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Microbial Community from Tannery Wastewater in Microbial Fuel Cell

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A microbial fuel cell (MFC), a bio-electrochemical device, can simultaneously remove carbonaceous and nitrogenous pollution, while generating bioelectricity. The batch condition was designed with a temperature of 37°C, pH 7.0, and fixed external resistance at 1,000 ohms. At steady state operation, the extent of organic pollutants removed from artificial nitrogen-rich and tannery wastewaters was 590 and 700 mg COD L-1. The nitrogen removal from the artificial wastewater was 384 mg L-1 after 1-d operation and for tannery wastewater the value was 214 mg L-1 after 4-d operation, respectively. Alphaproteobacteria, Gammaproteobacteria, and Deltaproteobacteria are the dominant bacterial groups at the steady state operation. Gammaproteobacteria and Deltaproteobacteria (exo-electrogenic bacteria) were previously reported as electricity producing bacteria. These results confirmed that the MFC could be used for treating wastewater and generating electricity in a single reactor.
Keyword: Microbial fuel cell, Exo-electrogenic bacteria, Nitrifying bacteria, Denitrifying bacteria, Next generation sequencing

1. Introduction

Microbial fuel cells (MFCs) are sustainable technology that potentially combines wastewater treatment and bioenergy production in a single step. MFCs generate electricity directly under anaerobic conditions in an anode chamber and under aerobic conditions in a cathode chamber. The presence of organic substrates (wastewater) results in a redox gradient between the aerobic and anaerobic conditions. Thus, the performance of MFCs depends on the redox gradient between the anode and the cathode in the reactor (Corbella et al., 2017). The key factor for MFC operation is the microbial community, which consists of exo-electrogenic bacteria and fermentative bacteria. Recently, the microorganisms known to be electrochemical bacteria provide electricity generation in MFCs. Those microorganisms are proven to generate electron transfers, working without a mediator (Wei et al., 2014). The various ways used to transfer electrons to the anode consist of: metabolic mediates, redox mediators, conductive nanowire, and cytochromes (He et al., 2017). Microorganisms were fed in several types of substrates such as leachate (Zhang et al., 2008), brewery (Feng et al., 2008), and starch processing (Liu et al., 2009), obtaining current densities of 0.15, 0.2, and 0.09 mA cm-2, respectively. MFCs have successfully treated domestic and brewery wastewaters, but the treatment of highly polluted tannery wastewater was not successful. Therefore, this technology can simultaneously treat organic pollution and generate electricity in a single reactor. In the other hand, traditional energy production technologies (such as biogas production technology) cannot directly be used for electricity production since it needs the generator to convert energy to electricity production. This innovative technology is attractive for sustainable bioenergy production and waste treatment. The best understanding of exo-electrogenic bacteria in MFCs uses the pyrosequencing technique. This study aimed to provide useful information about the possibility

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of simultaneous nitrogen treatment and electricity generation in MFCs with respect to microbial diversity, using a pyrosequencing technique (Next Generation Sequencing, or NGS) in the MFCs fed with tannery wastewater.

2. Materials and Methods

2.1 MFC Design and operation

The design of MFCs refers to Liu and Logan (2004). The volume of MFCs was 28 mL as indicated in Figure 1. The electrodes (anode and cathode) were made from carbon cloth. A proton exchange membrane (PEM) was not used because MFCs without PEMs can achieve greater electricity generation when compared to MFCs with PEMs (Nevin et al., 2008). There are two conditions in one reactor: the anode was maintained in anaerobic conditions and the cathode was open to the air. The MFC operation used artificial and tannery wastewater for the substrate. Artificial and tannery wastewater was high in nitrogen (N) and chemical oxygen demand (COD), therefore COD: N of tannery wastewater was low. In terms of external resistance, this was fixed at 1,000 ohms. (Sawasdee V., and Pisutpaisal N., 2016).

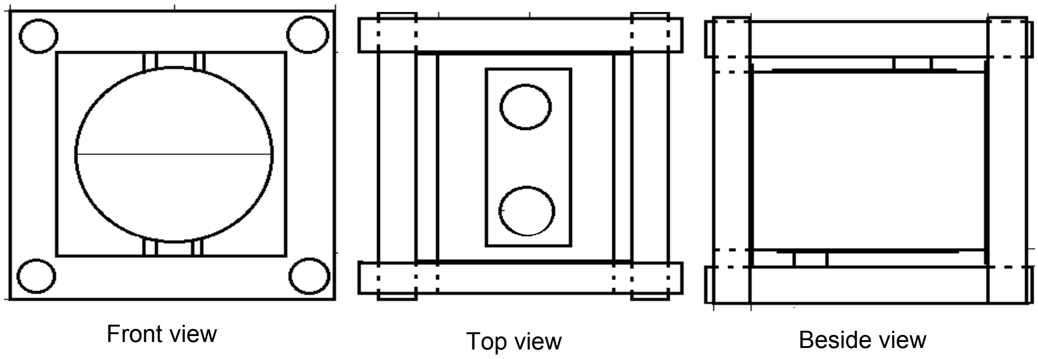

Figure 1: Diagram of MFC system

2.2 Chemical and electrochemical analysis

Voltage (V) was measured with a data acquisition system (Model 2700; Keithly) and recorded on personal computer. Current was calculated from Ohm's law. Oxidation reduction potential (ORP) was measured with ORP electrode. Nitrogen and COD was measured with Standard Method of American Public Health Association (APHA).

2.3 DNA Preparation and Purification

DNA preparation for next generation sequencing (NGS) analysis investigated the microbial communities in anode and cathode biofilm. DNA was extracted from the biofilm in both anode and cathode chambers using the sludge sample method (Zhou et al., 1996). The pieces of carbon cloth from the anode and cathode chambers were sampled for DNA extraction into a centrifuge tube with pellets and 5 mL of extract buffer. Lysozyme was added in the centrifuge, mixed, and incubated at 37°C for 60 minutes. Then, 20% sodium dodecyl sulfate (SDS) was added, incubated at 70°C for 30 minutes, centrifuged at 12,000 rpm for 5 minutes, and the supernatant was transferred to a new tube. Iso-amyl was added to the DNA solution, centrifuged, and the liquid layer removed to next tube. Then, the DNA solution was precipitated by iso-propanol and incubated on ice for 2 hours. Finally, the DNA was washed with 1 ml of 70% ethanol and 30 ul of DI water. The preparation of the DNA was followed by DNA purification. Then 7 V of Polymerase Chain Reaction (PCR) buffer was added to the DNA sample, then vortex and wash buffer 2-3 times before being centrifuged at 12,000 rpm for 3 minutes. Finally, the DNA sample was eluted with 20-50 ul of elution buffer.

2.4 Pyrosequencing (Next Generation Sequencing, NGS)

Pyrosequencing is a next generation sequencing technique that identifies microorganisms. The diversity of the microbial community was thus investigated by NGS. It is a new technology to investigate high throughput base pairs for microbial community analysis. This technology can be analyzed at low cost and with high quality for the microbial community. Database species annotation is used for NGS analysis: the databases that are used for microbial community reference are 16S rDNA (bacterial and archaea) and 18S rDNA (fungal community).

3. Results and discussions

3.1 Electricity generation

The electricity was shown in terms of power and current density. In this study, the end of electricity generation is observed as a drop in current and power density to below 75 mA m-2 and 4 mW m^{-2}, respectively, in a single batch cycle (Figure 2), while the highest current and power density was 200 mA m^{-2} and 29 mW m^{-2}, respectively. Hampannavar et al. (2011) studied electricity generation from distillery wastewater (nitrogen rich) in a single chamber MFC, which obtained power generation of 28.15 mW m^{-2}. Generally, the electricity generation from nitrogen rich wastewater is low, while this study obtained high electricity generation (power and current density). These results can be confirmed with the microbial communities by class level (Figure 4).

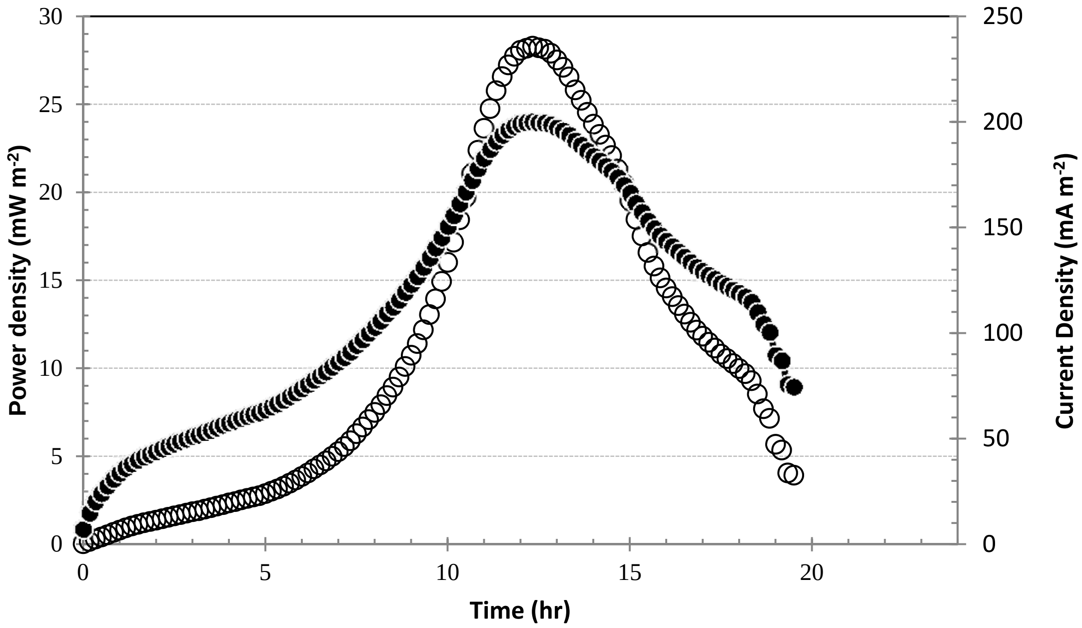

Figure 2: Power (O) and Current density (●) from tannery wastewater in MFC

3.2 Chemical and electrochemical analysis

The COD and nitrogen removal from tannery wastewater achieved the removal of approximately 70% and 50%, respectively. Figure 3 shows the relationship between voltage and COD removal after 20 hours. In terms of other research studies, these obtained low COD removal; for example, Huang and Logan (2008) found that paper recycling wastewater can achieve COD removal efficiency of 51%, while Min and Logan (2004) found that domestic wastewater can achieve COD removal efficiency of 42%. When MFCs were operated with tannery wastewater, the biodegradable organic compounds could be consumed not only by fermentative bacteria but also by exo-electrochemical active bacteria. ORP showed the transfer of electrons in the system, which obtained 27 mV. The ORP values of the denitrification reaction were between 50 to -50 mV (Higgins, 2013); this study obtained ORP 27 mV, and therefore the denitrification system occurred. This system (MFC) offered low operational cost and can easily be operated for pollution removal and energy production.

3.3 Microbial communities

Class levels for which abundance is less than 0.5% in all samples were classified into 'others' in other ranks. There are 18 classes that were found in the anode and cathode chambers. The identification of microbial communities by NGS showed Gammaproteobacteria, Alphaproteobacteria, and Betaproteobacteria that play roles in the waste treatment and electricity generation (Parot et al., 2009). The lists of microorganisms are shown in Figure 4. The nitrogen removal and electricity generation in MFCs were confirmed with the microbial community's data. Nitrosomonas and Nitrobacter were present in the Class Betaproteobacteria. The denitrifying bacteria were present in Class Gammaproteobacteria. The nitrifying bacteria can convert NH_4 to NO_2 and convert NO_2 to NO_3. The denitrifying bacteria can convert NO3 to N2. The exo-electrochemical active bacteria such as Gammaproteobacteria, Alphaproteobacteria, and Deltaproteobacteria were also present in the anode and cathode chambers.

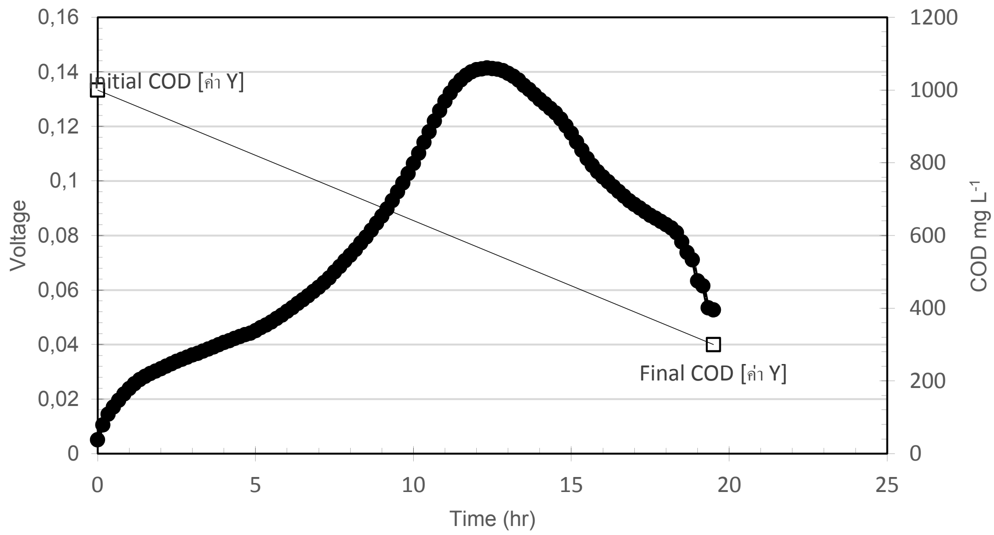

Figure 3: The relationship between voltage and COD removal from tannery wastewater in MFC.

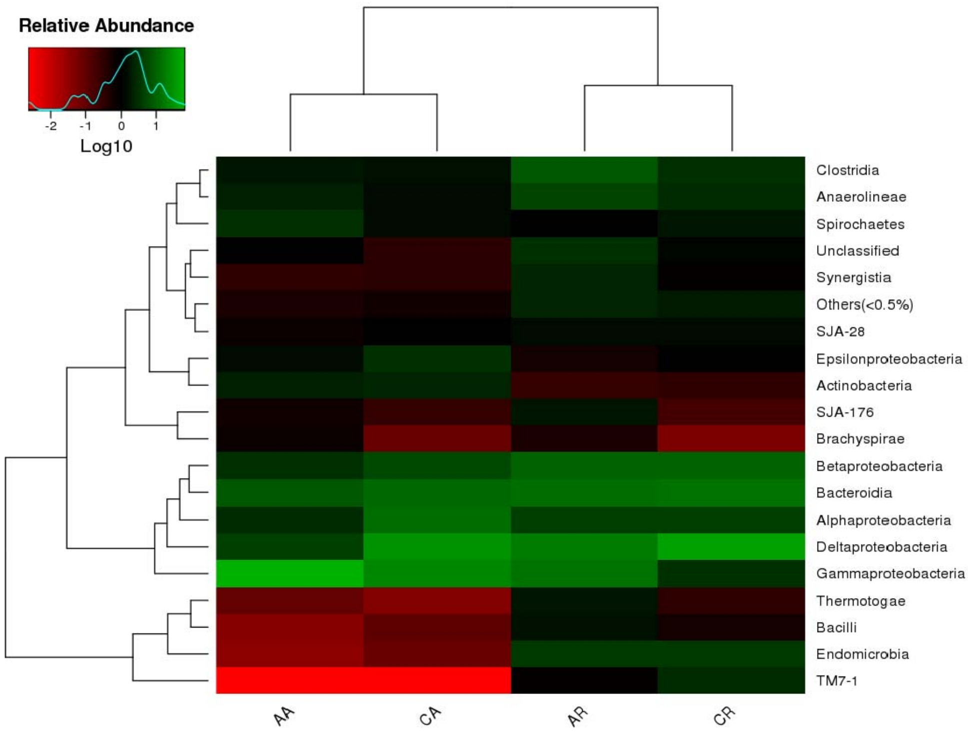

Figure 4: Heat map (Taxonomy) of Class which occurred in MFCs. The abbreviation of histogram; AA is artificial wastewater in anode, CA is artificial wastewater in cathode, AR is real wastewater in anode, and CR is real wastewater in cathode.

Commonly, Deltaproteobacteria was the most abundant class in all samples (Figure 4). The next most abundant classes were Betaproteobacteria and Gammaproteobacteria respectively. Exo-electrochemical active bacteria are used to generate electricity from the oxidation reactions of organic matter with MFC. The taxonomy of exo-electrochemical active bacteria was showed in Table 1.

Table 1. Taxonomy of exo-electrochemical active bacteria in MFC (Zhang et al., 2015)

Taxonomy	Microorganisms	Current or power output
Alphaproteobacteria	*Rhodopseudomonas palustris*	2720 mW m^2
	Acidiphilium cryptum	12.7 mW m^2
Gammaproteobacteria	*Pseudomonas aeruginosa*	< 5 mW m^2
	Escherichia coli	100-600 mW m^2
Deltaproteobacteria	*Geobacter sulfurreducens*	1880 mW m^2

Recently, studies have shown the diversity of the microbial community in an MFC. There are more than 20 exo-electrochemical active bacteria. This study has shown the class of exo-electrochemical active bacteria in MFCs. The non-exo-electrochemical active bacteria are also important for electricity generation (Wei et al., 2014). The exo-electrogenic bacteria have been categorized in diverse groups such as Alphaproteobacteria (Rhodopseudomonas palustris, Ochrobactrum anthropic, and Acidiphilium cryptum), Deltaproteobacteria (Geobacter sulfurreducens, Geobacter metallireducens, and Desulfobulbus propionicus). For example; Geobacter sulfurreducens belong to the Deltaproteobacteria group distributed in the sludge that can serve as an electron donor and electron acceptor in anaerobic conditions (Zhang et al., 2015). Acidiphilium cryptum belongs to Alphaproteobacteria as exo-electrochemical active bacteria for MFCs in acid conditions (Zhang et al., 2015). These results suggest that the class level shown in artificial and tannery wastewater can be simultaneously performing COD and nitrogen removal while also generating electricity.

4. Conclusion

This study has demonstrated effective simultaneous pollution treatment and electricity generation from MFCs with tannery wastewater. MFCs were acclimated with nitrogen and glucose, then acclimated with tannery wastewater to obtain pollution treatment and electricity generation. The microbial diversity confirmed that the system can provide pollution treatment and electricity generation in a single process. Future work should be scaled up to contain more wastewater and achieve more energy production.

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Reference

APHA Standard Methods for the Examination of Water and Wastewater, 21st ed., Washington, DC, USA: American Public Health Association, American Water Works Association, Water Environment Federation, 2005.

Cheng S., Liu H., Logan B.E., 2006, Increased performance of single-chamber microbial fuel cells using an improved cathode structure, Electrochemistry Communications 8 489–494.

Corbella C., Steidl R.P., Puigagut J., and Reguera G., 2017, Electrochemical characterization of Geobacter lovleyi identifies limitations of microbial fuel cell performance in constructed wetlands, International microbiology, 20 (2): 55-64.

Feng, Y., Wang, X., Logan, B.E., and Lee, H., 2008, Brewery wastewater treatment using air-cathode microbial fuel cells, Applied and Environmental Microbiology, pp. 873-880.

He L., Du P., Chen Y., Lu H., Cheng X., Chang B., and Wang Z. 2017. Advances in microbial fuel cells for wastewater treatment. 71: 388-403.

Huang LP, Logan BE., 2008, Electricity generation and treatment of paper recycling wastewater using a microbial fuel cell. Appl Microbiol Biotechnol;80: 349-55.

Logan, B.E., 2009. Exo-electrogenic bacteria that power microbial fuel cells, Nature Review Microbiology, 7(5): 375-381.

Lovley, D.R., 2011. Powering microbes with electricity: direct electron transfer from electrodes to microbes. Environmental Microbiology Reports, 3(1):27-35.

Mardis E.R., The impact of next-generation sequencing technology on genetics, Trends in Genetics, 2008 24 (3).

Min B, Kim JR, Oh S, Regan JM, Logan BE., 2005, Electricity generation from swine wastewater using microbial fuel cells. Water Resour, 39: 4961-8.

Nevin K.P.,Richter H., Covalla S.F., Johnson J.P., Woodard T.L., Orloff A.L., Jia H., Zhang M., and Lovely D.R., 2008, Power output and columbic efficiencies from biofilms of Geobacter sulfurreducens comparable to mixed community microbial fuel cells. Environmental Microbiology, doi:10.1111/j.1462-2920.2008.01675.x.

Park Y., Cho H., Yu J., Min B., Kim SH., Kim GB., and Lee T, 2017, Response of microbial community structure to pre-acclimation strategies in microbial fuel cells for domestic wastewater treatment, Bioresource Technology, pp 176-183.

Patrick Higgins, 2013, ORP Management in Wastewater as an Indicator of Process Efficiency, NEIWPCC's newsletter, Interstate Water Report

Santoro C., Arbizzani C., Erable B. (2017). Microbial fuel cells: From fundamentals to applications. A review. Journal of Power Sources, 356: 225-244.

Sawasdee V., and Pisutpaisal N., 2016, Simultaneous pollution treatment and electricity generation of tannery wastewater in air-cathode single chamber MFC, International Journal of Hydrogen Energy, 41: 15632-15637.

Wei Z., Zheng G., Zhen H., and Husen Z., 2014, Method for understanding microbial community structures and functions in microbial fuel cells: A review, Bioresource Technology, 461-468.

Zhang, J.N., Zhao, Q.L., You, S.J., Jiang, J.Q., Ren, N.Q., 2008. Continuous electricity production from leachate in a novel Upflow air-cathode membrane-free microbial fuel cell. Water Sci. Technol. 57 (7), 1017–1021.

Zhang Y.C., Jiang Z.H., and Liu Y., 2015, Application of Electrochemically Active Bacteria as Anodic Biocatalyst in Microbial Fuel Cells. Chinese Journal of Analytical Chemistry, 43 (1), 155-163.

Zhou J., Bruns M. A., and Tiedje J. M., 1996, DNA Recovery from Soils of Diverse Composition, Applied and Environmental Microbiology, 316-322.