■ RESEARCH ARTICLE

Enhancing the Efficiency of a Plant Microbial Fuel Cell through the Use of Methane-Oxidizing Bacteria

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ABSTRACT: Methane-oxidizing bacteria (MOB) and soil microorganisms (SMOs) are known to decompose methane and root exudates, respectively, to produce electrons and soil nutrients like ammonium. This investigation examines how the combined use of the two species may increase the efficiency of existing plant microbial fuel cells (PMFCs). It was found that power output increased by 10.2% when *Methylomonas sp*. (a type of MOB) was used with SMO A separated from local paddy soil. Additionally, SMO A was shown to enhance the growth of both cabbage and rice plants, while *Methylomonas sp*. had little influence on cabbage. When the two species were individually added to PMFCs, SMO A showed more sustained electrical production compared to that of *Methylomonas sp*. Power output of the PMFC including the MOB increased on days 2 and 3, but rapidly decreased as the oxygen composition of the soil decreased. For this reason, MOB seem to be ineffective as a primary, long-term electron source in PMFCs, though it can increase efficiency by providing nutrients from methane oxidation to *Geobacter*. MOB's combination with *Geobacter* seems promising as it creates an anoxic environment (suitable to *Geobacter*) by consuming oxygen.

KEYWORDS: Electrochemistry; Alternative Energy Sources; Microbial Fuel Cells; Plant Microbial Fuel Cells (PMFCs); Methane-Oxidizing Bacteria.

Introduction

With current advancements in technology, the global consumption of electricity is showing unprecedented growth. It was even predicted that electricity usage will increase by over 25% from 2017 to 2040.[1] However, current means of electricity production display several environmental and economic problems. At the current rate of population growth, the development of an efficient, eco-friendly, and economic energy source is of urgent need, and Plant Microbial Fuel Cells (PMFCs) seem to be a promising candidate.[2,3] Solely reliant on plants and microorganisms to decompose nutrients, PMFCs are beneficial in that they require minimal industrial input. The only practical caveat of PMFCs is their low efficiency (typically around 0.022%).[4]

A plant microbial fuel cell system involves multiple steps. First, when soil microorganisms oxidize organic substances from the plant in the PMFC by dissociating them near the cathode, electrons are produced. Then the electrons near the cathode move to the anode and reduce oxygen, an oxidizing agent; current is generated from the movement of electrons. The type of microorganism present in the soil can affect the oxidation reactions and thus the power efficiency of a PMFC. The following equations show examples of electrons being produced from the oxidation of glucose and acetate respectively.[5]

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

OR

$$CH_3COO^- + 3H_2O \rightarrow CO_2 + HCO_3^- + 8H^+ + 8e^-$$

Given the information above, this study examines the effects of introducing a new microorganism – specifically using the synergism between the existing soil microorganisms and methane-oxidizing bacteria – to enhance the power efficiency of the current PMFC model.

All MOB can be categorized into two groups: anaerobic and aerobic (or facultatively anaerobic). Anaerobic MOB have been incorporated in many PMFC-related studies using rice plants in paddy fields because the bottom layer of paddy fields is favorable to anaerobic bacteria and rice farming is a type of hydroponic system suitable for electrons to flow. Yet, aerobic MOB also have the potential to be involved in PMFC research because paddy fields have layers that consist of 10% oxygen. In essence, MOB go through the following oxidation reaction to produce electrons.[6]

$$CH_4 + 3O_2^- \rightarrow CO + 2H_2O + 6e^-$$

OR

$$CH_4 + 4O_2^- \rightarrow CO_2 + 2H_2O + 8e^-$$

In addition to producing electrons, this oxidation reaction produces biofuels, such as methanol, and other by-products, such as CH_2O and $HCOO^-$, that are involved in the oxidation reaction of Geobacter, producing even more electrons.[2] Consequently, the current is increased and thus power efficiency of the PMFC is enhanced, further illustrating the potential of aerobic MOB.

Moreover, certain types of MOB appear to be beneficial to plant growth as a previous study claims that treating peanuts with MOB aided the growth of their roots and root hairs.[7] Improvements in plant growth also can increase the power production of PMFCs as more organic material will be produced by the plant.

The aim of this investigation is to observe the effects of aerobic MOB and SMO on plant growth to develop a more power efficient PMFC model using the two microorganisms. First, the symbiosis of MOB and SMOs in local soil was investigated to determine the appropriate microbe pair and ratio for power output. Next, with the selected MOB and SMO pair, their effects on the growth of rice plants were

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observed. Lastly, the final model was applied to paddy soil with rice plants.

Methods

MOB Incubation and Methane Gas Production:

Initially, two types of MOB – *Methylosinus trichosporium* (KCTC 12760) and *Methylomonas sp*. (KCTC 62176) – were chosen to be used in this study because they can both survive in aerobic conditions and have been studied in the past. The bacteria culture medium that was used consisted of 2.94 g of ammonium mineral salt (AMS, MB cell MB-A0722) and 5 mL of methanol dissolved in 1 L of distilled water. The solution was autoclaved at 121˚C for 15 minutes and was cooled down to 22~25˚C before use. Each type of bacteria was streaked on the solid AMS culture medium in a petri dish using an inoculation loop and then was cultured at 30˚C in an incubator. Figure 1 shows the resulting bacterial colonies.

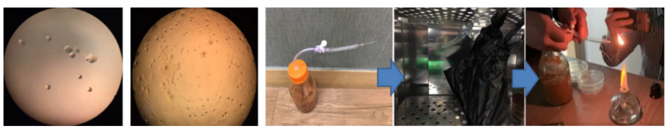

Figure 1: From the left: Incubated *Methylosinus trichosporium* colonies, incubated *Methylomonas sp.* colonies, methane gas synthesis using food waste, checking the presence of methane gas.

A series of preliminary experiments was then performed to design a system for supplying methane gas to the two MOB. The system involved attaching a rubber tube, a small valve, and an injection needle to the lid of a food waste bin and fermenting the waste for seven days. The end of the needle was lit on fire to confirm whether methane was being produced in the bin.

Selection of MOB:

While supplying methane to the MOB, the changes in the voltage of the cell and the growth rate of the rice plants were observed. Both sterilized and unsterilized paddy soil were used to examine the impact of existing soil microorganisms on the voltage of the cell. The electrical cell used in this experiment had a zinc plate as its anode and a copper plate as its cathode, as shown in Figure 2. To supply a consistent amount of methane, the food waste bin was connected to each cell using plastic pipes attached to one-way valves. Each cell contained the AMS culture medium, MOB 1 or 2, and a sponge with 5 rice seeds.

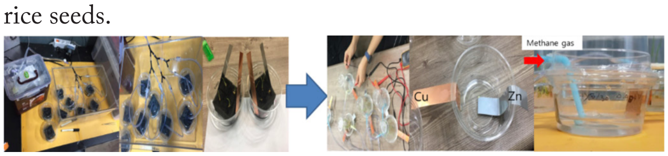

Figure 2: (Left) evenly supplying methane gas from the food waste bin to each container. (Right) Measuring voltage across each MOB container after supplying it with methane gas.

In the preliminary experiments, it was observed that fewer and shorter rice seeds sprouted in the cells containing MOB 1 than in those with MOB 2, as shown in Figure 3. The average voltage was also lower in the cells containing MOB 1 than in the control groups or those with MOB 2. Moreover, the average voltage was higher in the cells containing soil microorganisms and was increased even further when MOB was added. Consequently, the soil microorganisms and MOB 2 were used in the subsequent experiments, and MOB 1 was eliminated.

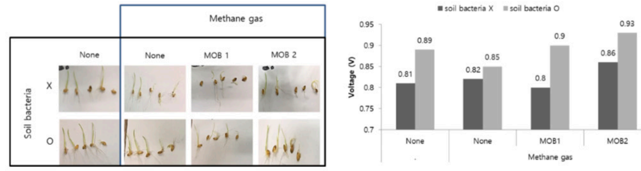

Figure 3: Different combinations of SMO and MOB species and resulting effects on rice plant growth.

Classifying SMOs Based on Electrochemical Characteristics:

The soil microorganism most favorable to the experiment was found by isolating each type of bacteria in the paddy soil that was brought from South Jeolla Province and examining the effect of each type of bacteria on the voltage of the cell and growth of rice plants. 1 g of paddy soil and 10 mL of distilled water were mixed and streaked with an inoculation loop on a solid NB culture medium. The bacteria were grouped into five types based on appearance as shown in Figure 4.

The five types of soil bacteria were separately cultured again on the NB medium. On Day 0 and Day 3 of culturing, the absorbance of the bacteria and the voltage of the cell were observed using a spectrophotometer and multimeter, respectively.

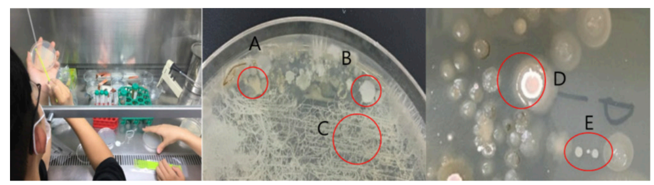

Figure 4: Incubating and separating soil microorganism colonies into 5 groups (A~E).

Electrochemical Behavior of SMOs when Incubated with MOB:

The initial concentrations of SMO A~E were set to be equal at absorbance 0.1 AU before being cultured for 3 days. The NB medium with the cultured SMOs was placed in a 30˚C water bath. A salt bridge was created by heating a 2 M KNO3 solution with agar to measure electrical properties of the solution. A 100 Ω resistor was connected in series to the SMO electrochemical cell to measure power output for 5 minutes each. Circuit diagrams are shown in Figure 5.

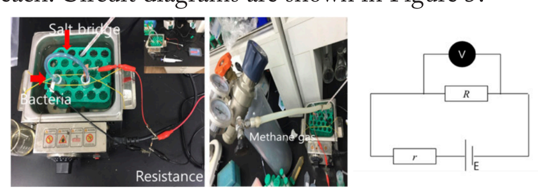

Figure 5: Measuring power output of each cell containing SMO and MOB.

It was found that power output was the highest for the cell containing SMO A. Increase in power of SMO A in a NB medium was measured. As the addition of microorganisms could have influenced the electrical properties (i.e., conductivity) of the solution, its impact was monitored by measuring power output directly from the terminals of the cells without the resistor. Finally, methane gas was readily injected into a solution containing NB, AMS, and MOB (Methylomonas sp.) was cultured, as cultured SMO A was periodically introduced.

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The addition of SMO A in the solution was shown to increase total power output.

Symbiosis of SMO and MOB Based on Effects on Plant Growth and Electrochemical Behavior:

To determine the optimal ratio and concentration of the solution containing soil bacteria A and MOB, three ten-fold serial dilutions (1:10, 1:100, 1:1000) were performed. Then each diluted solution was treated onto a solid 0.7% agar medium in a petri dish, and 16 cabbage seeds were planted in each dish. A week later, the chlorophyll concentration of the cabbage plants and the voltage of each petri dish containing the different solutions were measured.

Application of SMO+MOB Microbial Fuel Cells to Rice Plants in Paddy Soil:

Rice plant seeds were germinated in 0.7% agar medium in advance and replanted in paddy soil. A week after treating SMO A and MOB in each plastic container with paddy soil as shown in Figure 6, the length of the rice plants in and the voltage of each container were measured. However, there was no significant change in the voltage, so the voltage of each container was measured again but on a daily basis for a week. Starting with this experiment, a pure methane gas container was used instead of the food waste bin to supply methane to the fuel cell.

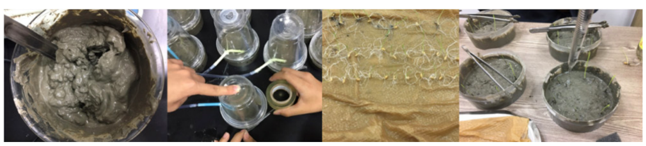


Figure 6: Preparing rice paddy and rice plant containers with SMO A and MOB (*Methylomonas sp.*).

The pH level of the paddy soil in each container was measured using a pH meter and once again using phenol red. The phenol red test was performed by measuring the absorbance of the paddy soil in each container at a wavelength of 415 nm using a spectrophotometer.

Results and Discussion

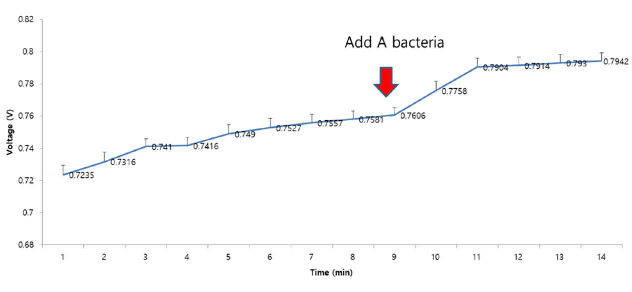


Figure 7: Electrochemical behavior of SMOs when incubated with MOB: potential difference across the 100Ω resistor by time.

Before the addition of MOB and SMO A, voltage remained at around 0.741 V. With the addition of MOB then SMO A, voltage slightly increased to 0.750 V on average then to around 0.794 V, as shown in Figure 7. The addition of MOB caused a 7.0×10^{-5} mW increase in power and 1.1×10^{-5} mW when SMO A was added to it. It was noted that the voltage reading dropped when bacteria were added. These changes were likely due to surface disturbances as the bacteria were added. Such effects were removed from the data as outliers.

The results of the experiment support the initial hypothesis that the presence of both SMO and MOB increases electrical output. However, with further considerations, the reasoning seemed misleading. The voltage of the cell must be constant regardless of biological activity as there is a set potential difference for every cathode-anode pair. Hence, it was that, with more free electrons in the solution due to the oxidation, the conductivity of the solution increased, decreasing the cell's internal resistance. After the experiment, it seemed likely that the increase in voltage measurements of the cell was a result of this decrease in internal resistance: $V=\varepsilon - IR_{internal}$. This new explanation is valid in the sense that less energy (potential drop) is dissipated by the internal resistance, and power output increases when SMO and MOB are used together.

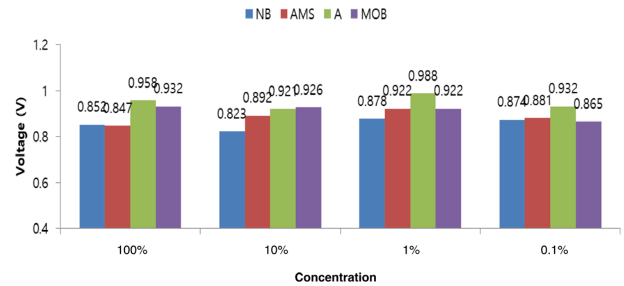


Figure 8: Symbiosis of SMO and MOB based on electrochemical behavior: voltage of PMFCs (petri dishes) with differing concentrations of NB & SMO A (red & blue) and AMS & MOB (green & purple).

As shown in Figure 8, the voltage measurements of the petri dishes with undiluted A and MOB (with concentrations of 100%) were greater than that of the petri dishes with undiluted NB and AMS, respectively. The same applies to the petri dishes with the bacteria/culture-media with concentrations of 10%. In short, the use of SMO A and MOB does increase the voltage of a PMFC when compared to the control groups with the NB and AMS culture media only.

However, there is not a clear relationship between the concentration of bacteria and voltage as the voltage measurements for all concentrations are not noticeably different. Yet, as mentioned above, higher concentrations of SMO A and MOB increase the voltage of the cell.

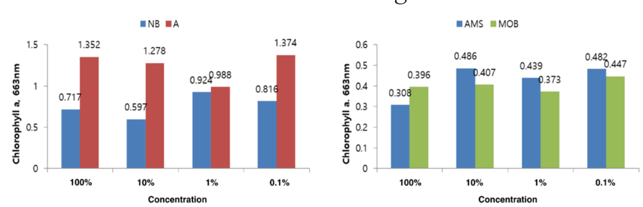


Figure 9: Symbiosis of SMO and MOB based on plant growth: chlorophyll a content in cabbage leaves grown in the agar medium treated with differing concentrations of NB & SMO A (left) and AMS & MOB (right).

As shown in Figure 9, chlorophyll a content in the cabbage leaves treated with SMO A was always higher than in the cabbage leaves treated with NB culture medium only. The difference in the chlorophyll content of the cabbage leaves treated with SMO A and NB culture medium only was the greatest for the undiluted, original solutions with concentrations of 100%. Chlorophyll a content in the cabbage leaves treated with MOB was greater than in the cabbage leaves treated with AMS culture medium only for the undiluted, original solutions with concentrations of 100%. For all other concentrations, chlorophyll content was greater in the container with AMS medium only.

These results show that SMO A always had a positive effect on plant growth in terms of chlorophyll a content regardless of the concentration of the bacteria used. This is because the cabbage leaves treated with NB only were acting as the control group, and the cabbage leaves treated with SMO A along with NB displayed higher chlorophyll content. For similar reasons, the results show that MOB had a positive effect on plant growth in terms of chlorophyll a concentration but only when the solutions with the bacteria are not diluted.

The concentration of the bacteria did not seem to have a significant relationship with plant growth. Instead, the presence in itself, regardless of the concentration, of the bacteria (SMO A and MOB) affected plant growth. SMO A aided plant growth, while MOB hindered plant growth when it was diluted. In short, SMO A could be used at all concentrations and MOB should only be used without dilution.

Taking these results into consideration, the undiluted samples of SMO A and MOB were used in the subsequent experiment with paddy soil.

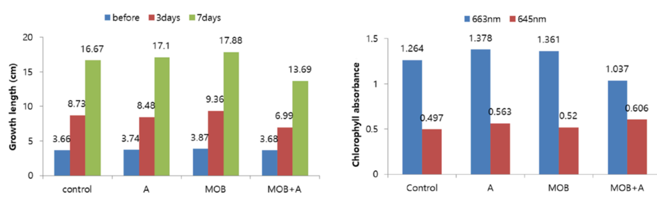
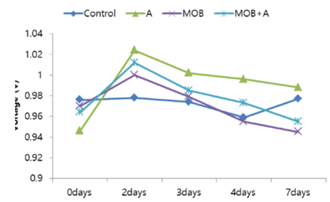


Figure 10: Height and chlorophyll content of the rice plants for varying combinations of SMO A and MOB (*Methylomonas sp.*).

The chlorophyll content and the height of the rice plants were monitored on days 0, 3, and 7 after treating them with SMO A and MOB, as shown in Figure 10. For both plant height and chlorophyll content, the two bacteria seemed to enhance the growth of rice plants when treated separately, with taller rice plants in their respective containers. However, rice plants showed slow growth when treated with both. This was likely due to the over-supply of nitrogen and other nutrients from the oxidation processes of the two bacteria, or the competition between SMO A and MOB.[8]

The rice plants did not grow best when both SMO A and MOB were present in the cell; it was when only MOB was added to the cell. But, all of the experimental conditions allowed for the growth of the rice plants, and none completely hindered the growth of the plants.

As shown in Figure 11, voltage peaked on day 2 for most PMFCs, with the SMO A container showing the largest increase of 0.946 V to 1.024 V. It can therefore be concluded that SMO A's oxidation process is the most rapid, having increased in conductivity the fastest and resulting in lower internal resistance compared to the control group on day 2. Containers with MOB seemed to decrease in voltage measurements rapidly, indicating that the soil did not have



	Control	A	MOB	MOB+A
0days	0.976	0.946	0.97	0.964
2days	0.978	1.024	1.000	1.012
3days	0.974	1.002	0.979	0.985
4days	0.959	0.996	0.955	0.973
7days	0.977	0.988	0.945	0.955

Figure 11: Change in voltage over time in rice plant containers (cells) with different combinations of SMO and MOB.

sufficient methane that can be oxidized. As determined in previous experiments, the container with both MOB and SMO A had higher voltage readings than that of the MOB-only PMFC. However, the container with SMO A was still at a higher voltage than the one with MOB and SMO A, disproving the initial hypothesis. This can be explained using the results from the previous experiment. As plant growth was deterred with the use of SMO A and MOB together, root exudates would have been insufficient for SMO A to oxidize, ultimately leading to smaller decreases in internal resistance (smaller net electrical output).

Conclusion

The aim of this study was to implement aerobic MOB in existing plant microbial fuel cell systems to increase their electrical efficiency. In regard to power output, *Methylomonas sp.* (MOB) and SMO A extracted from local rice fields increased the power output of the PMFC by 10.2% when used together. In regard to plant growth, it was observed that both SMO A and MOB enhanced the growth of rice plants, and that SMO A also aided the growth of cabbage. Treating rice plants with both bacteria prevented proper growth, likely a result of excess nutrients in the soil provided by the MOB and SMOs.

Thus, the synergism of MOB and the existing PMFC model is promising in that MOB enhances the power output when they are in an appropriate ratio with the soil microorganisms in the PMFC. Furthermore, MOB also improves the growth of plants in the PMFC, further enhancing the power output of the cell by helping the plant produce more organic material for the soil microorganisms to oxidize. However, the ratio of MOB to SMO may change as the two species proliferate in the paddy soil, possibly limiting electrical performance and plant growth over time. To prevent the addition of MOB to PMFC from counteracting its purpose of enhancing the cell's performance, more research must be conducted to find a way to control the ratio of SMO and MOB in a paddy field.

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