilled water in an

ultrasonic bath (US-102, SND Ltd., Nagano, Japan) before each measurement. The potential scan was started at +0.5 V toward positively at 5 mV/s of the scan rate at room temperature. In addition, the alive cell before and after keeping the cell solution for 24 h was observed by the CFU on LB agar plates.

3.3 RESULTS AND DISCUSSION

3.3.1 MFC operation Fig. 3.1 shows the current generation of the MFC cultivated over five days with and without *C. fimi*. The current density of the MFC cultivated with *C. fimi* increased gradually and reached the maximum value of 10.0 ± 1.8 mA/m^2 whereas the current density without *C. fimi* was 1.6 ± 0.8 mA/m^2. The result indicated that the cellulose-fed MFC with *C. fimi* generated electricity even though the cell from pre-cultured medium was washed with physiological saline to eliminate the components of LB medium containing riboflavin and other flavin compounds in yeast extract serving as mediator (17,18). Therefore, the electric generation of the MFC demonstrated that metabolite of *C. fimi* facilitate electron transfer or *C. fimi* secreted mediator-like compounds to the outer electron transfer system (19).

As aforementioned, the electrical performances of the cellulose-fed MFC using *C. fimi* with anthraquinone-2,6-disulfonate as an electron shuttle had been reported. Even though the current generation was relatively higher than that observed in the present study, this study has revealed that the cellulose-fed MFC using *C. fimi* generated electricity without an additional mediator.

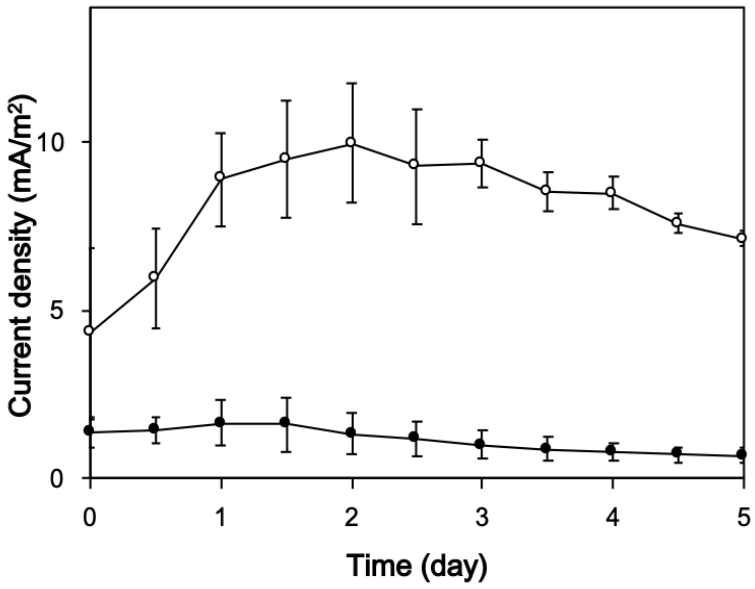

Fig. 3.1 Current generation of MFC with cellulose fuel solution containing 0.5 g of cellulose powder and 1.0 g of NaCl in 100 mL of phosphate buffer (0.1 M, pH 7.0). Open cycles indicate inoculated OD_{600} = 0.2 of washed cells, while closed cycles indicate no cells. MFCs are incubated at 30°C. Error bars indicate the standard deviations of the three-time experiments.

3.3.2 Polarization A polarization curve of the MFC is consisted of three characteristic regions located at different current ranges: The charge transfer overpotential is in the region of low current density; the ohmic overpotential (ohmic resistance) is in a region of intermediate current density; lastly, the mass transfer overpotential is in a region of relatively high current density. The ohmic resistance is caused by ionic resistance in electrolyte, membrane, and by electronic resistance in the electrodes, current collectors, interconnects, and the electronic components (20). The ohmic resistance of the MFC can be calculated from the linear portion of the slope in the region of the straight line of the voltage versus current density of the MFC and this ohmic resistance is used as an evaluation parameter. The polarization curve of the MFC was shown in Fig. 3.2. The maximum power density was 0.74 ± 0.07 mW/m^2 with the ohmic resistance of 6.9 kΩ estimated from the linear portion (0.015 − 0.039 mA).

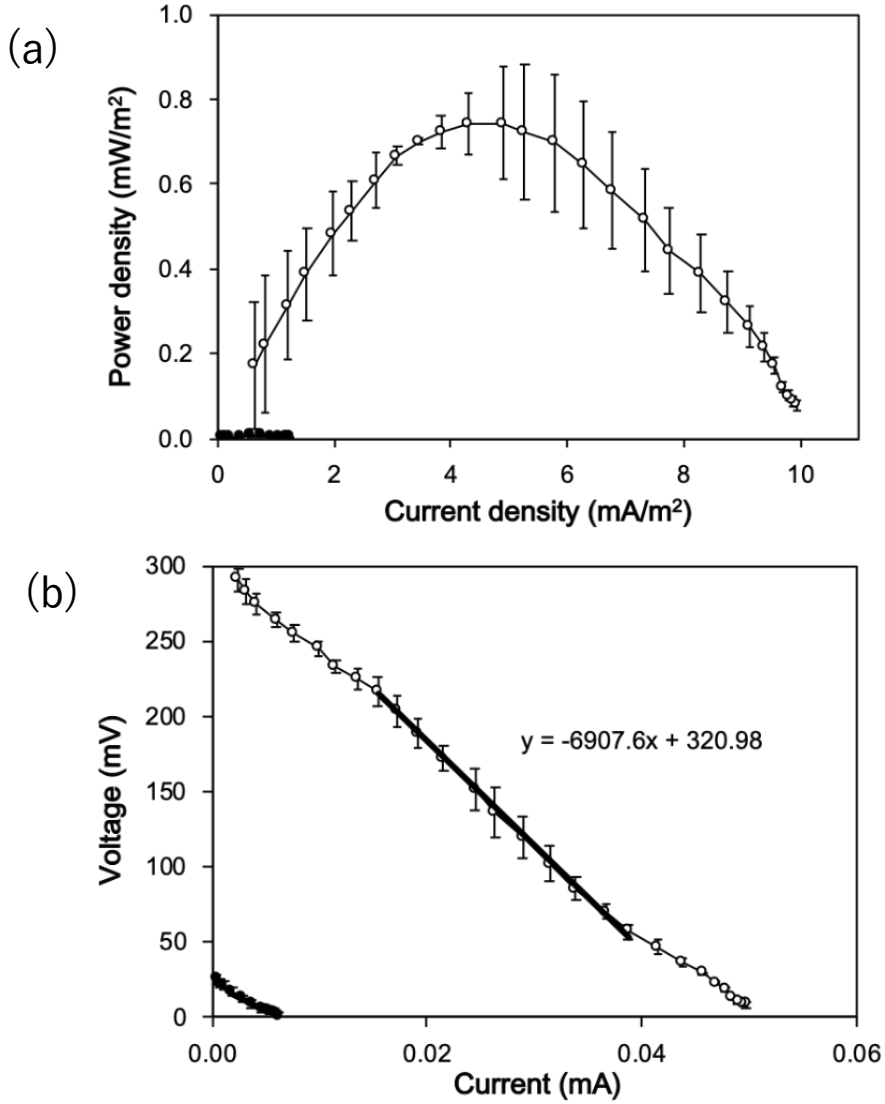

Fig. 3.2 Performance of the cellulose-fueled mediator-less MFC. (a) Potential of MFC and (b) polarization curves of MFC. Ohmic resistance is estimated from the linear portion of the curve (0.015 – 0.039 mA). Error bars indicate the standard deviations of the three-time experiments. Open symbols indicate containing cells (OD_{660} = 0.2) and closed indicate without cells.

3.3.3 Cyclic voltammetry The electron transfer classification of microorganisms can be classified into three types; Firstly, artificial mediators such as anthraquinone-2,6-disulfonate, *potassium ferricyanide* or neutral red; secondly, the bacteria-itself secreted mediators such as quinone or flavin derivatives; and finally, direct electron transfer from the cell surface to an anode (21).

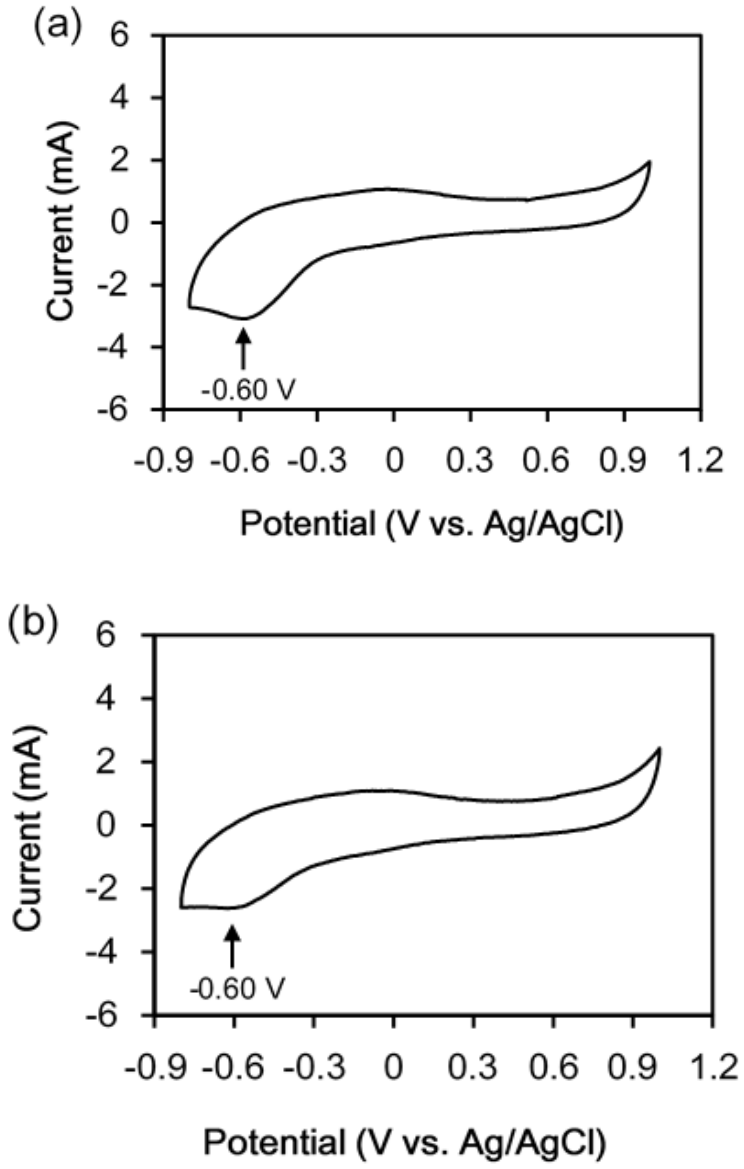

Fig. 3.3 Cyclic voltammograms of the cellulose fuel cell solution without N_2 bubbling. (a) Filtrated at the start of the operation, (b) filtrate obtained after 5 days MFC operation. The reduction peak of oxygen at –0.60 V vs. Ag/AgCl was observed from both measurements.

Fig. 3.3 shows the cyclic voltammograms of cellulose fuel solution without N_2 bubbling at the initial step of MFC operation (Fig. 3.3a) and after the 5-day operation (Fig. 3.3b). The reduction peak of oxygen at –0.60 V vs. Ag/AgCl (–0.40 V vs. NHE) was observed from both of CV measurements (22). Therefore, oxygen performed as the electron acceptor in the MFC in the cellulose fuel solution. For the reason, the fuel solutions were bubbled by N_2 from now

on in this study. No peak was observed from the cyclic voltammogram of the cellulose fuel solution alone (Fig. 3.4a) therefore, this demonstrated that there was no electron transferring compounds in the cellulose culture. Similarly, no peak appearance was also observed from the filtrated phosphate buffer containing the washed cell (Fig. 3.4b). Thus, *C. fimi* was assumed not to secrete mediator-like compound whereas the washed cell-containing phosphate buffer provided oxidation peak at the potential of +0.68 V vs. Ag/AgCl (+0.88 V vs. NHE), but reduction peak was not observed (Fig. 3.4c). The peak observed at +0.68 V is due to the oxidation of the reduced form of cytochrome *c* (Fe^{2+}) which is usually present in the reduced form in an anaerobic condition as this experiment. No peak appearance during the negative scan indicated that this reaction is almost irreversible and similar to the irreversible oxidation of ascorbic acid at the electrode on which the reduction of dehydroascorbic acid never proceeds (23,24). The phenomenon may be caused by the low reduction of the oxidized cytochrome *c* (Fe^{3+}) at the electrode without any mediator. In the 3-time scanned cyclic voltammograms, the oxidation peaks gradually decreased except not adding the cell (Fig. 3.4a), the occurrence was probably caused by the adhesion of cell on the electrode surface and the cell was still alive during the CV measurement (Fig. 3.5). These facts demonstrated that the alive cell donated electron to the electrode and oxidation reaction occurred during the metabolism of *C. fimi*. Similarly, the gram-positive organisms, the same phylum as *C. fimi*, had been revealed to transfer electrons from the inner membrane to the cell wall or membrane via periplasmic proteins involving the extensive scattering of soluble cytochrome *c* on the inner membrane (25,26), therefore, *C. fimi* has the possible ability of direct electron transfer from cell membrane surface to electrode. In other words, the electro-active enzymes in the membrane enable the transfer of electrons generated during metabolism to the electrode without external mediator.

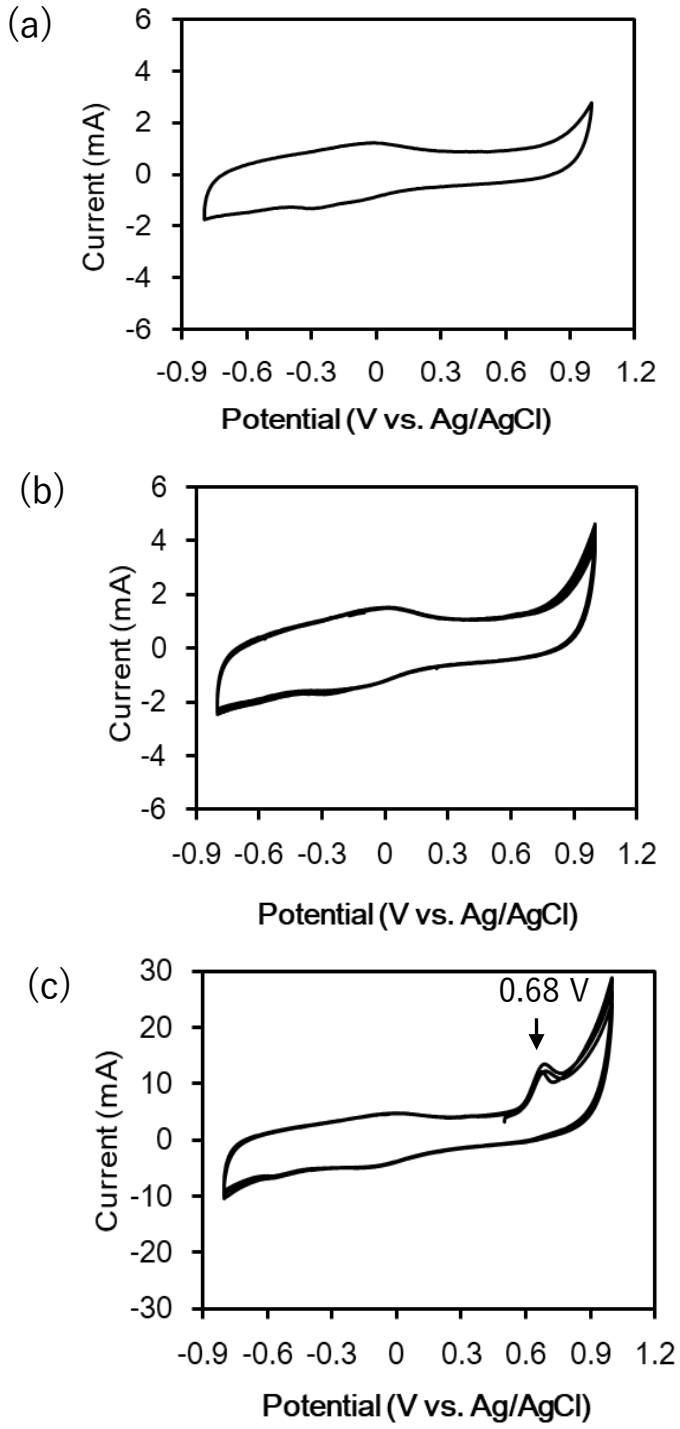

Fig. 3.4 Cyclic voltammograms of the cellulose fuel solution without N_2 bubbling at room temperature. (a) Filtrated cellulose fuel solution, (b) washed cell was added in phosphate buffer (0.1 M, pH 7.0) and then filtrated, (c) washed cell was added in phosphate buffer. Each CV was performed in three cycles. Potential scan started at +0.5 V in the positive direction at a potential scan rate of 5 mV/s. Oxidation peaks gradually decreased at +0.68 V vs. Ag/AgCl.

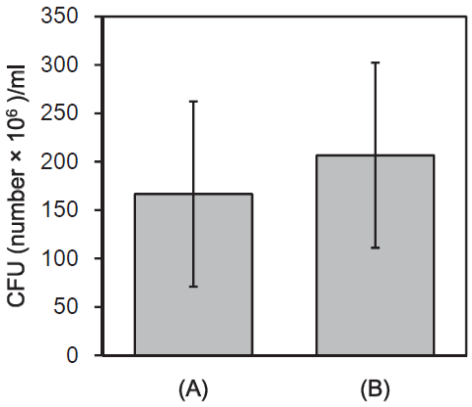

Fig. 3.5 Colony forming unit of the living cell on LB agar plate. (A) Immediately after suspended in phosphate buffer (0.1 M, pH 7.0), and (B) keeping 24 h just before measuring by cyclic voltammetry. Error bars indicate standard deviations of the three-time experiments.

3.3.4 Antibiotics effect on electron transfer A previous study had reported that cytochrome *c* can enable direct electrical contact with electrode (27). For instance, *C. fimi* was assumed to possess cytochrome *c* on its outer membrane surface which enables direct transfer electron in the MFCs. Moreover, *G. sulfurreducens* was disrupted to demonstrate the existence of cytochrome *c* (27). However, such method described in that previous study could not measure cytochrome *c* on the cell membrane surface of alive cell. The measurement of cytochrome *c* from alive cell was assumed to be more efficient than dead cell or bacterial cell membranes in the present study because of its efficient performance in current generation. Notably, the detection of cytochrome *c* from alive cell was suggested via bacterial biofilm with spectroscopic analysis (28,29). Herein, an alternative technique was proposed to study the presence of cytochrome *c* from alive cell.

Antimycin A binds to the quinone reduction site of the cytochrome bc_1 complex and inhibits the electron transfer (30). The sample without adding antimycin A shows the peak position at around +0.68 V vs. Ag/AgCl. The oxidation peaks were supposed to be the electrochemical response of cytochromes of *C. fimi*. In the case of adding antimycin A, the peaks decreased obviously even in the addition of 1 μg of antimycin A (Fig. 3.6).

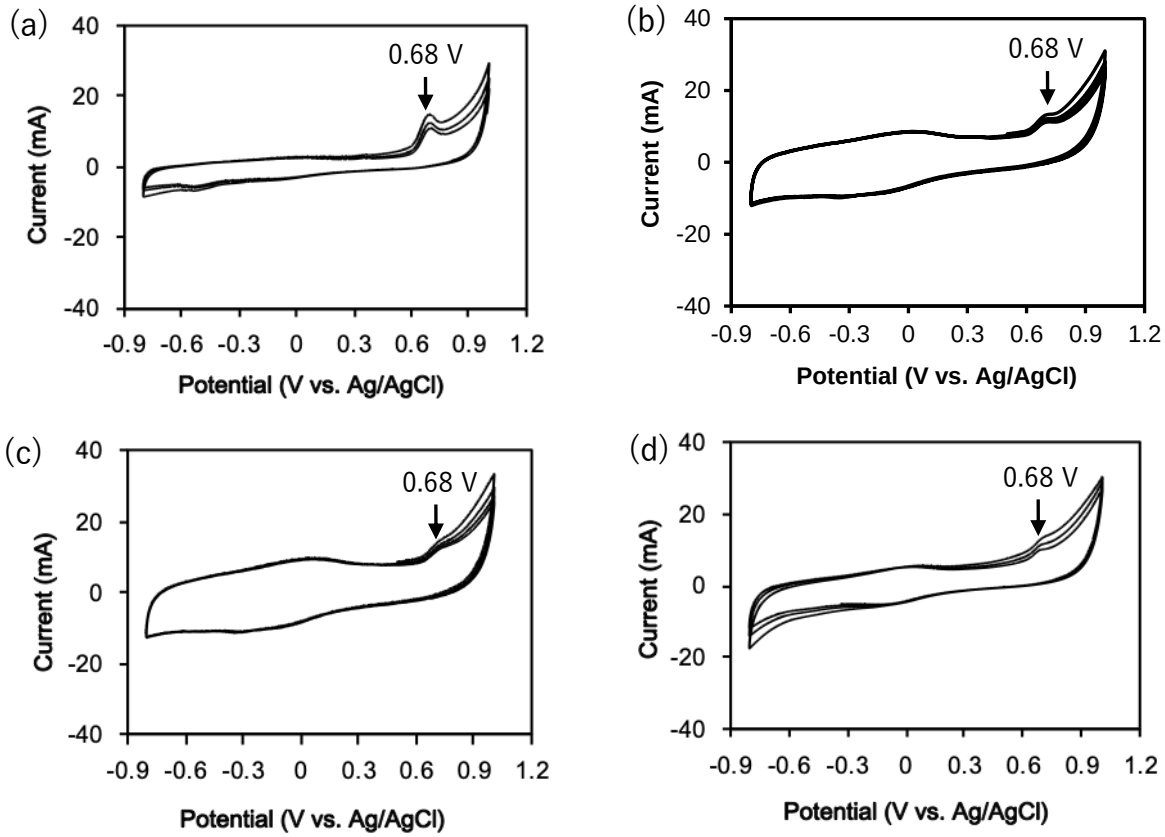

Fig. 3.6. Cyclic voltammograms of 5.0 mL of phosphate buffer (0.1 M, pH 7.0) containing 0.5 g of washed cells (a) without antimycin A, (b) with 1 µg of antimycin A, (c) with 10 µg of antimycin A, and (d) with 1 mg of antimycin A. Cyclic potential scan is repeated three times in each system. Potential scan started at +0.5 V in the positive direction at a potential scan rate of 5 mV/s. CV measurements are performed at room temperature. Oxidation peaks at +0.68 V vs. Ag/AgCl gradually decreased on each cyclic voltammogram.

The electron transfer activity of cytochrome *c* in the respiratory chain was assumed to be inhibited by antimycin A, and cytochrome *c* was present on the cell membrane surface. As well as Fig. 3.4, the oxidation peaks gradually decreased as shown in the 3 time-scanned cyclic voltammograms because of the cell adhesion onto the glassy carbon electrode. As aforementioned, the much cell adhesion became the resistance of electron transfer, thus electron may not be transferred to the entire cell membrane surface of the whole *C. fimi* in the CV sample. The cell survival after adding antimycin A into the samples was measured by the CFU and the result showed that the cell was alive (Fig. 3.7).

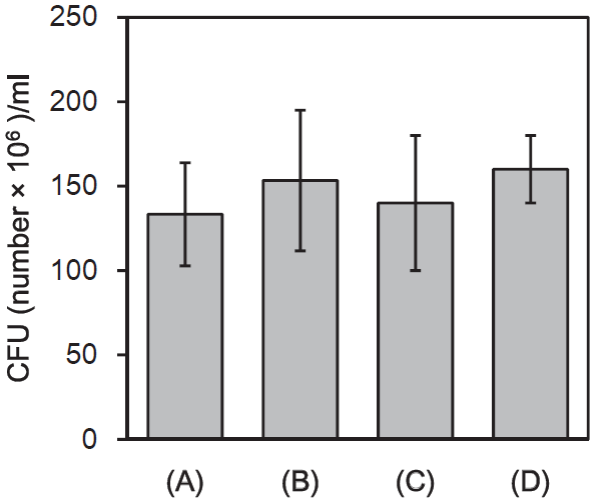

Fig. 3.7 Colony forming unit of living cell on LB agar plate of 5.0 mL of phosphate buffer (0.1 M, pH 7.0) containing 0.5 g of washed cells (A) without antimycin A, (B) with 1 µg of antimycin A, (C) with 10 µg of antimycin A, and (D) with 1 mg of antimycin A. Error bars indicate the standard deviations of the three-time experiments.

In this report, it is demonstrated that *C. fimi* generated electricity using cellulose as fuel without any electron mediator, and cytochrome *c* is strongly involved in the electron transfer from the cell surface to the electrode. In comparison with our previous research using the same MFC chamber and the strain except for the addition of 0.4 mM AQDS (2), the ohmic resistance was 99.3 Ω and the maximum power density was 37.8 mW/m^2 therefore, mediator was very effective to increase the maximum power of the MFC. However, the low efficiency of electric generation of the MFCs was still unsolved in this study. The possible solution may incorporate the expression of cytochrome *c* or cellulase via genetic engineering, MFC stacking, or increasing cell density.

References

1. **Huang, L., Cheng, S., Rezaei, F., and Logan, B. E.:** Reducing organic loads in wastewater effluents from paper recycling plants using microbial fuel cells, Environ. Technol., **30**, 499–504 (2009).
2. **Marsili, E., Baron, D. B., Shikhare, I. D., Coursolle, D., Gralnick, J. A., and Bond, D. R.:** *Shewanella* secretes flavins that mediate extracellular electron transfer. Proc. Natl. Acad. Sci. U. S. A. **105**, 3968–3973 (2008).
3. **Newton, G. J., Mori1, S., Nakamura, R., Hashimoto, K., and Watanabe, K.:** Analyses of current-generating mechanisms of *Shewanella loihica* PV-4 and *Shewanella oneidensis* MR-1 in microbial fuel cells. Appl. Environ. Microbiol. **75**, 7674–7681(2009).
4. **Gorby, Y. A., Yanina, S., McLean, J. S., Rosso, K. M., Moyles, D., Dohnalkova, A., Beveridge, T. J., Chang, I. S., Kim, B. H., Kim, K. S., and other 14 authors:** Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms, Proc. Natl. Acad. Sci. U. S. A., **103**, 11358–11363 (2006).
5. **Uno, M., Phansroy, N., Aso, Y., and Ohara, H.:** Starch-fueled microbial fuel cells by two-step and parallel fermentation using *Shewanella oneidensi*s MR-1 and *Streptococcus bovis* 148, J. Biosci. Bioeng., **124**, 189–194 (2017).
6. **Le-Xing, Y., Li-Dan, L., Yong, X., You-Fen, D., Bi-Lian, C., Yan-Xia, J., and Feng, Z.:** Flavins mediate extracellular electron transfer in gram-positive *Bacillus megaterium* strain LLD-1. Bioelectrochemistry., **119**, 196–202 (2018).
7. **Tadashi, M. and Tshiaki, N.:** Electrochemical classification of gram-negative and gram-positive bacteria. Appl. Environ. Microbiol, **50**, 238–242 (1985).
8. **Vernon, L. P.:** Bacterial cytochromes, I. cytochrome composition of Micrococcus denitrificans and Psudomonas denitrificans. J. Biol. Chem., **222**, 1035–1044 (1956).

9. **Ali, N., Anam, M., Yousaf, S., Maleeha, S., and Bangash, Z.:** Characterization of the electric current generation potential of the *Pseudomonas aeruginosa* using glucose, fructose, and sucrose in double chamber microbial fuel cell, Iranian J. Biotechnol., **15**, 216–223 (2017).
10. **Matsushita, K., Yamada, M., Shinagawa, E., Adachi, O., and Ameyama, M.:** Menbrane-bound respiratory chain of pseudomonas aeruginosa grown aerobically, J. Bacteriol., **141**, 389–392 (1980).
11. **Prasad, D., Arun, S., Murugesan, M., Padmanaban, S., Satyanarayanan, R. S., Berchmans, S., and Yegnaraman, V.:** Direct electron transfer with yeast cells and construction of a mediatorless microbial fuel cell, Biosens. Bioelectro., **22**, 2604–2610 (2007).
12. **Ren, Z., Ward, T. E., and Regan, J. M.:** Electricity production from cellulose in a microbial fuel cell using a defined binary culture, Environ. Sci. Technol., **41**, 4781–4786 (2007).
13. **Ishii, S., Shimoyama, T., Hotta, Y., and Watanabe, K.:** Characterization of a filamentous biofilm community established in a cellulose-fed microbial fuel cell, BMC Microbiol., **8**, 1–12 (2008).
14. **Rezaei, F., Richard, T. L., Brennan, R. A., and Logan, B. E.:** Substrate-enhanced microbial fuel cells for improved remote power generation from sediment-based systems, Environ. Sci. Technol., **41**, 4053–4058 (2007).
15. **Inoue, K., Ito, T., Kawano, Y., Iguchi, A., Miyahara, M., Suzuki, Y., and Watanabe, K.:** Electricity generation from cattle manure slurry by cassette-electrode microbial fuel cells, J. Biosci. Bioeng., **116**, 610–615 (2013).
16. **Rismani-Yazdi, H., Christy, A. D., Dehority, B. A., Morrison, M., Yu, Z., and Tuovinen, O. H.:** Electricity generation from cellulose by rumen microorganisms in

microbial fuel cells, Biotechnol. Bioeng., **97**, 1398–1407 (2007).

17. **Masuda, M., Freguia, S., Wang, Y. F., Tsujimura, S., and Kano, K.:** Flavins contained in yeast extract are exploited for anodic electron transfer by *Lactococcus lactis*, Bioelectrochemistry, **78**, 173–175 (2010).
18. **Sayed, E. T., Nasser A. M. B., Abdelkareem, M. A., H. Fouad, and Nakagawa, N.:** Yeast extract as an effective and safe mediator for the Baker's-yeast-based microbial fuel cell, Ind. Eng. Chem. Res., **54**, 3116−3122 (2015).
19. **Wigginton, N. S., Rosso, K. M., Lower, B. H., Shi, L., and Hochella, M. F.:** Electron tunneling properties of outer-membrane decaheme cytochromes from *Shewanella oneidensis*, Geochimica. et. cosmochimica. acta., **71**, 543–555 (2007).
20. **Zhao, F., Slade R. C. T., and Varcoe, J., R.:** Techniques for the study and development of microbial fuel cells: an electrochemical perspective, Chem. Soc. Rev., **38**, 1926–1939 (2009).
21. **Leropoulos, I. A., Greenman, J., Melhuish, C., and Hart, J.:** Comparative study of three types of microbial fuel cell, Enzyme Microbial. Technol., **37**, 238–245 (2005).
22. **Karatani, H., Shizuki, T., Halon, R., and Nakayama, E.:** A method for the electrochemical initiation of the in vitro bacterial luciferase reaction, Photochem. Photobiol. **61**, 422–428 (1995).
23. **Tsegaye, T.** and **Assefa, S.:** A Comparative Study on Electrochemical Determination of Vitamin C in Liver and Tomato Using Platinum and Glassy Carbon Electrodes, Biochem. Mol. Biol., **2**, 25–36 (2017).
24. **Koh, S. N., Wee, T. T., Zulkarnain, Z., Ruzniza, M. Z., Mohammed, Z.:** Voltammetry detection of ascorbic acid at glassy carbon electrode modified by single-walled carbon nanotube/Zinc Oxide. Int. J. Electrochem. Sci., **8**, 10557–10567 (2013).
25. **Melissa, R. C., Garret, S., Shanti, B., Kelsea, A. J., Frank, O. A., David, M., and**

Phillip, J. B.: The genome sequences of *Cellulomonas fimi* and ‘‘*Cellvibrio gilvus*’’ reveal the cellulolytic strategies of two facultative anaerobes, transfer of ‘‘*Cellvibriogilvus*’’ to the Genus *Cellulomonas*, and proposal of *Cellulomonas gilvus* sp. nov., PLoS ONE., **8 (1)**, e53954, (2013).

26. **Hans, K. C., Anthony, T. I., Amita, G., Boon, S. Y., Rosalie, T., Ryan, A. M., Richard, A. M., Manfred, A., and John, D. C.:** Surface multiheme c-type cytochromes from *Thermincola potens* and implications for respiratory metal reduction by gram-positive bacteria. PNAS., **109**, 1702–1707 (2012).
27. **Lovley, D. R.:** Electromicrobiology, Ann. Rev. Microbiol., 66, 391–409 (2012).
28. **Mori, S., Ishii, K., Nakamura, R., and Hashimoto, K.:** In vivo participation of artificial porphyrins in electron-transport chains: electrochemical and spectroscopic analyses of microbial metabolism. Inorg. Chem., **50**, 2037–2039 (2011).
29. **Nakamura, R., Ishii, K., and Hashimoto, K.:** Electronic absorption spectra and redox properties of C type cytochromes in living microbes. Angew. Chem. Int. Ed., **48**, 1606–1608 (2009).
30. **Huang, L., Cobessi, D., Tung, E. Y., and Berry, E. A.:** Binding of the respiratory chain inhibitor antimycin to the mitochondrial bc_1 complex: A new crystal structure reveals an altered intermolecular hydrogen-bonding pattern, J. Mol. Biol., **351**, 573–597 (2005).

Chapter 4

Electrical performance of palm kernel shell utilized as fuel for *Cellulomonas fimi* in microbial fuel cell

4.1 INTRODUCTION

Nowadays, global electricity consumption trends are annually increasing faster than other energy forms due to the electrification of energy use (1). Renewable resources of many kinds of plants accumulate light energy from the sun as chemical bonding energy of polysaccharides, including cellulose. A conventional method to convert these chemical energies to electrical energy is burning these materials to obtain thermal energy, which generates steam to rotate an electric generator. In other words, the heat transfer from high temperatures to low temperatures becomes work. However, this process is limited to the Carnot cycle, because high temperature could not over the heatproof for burning boiler, and low temperature could not realize that of the atmosphere. The microbial fuel cell (MFC) represents one of the important technologies that can convert chemical energy from organic matter or biomass to electrical energy using a microorganism as a biocatalyst in an anaerobic anode compartment, hence producing bioelectricity (2). In a previous study, we reported the MFCs with *Cellulomonas fimi* using commercially available cellulose powder (CP) as a fuel (3). Oil palm trees are grown in the plantations of tropical rainforests in West Africa, America, and Asian countries such as Malaysia, Indonesia, and Thailand to produce oil from mesocarp and kernel (4,5). Palm kernel shell waste is obtained from the crushed kernel after palm oil extraction (6,7). Palm oil demand is projected to increase from the present time to 2050 (8). Consequently, the abundant crushed palm kernel shell quantities are assumed to increase as well. A number of intensive studies on palm kernel shell reported its high calorific value, and consequently it is used as fuel in

cogeneration plants to generate both heat and electrical energy (9). However, to the best of our knowledge, there is no report to date employing palm kernel shell as fuel for MFCs. The problem of applying these cellulosic materials to MFCs should be solved while considering *C. fimi*, because the morphologies of cellulosic materials would affect its utilization by the microorganism.

In this study, three types of cellulosic fuel, kernel shell cellulose (KSC), kernel shell nanocellulose (KSN), and KSN residual (KSNR) were prepared, along with commercially available CP.

4.2 MATERIALS AND METHODS

4.2.1 Strain and pre-culture *Cellulomonas fimi* NBRC 15513 was employed for the MFCs as cellulose-degrading bacteria. A bacterial growth medium was prepared with Luria–Bertani (LB) medium consisting of 1 g of Tryptone (Nacalai Tesque Inc., Kyoto, Japan), 0.5 g of yeast extract (Nacalai Tesque), and 1 g of NaCl, the ingredients were dissolved in the 100 ml of distilled water, and sterilized by autoclaving at 120°C for 20 min. *C. fimi* was cultivated in the sterilized LB medium at 30°C for 48 h with simultaneously shaking at 120 rpm. Consequently, the pre-cultivated *C. fimi* was collected and then washed 3 times with physiological saline to eliminate the LB medium mixture by centrifugation (MX-301, Tomy digital biology Co., Tokyo, Japan) at 2,500 × g at 4°C for 5 min.

4.2.2 MFC configuration and operation condition The single-chambered plate MFC performed in this research had been reported in our previous research (3), (Fig. 4.1). The components of the MFC chamber were polycarbonate with the internal volume of 100 ml. The anode was a chamber-fit, circular-shaped carbon felt (LFP-210, Osaka Gas Chemicals Co., Osaka, Japan). A carbon paper (TGP-120, Toray Co., Tokyo, Japan) was used as a based material for making an air cathode. The air cathode had an effective area of 50 cm^2 coated with

layers as follows; a catalyst layer consisting of Pt-supported carbon (IFPC40-III, Ishifuku metal industry Co., Tokyo, Japan) with perfluorinated resin (Nafion 510211, Sigma–Aldrich, MO, USA), a polytetrafluoroethylene layer (PTFE, 60% dispersion, 31-JR, Du Pont–Mitsui Fluorochemicals Co., Tokyo, Japan).

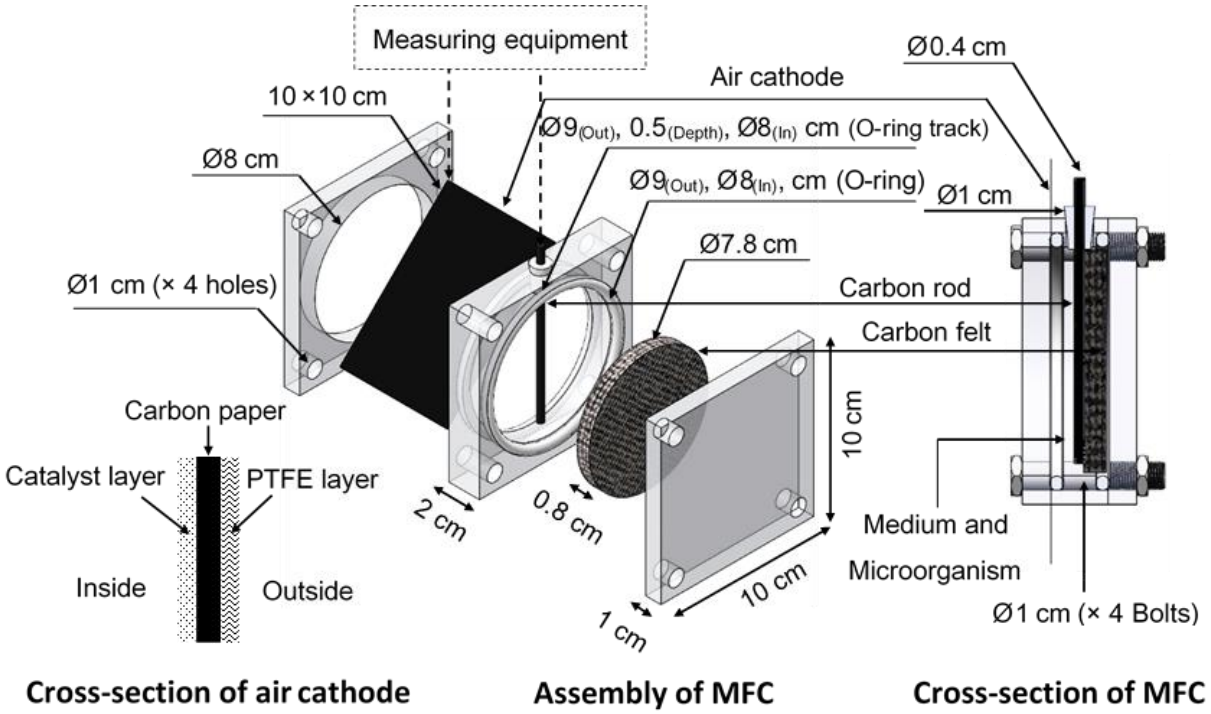

Fig. 4.1 Assembly and cross-section of the MFC and the air cathode. The chamber components are made of polycarbonate with the internal volume chamber of 100 mL. The carbon rod conducts to the carbon felt not the air cathode (3).

The MFC chamber and carbon rod (electron transferring bridge) were sterilized by irradiating UV light on disassembled components in a clean bench and the carbon felt anode was sterilized by autoclaving at 120°C for 20 min before assembling the MFCs. Each MFC was separately added with the 100 ml of the cellulose culture consisting of the washed *C. fimi* cell at an optical density (OD_{600}) of 0.2. The experiments were performed under an anaerobic condition by bubbling of N_2 at the rate of 100 mL/min for 3 min in the cellulose cultures already added in the MFCs. The MFCs were incubated in an incubator at 30°C for 14 days with simultaneously starting current measurement at the same time.

4.2.3 Electrical performance measurement The current generated by the MFCs was measured by the circuit used in our previous report (3), (Fig. 4.2) as follows; the current was monitored by a digital multimeter (KEW 1062, Kyoritsu Electrical Instruments, Tokyo, Japan) and simultaneously recorded by an application software (model 8241, Kyoritsu Electrical Instruments).

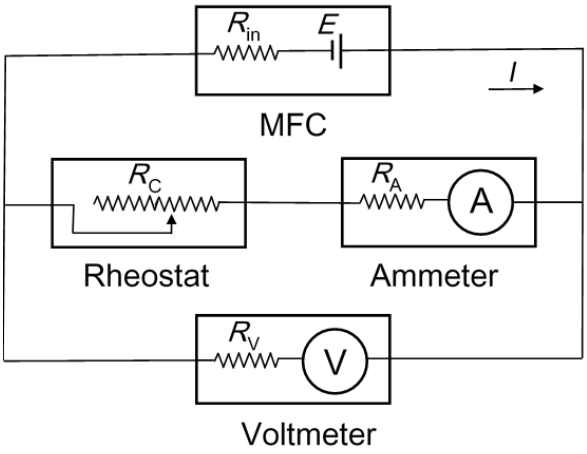

Fig. 4.2 The electric circuit to estimate an internal resistance of the MFCs. The R_{in}, R_C, R_A, and R_V represent the internal resistance values of MFC, rheostat, ammeter, and voltmeter, respectively. The "*I*" represents the current value in the line and "*E*" represents electromotive force. In our apparatus, R_A is negligibly small, and R_V is considered to be infinite (3).

The polarization curve and power density of the MFCs were measured when the current generation steadily reached the maximum point of the entire current generation. In this state, the current and voltage were recorded step by step after they stabilized (approximately 1 min) using a rheostat at various external resistances from 0 to 30 kΩ. The maximum power density and ohmic resistance of the MFCs were obtained by the polarization curves. The effective area of 50 cm^2 was used to calculate the current density (mA/m^2).

4.2.4 Preparation of fuel cellulose Palm kernel shell was obtained from Suksomboon Palm Oil Co. (Chonburi, Thailand). Okahisa et al. (2011) reported the production of cellulose nanofiber from bamboo. This method was modified and applied to kernel shell to prepare KSC,

KSN. The following is the points: To prepare KSC, 40 g of palm kernel shell was initially ground into small pellet by Hammer crusher (NH-34, Sansho Industry Co.) at 3450 rpm for about 5 min. The lipid removal process of the ground kernel shell was performed using a Soxhlet extractor with the mixed solution of 500 mL of toluene and 250 mL of methanol at 200°C for 12 h, then the mixed solution in the palm kernel shell pellet was removed and evaporated at room temperature. The lignin was then removed by adding 1 ml of CH_3COOH and 5 g of $NaClO_2$ every hour for 5 times into the lipid-removed palm kernel shell pellet suspended with 400 ml of distilled water, and heated at 70°C. Hemicellulose and starch were consequently eliminated with 5 wt.% sodium hydroxide (NaOH) solution at room temperature overnight, and then heated at 80°C for 2 h. The synthesized KSC was purified by washing with distilled water several times. While to produce KSN, KSC was disintegrated through a grinder (Masuko Co., Japan) which can provide nanocellulose in the fibrillated slurry at 1500 rpm with grinding stones (NKGC-80) to prepare KSN (10).

KSNR was obtained applying the method of Nakagaito et al. (2013) as follows KSN slurry containing 2.4 g of KSN in the 100 ml of water was added into Glass Round Bottom Flask equipped with Rotary Vacuum Evaporator (Tokyo, Rikakikai Co., Ltd.). The flask was soaked in a water bath heated at 80°C, 0.13 Pa with rotation at 40 rpm. for 30 min. The water in KSN slurry was slightly evaporated.

After the synthesis of the three celluloses (KSC, KSN, and KSNR) each fibrillated slurry was weighed to obtain the amount of cellulose existing in the slurry after water evaporation by using a moisture analyzer (MS-70, A&D Company, Ltd.) for calculating the exact amount of cellulose for the preparation of fuel solution. The fuel solution for MFCs were prepared as 0.5 g of these celluloses and CP (Wako Pure Chemical Industries, Ltd., Osaka, Japan) dissolving in 100 mL of 0.1 M phosphate buffer (pH 7.0) containing 1 g of NaCl and subsequently autoclaving at 120°C for 20 min.

4.2.5 Appearance and dispersion observation of the celluloses The observation of appearance and dispersion of each cellulose was simply observed by photographing the celluloses suspended in distilled water. The samples of cellulose suspension were separately prepared by mixing each cellulose with distilled water at the ratio of 0.5% (w/v). 3.0 mL of suspended cellulose was filled in a glass cell (1 × 1 × 4.5 cm, G-104, AS ONE Co.), and then photographed after precipitation at room temperature for 15 min.

4.2.6 Dynamic Light Scattering The samples were prepared by mixing each cellulose with distilled water at the ratio of 0.5% (w/v), and was then measured by a particle size analyzer (ELSZ-1000) at 25°C (11).

4.3 RESULTS AND DISCUSSION

4.3.1 Current generation by the MFCs Current was generated by the MFCs for 14 days with the cellulose cultures shown in Fig. 4.3. The MFCs utilizing KSN yielded the highest maximum current density, followed by CP, KSC, and KSNR, respectively.

4.3.2 Polarization and power generation A polarization curve is generally used to study the performances of MFCs. It consists of three regions as follows: first, the activation region in the rapid drop of voltage; second, the ohmic resistance region in a linear drop of voltage; and finally, the concentration polarization region in the range of the continuous increase of the current, with the rapid and non-linear drop of voltage (12).

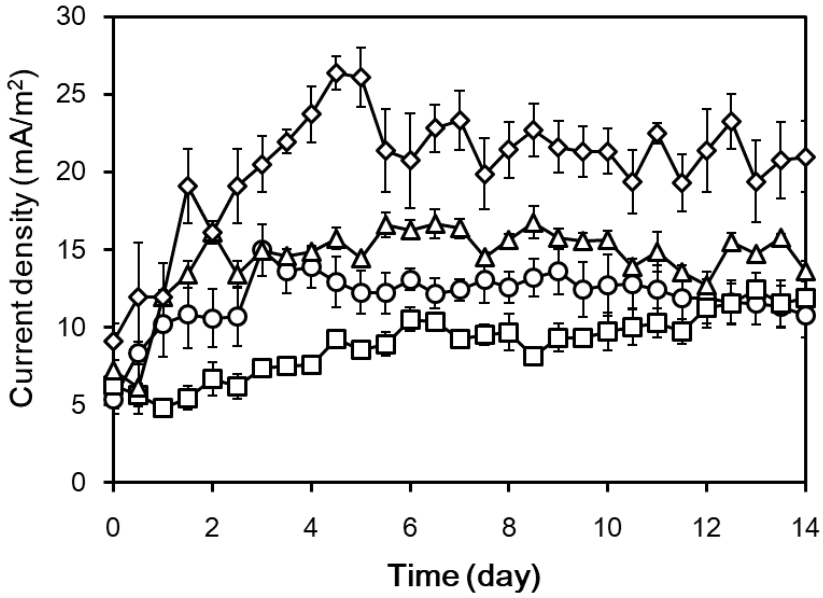

Fig. 4.3 The current generation of the MFCs cultured with the cellulose fuel solutions. Symbols: Kernel shell cellulose (KSC); (circles), kernel shell nanocellulose (KSN); (diamonds), kernel shell nanocellulose residual (KSNR); (squares), and cellulose powder (CP); (triangles). The error bars indicate the standard error of triplicated experiments.

The ohmic resistance of the MFCs was calculated from the slope of the linear region of the voltage versus current density graph of the MFC, and this ohmic resistance is used as an evaluation parameter. The obtained ohmic resistance of KSC, KSN, KSNR, and CP as fuel solutions were 6.3 ± 1.8, 2.7 ± 0.4, 7.9 ± 0.8, and 4.0 ± 0.4 kΩ, estimated from the linear ranges within 0.016–0.041, 0.021–0.061, 0.011–0.034, and 0.027–0.064 mA, respectively (Fig. 4.4a). The respective maximum power densities were 0.9 ± 0.2, 1.5 ± 0.3, 0.6 ± 0.3, and 1.3 ± 0.3 mW/m^2 (Fig. 4.4b). The MFCs showed the highest power density from the utilization of KSN, followed by CP, KSC, and KSNR, respectively.

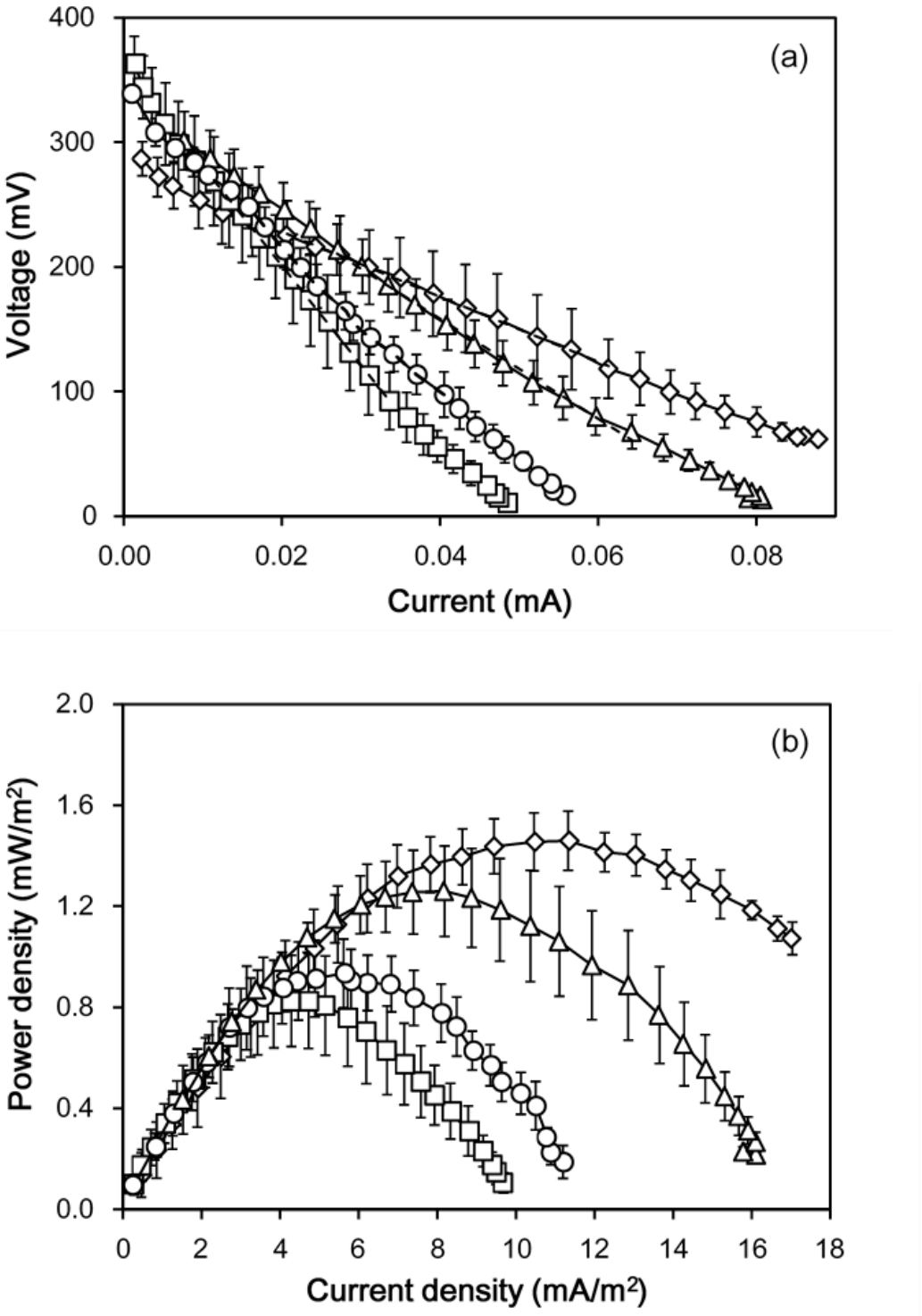

Fig. 4.4 The polarization curve (a) and power density curve (b) of the MFCs cultured with the cellulose fuel solutions. Symbols: Kernel shell cellulose (KSC); (circles), kernel shell nanocellulose (KSN); (diamonds), kernel shell nanocellulose residual (KSNR); (squares), and cellulose powder (CP); (triangles). The error bars indicate the standard error of triplicated experiments.

4.3.3 Appearance and dispersion Evidence of the entanglement phenomenon was obtained by photographs of the celluloses suspended in distilled water, as shown in Fig. 4.5. The suspended cellulose was left at room temperature for 15 min and subsequently photographed. KSC and CP were observed to precipitate to the bottom of the glass cell, whereas CP showed higher precipitation owing to the difference of the water dispersion ability. KSN and KSNR containing residual apparently dispersed in distilled water, giving rise to a slurry texture. Fibrillar cellulose was prepared by disintegrating cellulose fibers at high shear. This technique provided entangled networks of fibrillar cellulose, consisting of nanoscale elements and exhibiting a gel-like behavior in water (13). Furthermore, crystalline and semi-crystalline cellulose could also be obtained (14). Fig. 4.5 shows that the celluloses have a different dispersibility in water, which might affect the cellulose consumption of *C. fimi*, whereas the water removal process of KSN facilitated KSNR.

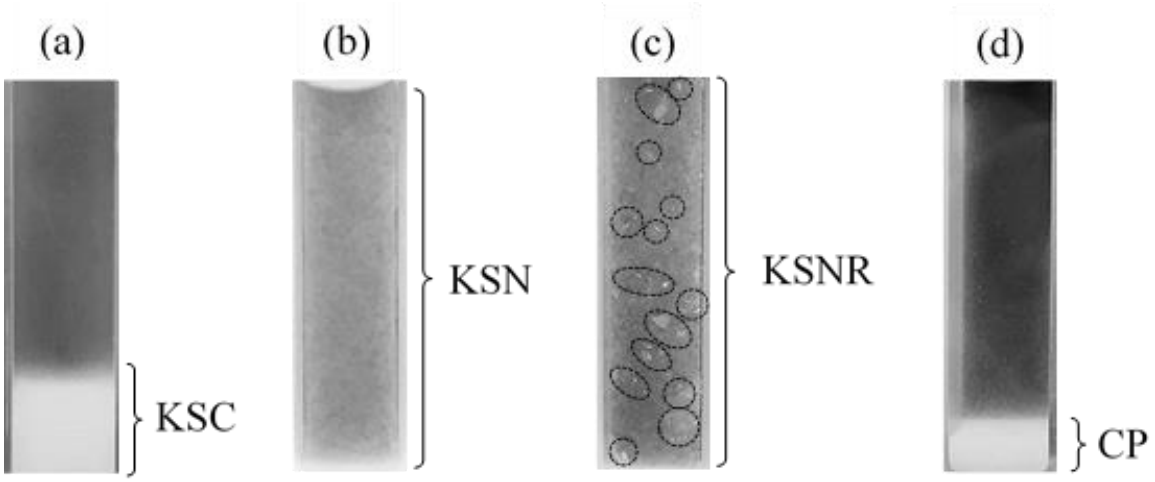

Fig. 4.5 The photographs of the celluloses suspended with distilled water (0.5% wt/v) in glass cells (1 × 1 × 4.5 cm). Kernel shell cellulose (KSC); (a), kernel shell nanocellulose (KSN); (b), kernel shell nanocellulose residual (KSNR); (c), and cellulose powder (CP); (d). Black is a background; the cloudy color is cellulose. The dotted cycles in (c) indicate the residuals of KSNR in sight.

As is well known, cellulose does not homogenously dissolve in water alone. However, Iotti et al. reported the dispersion phenomena of microfibrillar cellulose, which became a slurry in water (15). The observation of cellulose characteristics in water is necessary, as celluloses are suspended in distilled water upon their utilization as a fuel solution of the MFCs.

4.3.4 Particle size of each cellulose Finding the particle size of each cellulose is required for analysis, as it can influence the rate of cellulose decomposition by the microorganism (16,17) and thus affects the performance of the MFCs. The percent distribution of the particle size around the averages of KSC, KSN, KSNR, and CP was approximately 7850 ± 35, 964 ± 43, 47,024 ± 29,167, and 5883 ± 52 nm, respectively, as shown in Fig. 4.6. KSNR exhibited the largest particle size with the broadest distribution and standard deviation. The results indicated that the water removal process of KSN to KSNR formed three-dimensional networks, crosslinked by hydrogen bonds bridging the surface hydroxyl groups of nanocellulose (11,18). The obtained electrical performances indicate that each cellulose yields different values of current and power generation, although each fuel solution contained the same amount of the cellulose (0.5 g in 100 mL of fuel solution). In this study, the MFCs experiments were conducted under the same conditions, except for the case with the cellulose substrate. This indicates that the cellulose structure affects the enzymatic degradation of *C. fimi*, which secretes cellulose enzymes to break down the polysaccharide molecules (19) and thereupon metabolizes these molecules, resulting in electron generation. As shown in Table 1 and Fig. 4.6, the cellulose particle size is related to the ohmic resistances measured on the fifth day of the MFCs operation. The larger particle sizes exhibited high ohmic resistance, whereas the smaller particle sizes had a smaller ohmic resistance value. This might be due to the bacterial degradability of *C. fimi*, as the smaller particle size was easily degraded by enzymatic activity, resulting in the decrease of cellulose mass on the fifth day. This yielded lower ohmic resistance, because high organic matter in the anode could also influence a high resistance in

the MFCs (20). Another possible cause is that the characteristics of cellulose structure and dispersion affect the electrolyte mobilities in the fuel solutions, as shown in Fig. 4.5a and 5d; KSC and CP precipitated to the bottom of the glass cell, which similarly occurs in an in-MFC chamber, where the precipitate could cover the anode surface and turn into resistance. Ohmic resistance is caused by ionic resistances in the electrolyte, membrane, biofilm, and electronic resistances in the electrodes, current collectors, interconnects, and electronic components (21). The results of the ohmic resistance and maximum power density estimated from Fig. 4.4 are summarized in Table 4.1.

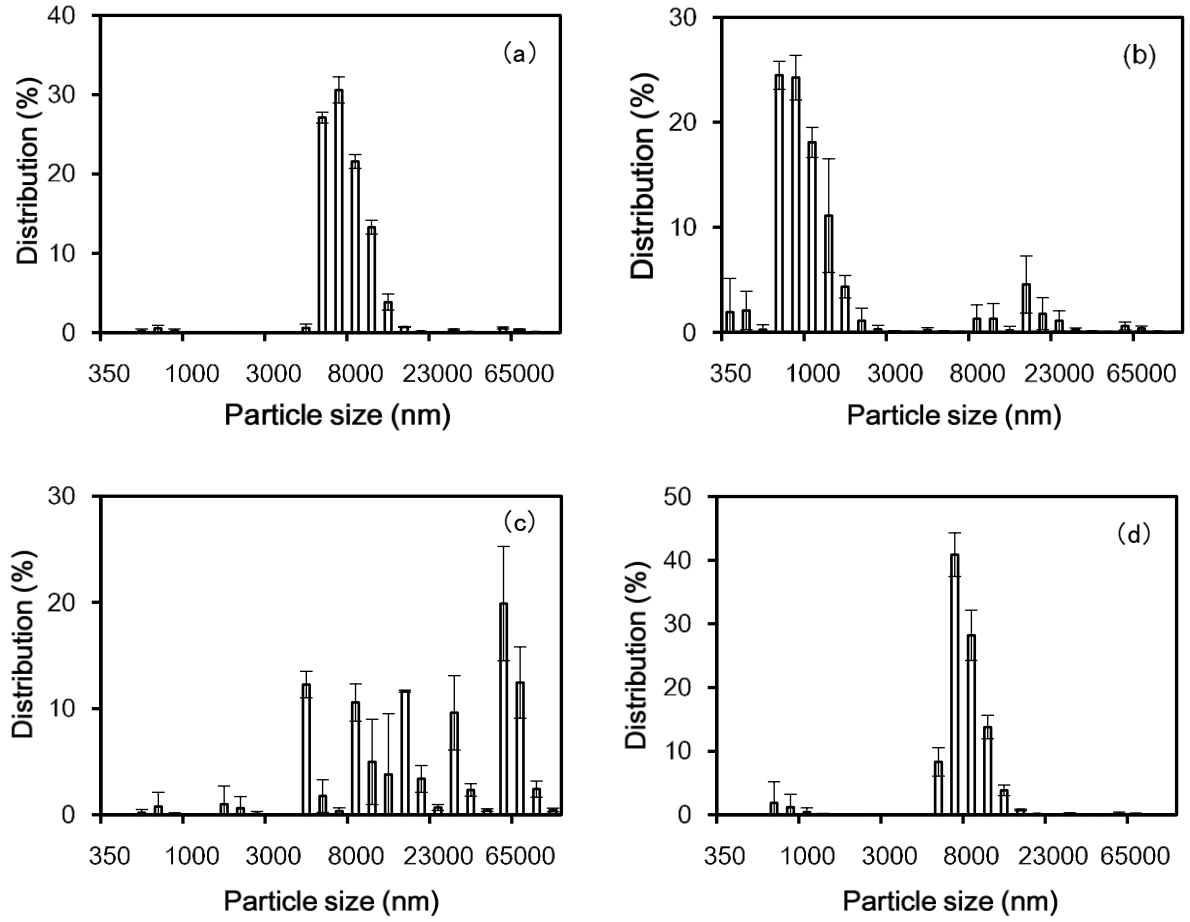

Fig. 4.6 The particle size distribution of the celluloses suspended with distilled water using dynamic light scattering. Kernel shell cellulose (KSC); (a), kernel shell nanocellulose (KSN); (b), kernel shell nanocellulose residual (KSNR); (c), and cellulose powder (CP); (d). The error bars indicate the standard deviation of triplicated experiments.

The internal power loss of the MFC is mainly caused by ohmic resistance (22). The efficient dispersion of cellulose in the fuel solutions also promotes microbial access to the cellulose by a three-directional access and facilitates bacterial activity for cellulose degradation, as enzymes can better access the cellulose surface.

In contrast, the dense cellulose aggregation in KSC and CP (Fig. 4.5a and 5d) could limit bacterial access and the motility of *C. fimi* (23,24) to move to other areas with more resources or nutrients (25). Thus, the enzymatic efficiency in degrading cellulose can be inhibited. Consequently, the current generation of KSC and CP were lower than in KSN, which has smaller particle size with efficient dispersion. KSNR exhibits efficient dispersion in the fuel solution (Fig. 4.5c), but the larger particle size with entanglement and residuals is difficult to degrade by enzymatic activity. Therefore, *C. fimi* cannot efficiently degrade KSNR, resulting in low current generation (26).

Table 4.1 Performances of the MFCs cultured with the cellulose fuel solutions.

Electrical performances	Source of cellulose			
	KSC[a]	KSN[b]	KSNR[c]	CP[d]
Maximum current density (mA/m^2)	15.0 ± 2.9	26.4 ± 1.8	11.9 ± 2.6	16.8 ± 1.8
Maximum power density (mW/m^2)	0.9 ± 0.2	1.5 ± 0.3	0.6 ± 0.3	1.3 ± 0.3
Ohmic resistance (kΩ)	6.3 ± 1.8	2.7 ± 0.4	7.9 ± 0.8	4.0 ± 0.4

The fuel solutions for MFCs were prepared as 0.5 g of these celluloses dissolving in 100 mL of 0.1 M phosphate buffer (pH 7.0) containing 1 g of NaCl and subsequently autoclaving at 120°C for 20 min. Standard deviation was calculated from triplicated experiments.

[a] KSC, Kernel shell cellulose

[b] KSN, Kernel shell nanocellulose

[c] KSNR, Kernel shell nanocellulose residual

[d] CP, Cellulose powder

4.4 CONCLUSION

The palm kernel shell waste from palm oil production can be used as the carbon source for *C. fimi* to generate bioelectricity by employing MFCs. The electric generation by cellulose from palm kernel shell was found to be similar to the commercially available cellulose; therefore, palm kernel shell can be used as an alternative commercial cellulose for electric generation. This research reveals that palm kernel shell can be used as renewable electrical energy; however, the different characteristics of cellulose should be considered, as they influence current generation. Nevertheless, current generation of the MCFs using palm kernel shell remains low; however, it is at a similar level of commercial CP. Another obvious benefit of this application is the reduction of waste of palm kernel shell in the palm oil production process.

References

1. **Enerdata** (2019, May 28). Global Energy Statistical Yearbook 2018. Retrieved from https://yearbook.enerdata.net/.
2. **Rahimnejad, M., Adhami, A., Darvari, S., Zirepour, A. and Oh, S. E.:** Microbial fuel cell as new technology for bioelectricity generation: A review, Alexandria Eng. J., **54**, 745–756 (2015).
3. **Takeuchi, Y., Khawdas, W., Aso, Y. and Ohara, H.:** Microbial fuel cells using *Cellulomonas* spp. with cellulose as fuel, J. Biosci. Bioeng., **123**, 358–363 (2017).
4. **Okoroigwe, E. C. and Saffron, C. M.:** Determination of Bio-Energy Potential of Palm Kernel Shell By Physicochemical Characterization, Niger. J. Technol., **31**, 329–335 (2012).
5. **Kevwe P.O, O. and anze N, N.:** Production of Bio-Char by Pyrolysis of Palm Kernel Shell, Int. J. Mech. Eng., **5**, 1–4 (2018).
6. **Kim, S. W., Koo, B. S. and Lee, D. H.:** Catalytic pyrolysis of palm kernel shell waste in a fluidized bed, Bioresour. Technol., **167**, 425–432 (2014).
7. **Itam, Z., Beddu, S., Mohd Kamal, N. L., Alam, M. A. and Ayash, U. I.:** The Feasibility of Palm Kernel Shell as a Replacement for Coarse Aggregate in Lightweight Concrete, IOP Conf. Ser. Earth Environ. Sci., **32**, 7–11 (2016).
8. **Febriansyah, H., Setiawan, A. A., Suryopratomo, K. and Setiawan, A.:** Gama stove: Biomass stove for palm kernel shells in Indonesia, Energy Procedia, **47**, 123–132 (2014).
9. **Bazargan, A., Rough, S. L. and McKay, G.:** Compaction of palm kernel shell biochars for application as solid fuel, Biomass and Bioenergy, **70**, 489–497 (2014).
10. **Yoko, O., Kentaro, A., Masaya, N., Nakagaito, A.N., Takeshi, N., Hiroyuki, Y.:** Effects of delignification in the production of plant-based cellulose nanofibers for optically transparent nanocomposites, Compos. Sci. Technol., **71**, 1342–1347 (2011).

11. **Mendoza, L., Batchelor, W., Tabor, R. F. and Garnier, G.:** Gelation mechanism of cellulose nanofibre gels: A colloids and interfacial perspective, J. Colloid Interface Sci., **509**, 39–46 (2018).
12. **Fan, Y., Sharbrough, E. and Liu, H.:** Quantification of the internal resistance distribution of microbial fuel cells, Environ. Sci. Technol., **42**, 8101–8107 (2008).
13. **Mariano, M., El Kissi, N. and Dufresne, A.:** Cellulose nanomaterials: size and surface influence on the thermal and rheological behavior, Polímeros, **28**, 93–102 (2018).
14. **Kane, S. D. and French, C. E.:** Characterisation of novel biomass degradation enzymes from the genome of *Cellulomonas fimi*, Enzyme Microb. Technol., 113, 9–17 (2018).
15. **Iotti, M., Gregersen, Ø. W., Moe, S. and Lenes, M.:** Rheological Studies of Microfibrillar Cellulose Water Dispersions, J. Polym. Environ., **19**, 137–145 (2011).
16. **Hu, Z. H., Yu, H. Q. and Zhu, R. F.:** Influence of particle size and pH on anaerobic degradation of cellulose by ruminal microbes, Int. Biodeterior. Biodegrad., **55**, 233–238 (2005).
17. **Maryanty, Y., Sumitro, S. B. and Tri, A.:** Effect of Particle Size and Pretreatment on Cellulose Degradation of Rice Straw from Agricultural Land in Malang, Int. J. ChemTech Res., **10**, 600–610 (2017).
18. **Nakagaito, A. N., Kondo, H. and Takagi, H.:** Cellulose nanofiber aerogel production and applications, J. Reinf. Plast. Compos., **32**, 1547–1552 (2013).
19. **Duedu, K. O. and French, C. E.:** Characterization of a *Cellulomonas fimi* exoglucanase/xylanase-endoglucanase gene fusion which improves microbial degradation of cellulosic biomass, Enzyme and Microbial Technology, **93**, 113–121 (2016).

20. **González Del Campo, A., Cañizares, P., Lobato, J., Rodrigo, M. and Fernandez Morales, F. J.:** Effects of External Resistance on Microbial Fuel Cell's Performance. Handb. Environ. Chem. (2016). doi:10.1007/698-2014-290

21. **Zhao, F., Slade, R. C. T. and Varcoe, J. R.:** Techniques for the study and development of microbial fuel cells: An electrochemical perspective, Chem. Soc. Rev., **38**, 1926–1939 (2009).

22. **ElMekawy, A., Hegab, H. M., Dominguez-Benetton, X. and Pant, D.:** Internal resistance of microfluidic microbial fuel cell: Challenges and potential opportunities, Bioresour. Technol., **142**, 672–682 (2013).

23. **Thayer, D. W.:** Motility and Flagellation of *Cellulomonads*, Int. J. Syst. Bacteriol., **34**, 218–219 (2009).

24. **Christopherson, M. R., Suen, G., Bramhamcharya, S., Jewell, K. A., Aylward, F. O., Mead, D., Brumm, P. J.:** The Genome Sequences of *Cellulomonas fimi* and 'Cellvibrio gilvus' Reveal the Cellulolytic Strategies of Two Facultative Anaerobes, Transfer of 'Cellvibrio gilvus' to the Genus Cellulomonas, and Proposal of *Cellulomonas gilvus* sp. nov, PLoS One, **8**, (2013).

25. **Jones, S. E., Ho, L., Rees, Christiaan, A, Hill, J. E., Nodwell, J. R., Elliot, M. A:** Streptomyces exploration is triggered by fungal interactions and volatile signals, Elife, **6**, 1–21 (2017).

26. **Muhamad, H. M. N., Mohd, F. M. M., Hassan, S. A. E., Norahim, I., Mohd, F. A.W., Zaharah, I.:** Bioelectricity generation in microbial fuel cell using natural microflora and isolated pure culture bacteria from anaerobic palm oil mill effluent sludge, Bioresour. Technol., **190**, 458–465 (2015).

Chapter 5
General conclusion

At the present day, the electric consumption is increasing annually. Renewable resources are expected to take place energy obtained from coal causing gas intoxication and greenhouse effect, etc. Cellulose is an abundant renewable resource on the earth, and has been widely researched in several research fields. One of the most important technologies for generating electricity is a microbial fuel cell (MFC). MFC is a device that can generate electricity using microorganism as a biocatalyst for the current generation. The thermal power generation of the Carnot cycle showed efficiency obtained from burning fuels by just only 40% from chemical energy from burnt materials, whereas MFC has no limitation on converting chemical bond energy of organic substrate to electric energy. Cellulose-fed MFC has also been being studied as an alternative current generating device. However, many factors influence the current generation such as organic matter, suitable microorganism for organic matter, electrodes (anode and cathode), electron mediator, etc. In this study, the authors have studied and developed the single-chamber MFC using *C. fimi* and focused on the suitable organic matter for *C. fimi*, and enhanced the electron transfer ability of *C. fimi* using antimycin A as an electron transfer inhibitor. Furthermore, palm kernel shell waste was revealed to be able to use as an alternative renewable source as its similar the electrical performances compared to commercially available cellulose.

All studies aforementioned, the single-chambered MFC was performed throughout our study using *C. fimi* with different kinds of fuel sources. The electric generation of all MFCs is satisfiable compared to those of cellulose-fed MFC both mediator-less and mediator MFC

previously reported. The direct electron transfer ability of *C. fimi* which has not been known was also first revealed in this which is an interesting new finding for the cellulose-fed, mediator-less MFC. Each study is concluded as the followings;

In the first study *Cellulomonas fimi*, *Cellulomonas biazotea*, and *Cellulomonas flavigena* are cellulose-degrading microorganisms chosen to compare the degradation of cellulose. *C. fimi* degraded 2.5 g/L of cellulose within 4 days, which was the highest quantity among the three microorganisms. The electric current generated by the microbial fuel cell (MFC) using the cellulose-containing medium with *C. fimi* was measured over 7 days. The medium in the MFC was sampled every 24 h to quantify the degradation of cellulose, and the results showed that the electric current increased with the degradation of cellulose. The maximum electric power generated by the MFC was 38.7 mW/m^2, and this numeric value was 63% of the electric power generated by an MFC with *Shewanella oneidensis* MR-1, a well-known current-generating microorganism. Our results showed that *C. fimi* was an excellent candidate to produce the electric current from cellulose via MFCs.

In the second study, the strain of *C. fimi* NBRC 15513 can generate electricity with cellulose as fuel without mediator using a single chamber type microbial fuel cell (MFC) which had 100 mL of the chamber and 50 cm^2 of the air cathode. The MFCs were operated over five days and showed the maximum current density of 10.0 ± 1.8 mA/m^2, the maximum power density of 0.74 ± 0.07 mW/m^2 and the ohmic resistance of 6.9 kΩ. According to the results of cyclic voltammetry, the appearance of the oxidation or reduction peak was not observed from the cell removed solution. The fact is that *C. fimi* does not secrete mediator-like compounds, while the oxidation peak was observed at +0.68 V from the phosphate buffer containing the washed cell. The peak appearance was caused by the electron transfer activity of which corresponds to cytochrome *c*, and disappeared after adding antimycin A which inhibits the electron transfer activity. The cell was alive throughout the experiment as the

result of a colony forming unit on Luria–Bertani agar plates. This was thought that cytochrome *c* was on the membrane surface of the living cell and played a role in the direct electron transfer between the cells and anode.

In the third study, kernel shell cellulose, kernel shell nanocellulose, and kernel shell nanocellulose residual synthesized from palm kernel shell were utilized as fuel for microbial fuel cells (MFCs) with *C. fimi*. Commercially available cellulose (cellulose powder) was also performed to compare the performances of the MFCs. Kernel shell nanocellulose provided the highest current generation with the maximum current density of approximately 26.4 ± 1.9 mA/m^2 followed by cellulose powder, kernel shell cellulose, and kernel shell nanocellulose residual with the value of 16.8 ± 1.8, 15.0 ± 2.9, and 11.9 ± 2.6 mA/m^2, respectively. The current generation of the palm kernel shell was also similar to the electrical performances of commercially available cellulose, and depended on the characterization of each cellulose.

Even though the current generation of the MFCs in this study in the case of a non-addition of mediator was still low, but it indicated that the current could be generated with no electron mediator due to self-produced electron mediator of *C. fimi*. The author would develop the cellulose-fed, mediator-less MFC for practical use with The possible solution may incorporate the expression of cytochrome *c* or cellulase via genetic engineering, MFC stacking, or increasing cell density to enhance high electric generation in the future.

Publication list

1. **Yuya Takeuchi, Wichean Khawdas, Yuji Aso, and Hitomi Ohara.:** Microbial fuel cells using *Cellulomonas* spp. with cellulose as fuel, Journal of Bioscience and Bioengineering, **123**, 358–363 (2017). DOI: 10.1016/j.jbiosc.2016.10.009
2. **Wichean Khawdas, Keigo Watanabe, Hajime Karatani, Yuji Aso, Tomonari Tanaka, and Hitomi Ohara.:** Direct electron transfer of *Cellulomonas fimi* and microbial fuel cells fueled by cellulose, Journal of Bioscience and Bioengineering, **128 (5)**, 593–598 (2019). DOI: 10.1016/j.jbiosc.2019.05.001
3. **Wichean Khawdas, Yuji Aso, Tomonari Tanaka, Yoko Okahisa, Iori Kazama, and Hitomi Ohara.:** Electrical performance of palm kernel shell utilized as fuel for *Cellulomonas fimi* in microbial fuel cell, Journal of Chemical Engineering of Japan, **53 (4)**, 146–152 (2020). DOI: 10.1252/jcej.19we125

Other publications

1. **Keiichiro Nomura, Ryugo Shinmura, Weraporn Pivsa-Art, Wichean Khawdas, Yuji Aso, and Hitomi Ohara.:** Interactions between cyclic oligo(l-lactic acid) and alkali metal ions in organic solvent, Journal of Polymer research, 22, (2015). DOI:10.1007/s10965-015-0826-z
2. **Nichanan Phansroy, Wichean Khawdas, Keigo Watanabe, Yuji Aso, and Hitomi Ohara.:** Microbial fuel cells equipped with an iron-plated carbon-felt anode and *Shewanella oneidensis* MR-1 with corn steep liquor as a fuel, Journal of Bioscience and Bioengineering, **126**, 514–521 (2018). DOI: org/10.1016/j.jbiosc.2018.04.011

Acknowledgment

The present research has been carried out under the guidance of Professor Dr. Hitomi Ohara, Department of Biobased Materials Science, Kyoto Institute of Technology, from 2016–2019. The author would like to express his sincere thanks to Professor Dr. Hitomi Ohara for his constant technical advice, valuable discussion, and kind support on this research and explanation of extensive knowledge on a microbial fuel cell.

The author would like to express his sincere gratitude to Associate Prof. Yuji Aso for his encouragement and valuable guidance, experimental discussion, kind help, and support during his doctor's course.

The author would like to express his appreciation to Assistant Professor Yoko Okahisa and her student Yano Kaori, Research assistance for discussion, suggestion, and for supporting materials and equipment for synthesizing cellulose of palm kernel shell.

The author would like to express his deep appreciation to Professor Hajime Karatani, Department of Biomolecular Engineering, Kyoto Institute of Technology, at for valuable discussion, warm encouragement, and kind support on this research and explanation of extensive knowledge on cyclic voltammetry measurement.

The author would like to express his deep appreciation to Dr. Sommai Pivsa-Art and Dr. Weraporn Pivsa-Art from Department of Materials and Metallurgical Engineering, Rajamangala University of Technology Thanyaburi for always giving valuable chance and encouragement.

The author wishes to express his thanks to his tutors and members of the laboratory for their cooperation and assistance.

The author would like to express his deep appreciation to teachers who the author studied with, for giving valuable knowledge in several fields such as academic research, languages,

living in Japan, using the equipment for research, giving good experiences, etc.

The author would like to thank Suksomboon Palm Oil Co., Chonburi, Thailand for providing palm kernel shell for performing the experiment until completed.

The author exceedingly appreciates valuable chance from Kyoto Institute of Technology and Japanese government for supporting credit fee and scholarship (MEXT scholarship) thought out his study.

Finally, the author would like to thank his family member, Mr. Sangwan Khawdas and Miss Noy Khawdas, his two brothers and relatives, for their constant understanding and encouragement through this research.

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September, 2019