Development of Microbial Fuel Cells using Microorganisms Not Eliminated by Indigenous Bacteria

Kengo INOUE

Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, University of Miyazaki

Research objective

Microbial fuel cells are energy conversion technologies that utilize microorganisms capable of transferring electrons to electrodes, allowing power generation while treating organic compounds in organic wastewater (Fig. 1). In our previous study, microbial fuel cells using livestock waste, such as cattle manure, has been established [1]. By utilizing microbial fuel cells, electricity can be generated while maintaining the treatment efficiency of the most common wastewater treatment technology, aerobic treatment (activated sludge method).

In this study, the long-term operation of microbial fuel cells using swine wastewater was conducted. Microorganisms were isolated from the anode biofilm of microbial fuel cells to develop high-performance microbial fuel cells using the isolate, which was not eliminated by indigenous bacteria in swine wastewater.

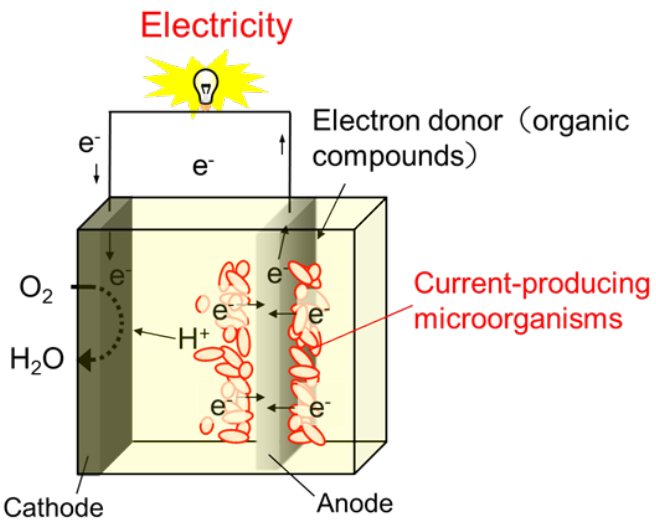

Figure 1. Overview of the Microbial Fuel Cell. Microorganisms in swine wastewater break down and metabolize organic compounds, transferring electrons to the anode electrode in the process. At the cathode, oxygen from the ambient air serves as an electron acceptor, allowing the generation of electrical current through a reaction that involves electrons from the anode and protons from the electrolyte (swine wastewater) to produce water. In this study, we have improved the efficiency of organic compound treatment by using microbial fuel cells while aerating the electrolyte.

Methods

i) Long-term operation of swine wastewater microbial fuel cells: Microbial fuel cells using swine wastewater were operated for 183 d. External resistances ranging from 5.1 to 100 Ω were installed, and the voltage was recorded every minute using a data logger. Swine wastewater was subjected to solid-liquid separation using only the liquid portion. The swine wastewater was replaced approximately once a week. During the operation period, aeration was performed in the swine wastewater while processing the reactor, including the microbial fuel cells. The treatment efficiency was evaluated by measuring the chemical oxygen demand (COD) of swine wastewater in the reactor. Additionally, samples of swine wastewater from the reactor were collected at intervals of 3–4 d, and the pH, nitrate ion concentration, and electrical conductivity were measured.

ii) Microbial community structure analysis: Swine wastewater samples before introduction into the microbial fuel cells, anode biofilms on day 28 of operation, and anode biofilms on day 137 of operation were collected, and DNA was extracted. The extracted DNA was used as a template to amplify the 16S rDNA using PCR. Nucleic acid sequences were determined using next-generation sequencing.

iii) Isolation of microorganisms from anode biofilm samples and identification of isolated strains: To isolate anaerobic electricity-generating bacteria, media containing lactate-sulfate for nitrate-reducing bacteria, acetate-carbonate for methanogens, R2A, and modified GAM were prepared under anaerobic conditions. Noble agar or gelatin gum was added to the solid medium. Biofilm samples scraped from the anode surface were diluted in phosphate buffer to 10^{-4}–10^{-8} under anaerobic conditions and spread onto solid media. The plates were incubated at 30 °C in an anaerobic chamber for several months. Colonies that appeared during the culture period were subjected to colony PCR using universal primers for bacteria and archaea to amplify the 16S rDNA. The obtained DNA fragments were sequenced to identify the genus and species.

Results

During the 183-d operation of microbial fuel cells using swine wastewater, the average power density was 40.4 mW/m^2 (per electrode projection area), and the maximum power density was 83.9 mW/m^2. The organic matter processing rate averaged 432.2 mg-COD/L·day, and the processing efficiency (average processing over 8.7 d) after each swine wastewater replacement was 67.8%.

As considerable output and processing efficiency were achieved, anode biofilm samples were collected from the microbial fuel cells on days 28 (power density 51.4 mW/m^2) and 137 (60 mW/m^2) after the start of operation. Microbial community analysis revealed that the dominant microbial species changed significantly. Before the operation, *Sphirochaetaceae*

(19%), *Clostridiaceae* (14%), *Prevotellaceae* (6.8%), *Ruminococcaceae* (5.2%), and other families were relatively abundant in swine wastewater at the family level. In the anode biofilm on day 28, *Bacteroidales* (11.6%), *Porphyromonadaceae* (8.9%), and *Pelobacteraceae* (8.7%) were predominant, indicating a significant shift in the dominant microorganisms. On day 137 of operation, *Clostridiaceae* (16%), *Methanosaetaceae* (7.4%), and *Bacteroidales* (6.5%) were predominant. Microorganisms belonging to the *Pelobacteraceae* and *Methanosaetaceae* families, which were almost undetectable in the original swine wastewater before treatment, have been suggested to contribute to the decomposition of organic matter and electricity generation in the microbial fuel cells using swine wastewater. Additionally, microorganisms with a high potential for sulfate reduction, such as those from the *Desulfubulbaceae* and *Desulfobacteraceae* families, significantly increased after microbial fuel cell operation. Interestingly, *Geobacteraceae*, which are often dominant in microbial fuel cells near neutral pH, were not detected before or after operation.

After collecting the anode biofilm samples from the microbial fuel cells after operation, the samples were diluted and spread onto various solid media (as mentioned above) under anaerobic conditions. Although numerous colonies were observed in each medium, diversity was not clearly observed in colony morphology. Analysis of the 16S rDNA sequences of the 11 isolates revealed that they belonged to the genera *Bacillus*, *Brachymonas*, *Corynebacterium*, *Parabacteroides*, and *Rhodococcus*. These microorganisms did not match the 16S rDNA sequences of the dominant microorganisms in the anode biofilm after microbial fuel cell operation and were not closely related to them.

Conclusion

In the anode biofilm of microbial fuel cells using swine wastewater, no dominant microorganism species were found after the operation. Therefore, it is difficult to predict the microorganisms with a high likelihood of contributing to electricity generation by comparing microbial community structures. Consequently, despite attempts to isolate microorganisms from various solid media, the isolated strains did not match the target microorganisms. In the future, a narrower focus will be placed on microbial species that are speculated to be highly important, such as methanogens and sulfate-reducing bacteria, and isolation efforts will be conducted using specialized media for each species.

Reference

1) Inoue, K., Ito, T., Kawano, Y., Iguchi, A., Miyahara, M., Suzuki, Y., and Watanabe, K. (2013) Electricity generation from cattle manure slurry by cassette-electrode microbial fuel cells. *J. Biosci. Bioeng*. **116**: 610–615.