*Research article*

# Effects of Aeration and pH on the Performance of Lactic Acid Bacteria-Attached Carbon Fiber Electrode

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**Abstract** Lactic acid bacteria (LAB) and iron ions dissolved from steelmaking slag (SS), when attached to an electrode surface, improve the performance of carbon fiber electrodes. However, the electrode potential decreases during the attachment (fermentation without aeration), leading to decreased electrode performance when used as a cathode. Additionally, SS dissolution increases the solution pH, which can affect the survival rate of LAB. This study examines the effects of aeration on an electrode potential and solution pH on the survival rate of LAB in solution during fermentation. In the experiments, SS, LAB beverage, bamboo powder, and carbon fiber electrodes were placed in a bottle with and without aeration. Temporal measurements of the solution pH, iron ion concentrations, adenosine triphosphate (ATP), and electrode potential were performed. The results showed that aeration could prevent a 0.5-fold decrease in the electrode potential due to fermentation. The solution pH temporarily increased and exceeded eight during the fermentation, suggesting that SS had been dissolved. ATP began to decrease when the solution pH exceeded 8, indicating that the solution pH influences the survival rate of LAB. It is recommended that the fermentation should be conducted within three days. Furthermore, to improve the performance of a sediment microbial fuel cell (SMFC), the electrodes with and without aeration should be used as the cathode and anode of SMFC, respectively.

**Keywords** Carbon fiber electrode, steelmaking slag, lactic acid bacteria, aeration, pH

## INTRODUCTION

Microbial fuel cell technology (MFCT), which can be used to treat organic waste, household sewage, and agricultural wastewater, as well as supply electricity to homes and recycle resources back on farmlands, has drawn the interest of many researchers. It is believed that MFCT will provide numerous benefits for addressing and solving social issues such as organic waste disposal and energy scarcity, particularly in developing countries. However, the performance of MFCT remains low and must be improved before it can be used in practice. Increasing electrode performance is one way to improve the MFCT performance. According to Yamasaki et al. (2018), various potential losses occur during electron flow to an electrode. One of these is activation loss, which significantly impacts electrode performance. Pretreating the electrode is widely used to minimize activation loss.

Adsorption of bacteria or metal ions on an electrode surface is one way to improve electrode performance. Wang et al. (2009) successfully enhanced electrode performance by activating bacteria at the electrode, resulting in lower potential losses. The improved electrode performance caused by bacterial attachment and biofilm formation has been widely studied using cyclic voltammetry (Carmona-Martinez et al., 2011; Kang et al., 2012). Nishimura et al. (2018) reported that ferric ions dissolved from steelmaking slag (SS) improved sediment microbial fuel cell (SMFC) performance. Using SS and lactic acid bacteria (LAB) in the anode of SMFC could enhance SMFC performance (Touch et al., 2020).

On this basis, research into the changes in the performance of carbon fiber electrodes caused by the attachment of iron ions (Fe) dissolved from SS and LAB on the electrode surface has begun (Touch et al., 2022). The performance of the carbon fiber electrode improved after attaching (fermentation without aeration) LAB and Fe dissolved from SS on the electrode surface. However, the electrode potential decreases during the fermentation without aeration, decreasing electrode performance when used as a cathode. Therefore, aeration should be provided during the fermentation. Additionally, the dissolution of SS increased the solution pH, which may impact the survival rate of LAB.

## OBJECTIVE

This study investigates the effects of aeration on an electrode potential and solution pH on the survival rate of LAB in the solution during fermentation. Changes in the electrode potential owing to the fermentation are subsequently investigated. Moreover, polarization (current–voltage relation) is measured to detect alterations in SMFC performance when the fermented electrodes are used as the cathode and anode for the long-term current generation.

## METHODOLOGY

### Experimental Materials and Procedures

Touch et al. (2022) proposed a simple attachment process of iron ions dissolved from SS and LAB on the surface of carbon fibers. An attachment process similar to Touch et al. (2022) was used in this study.

Three liters of tap water were filled into cylindrical bottles with inner diameters and heights of 158 and 193 mm, respectively. Fig. 1 shows a schematic of a bottle with a 20-mm depth of SS and a 10-mL lactic-fermenting beverage (a commercial product). 3g of bamboo powder was added to the bottles, and fermentation reduced the solution pH. This process facilitates the dissolution of iron from SS, and nutrients from bamboo powder can activate LAB. An air stone was placed on the SS layer in the case with aeration, and carbon fiber electrodes were positioned 90 mm from the bottle bottom.

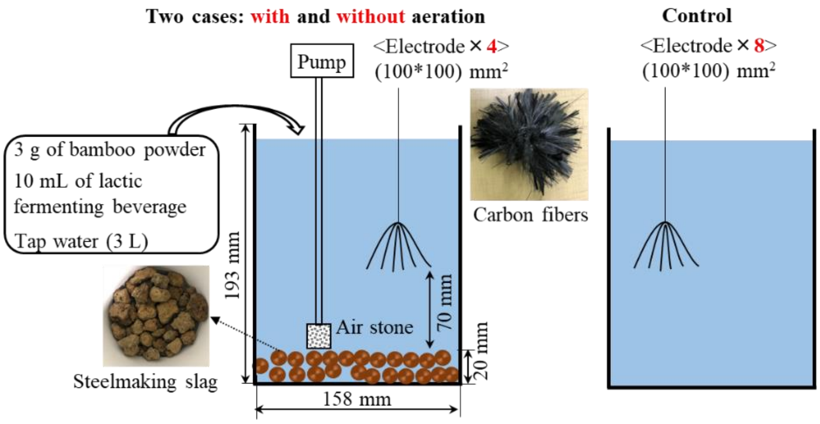



**Fig. 1 Experimental devices and materials**

Carbon cloth (News Company, PL200-E) was used as the electrode material, and it was preheated at 500°C for 1 h to improve its performance (Nagatsu et al., 2014). The heated carbon cloth with a 100-mm width and length was separated into fibers to form a brush-type electrode (Fig. 1).

Four electrodes were placed in a bottle without aeration, four electrodes in a bottle with aeration, and eight electrodes in a bottle filled with only tap water (Fig. 1, Control).

## Operations and Measurements

Experiments were conducted for 22 days following the schematic in Fig. 2. At 3, 7, 10, and 22 days after the experiment began, the potential of each electrode was measured using a voltmeter and an Ag/AgCl reference electrode.

The pH, redox potential (ORP), Fe concentration, and adenosine triphosphate (ATP) of the solution were measured on the day of measuring electrode potential. The pH and ORP were measured using a pH/ORP meter (Horiba, D-50), Fe concentration was measured using PACKTEST (Kyoritsu Corp., WAK), and ATP was measured using a lumitester (Kikkoman, Lumitester-Smart).

The fermented and control electrodes extracted from the bottles on Day 3 were used as the cathode and anode of SMFCs to examine the long-term performance of SMFCs for electricity generation (Fig. 2b). For the electrical current generation, an external resistance of 1 Ω was loaded between the anode and cathode. Several polarization measurements were performed throughout the electricity generation using the circuit in Fig. 2a to examine the performances of SMFCs. In the polarization measurement, an external resistance with 1 Ω–9.1 kΩ was loaded between the anode and cathode. Cell voltage was recorded 1 min after loading each external resistance and used to calculate the current according to Ohm's law: $I = U/R_{ex}$, where $U$ [V] is the voltage, $I$ [A] is the current, and $R_{ex}$ [Ω] is the external resistance. The power $P$ was calculated using $P = IU$. Current and power densities were obtained by dividing these values by the surface area of the electrode, i.e., 0.01 $m^2$. The maximum current density was also determined using polarization, as Nagatsu et al. (2014) described.

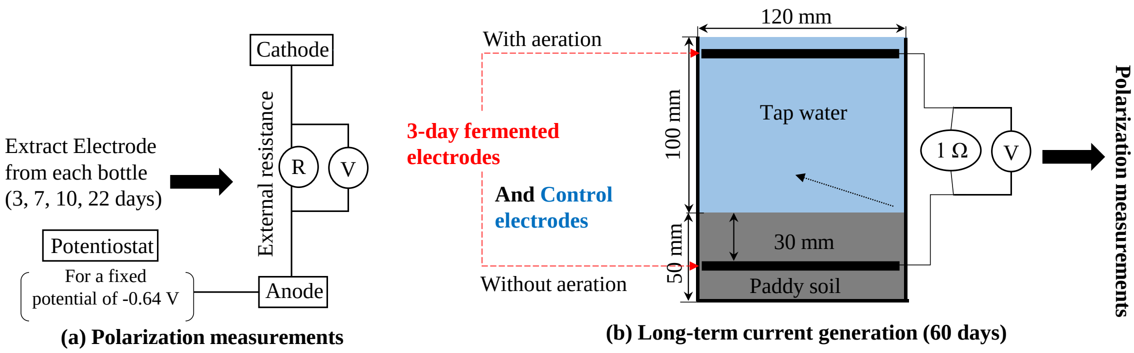



**Fig. 2 Operation and measurement methods**

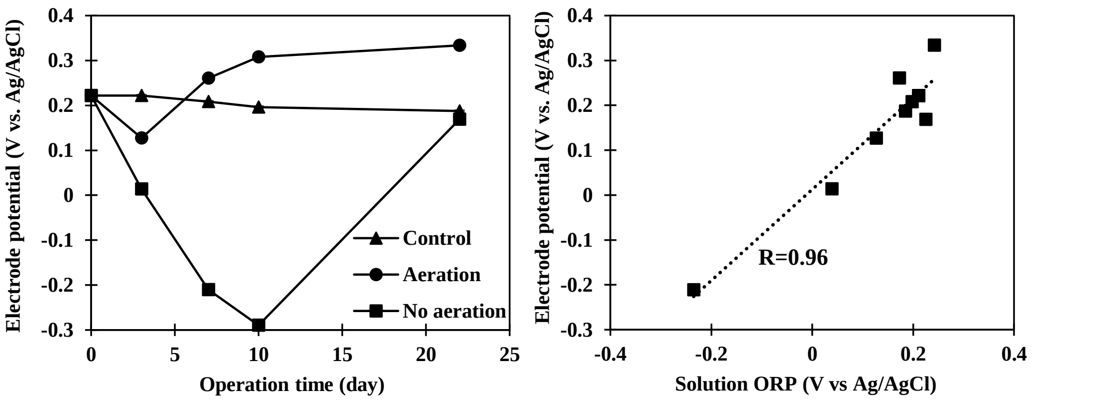
## RESULTS AND DISCUSSION

## Effects of Aeration on the Electrode Performance

Touch et al. (2022) discovered that the electrode potential decreased during fermentation without aeration. However, they were unclear about the decreasing characteristics of the electrode potential under the fermentation with and without aeration. Fig. 3 showed a comparison of the electrode potential and the solution ORP.

The electrode potential was 0.21 V on average for the control electrode during the experiment. On Day 3, the electrode potential decreased to 0.13 and 0.01 V for the electrodes fermented with and without aeration, respectively (Fig. 3a). Although aeration was performed, electrode potential decreased due to the fermentation of the bamboo powder. However, a 0.05 V decrease in the electrode potential was observed when aeration was performed, whereas a 0.11V decrease in the electrode potential was observed without aeration. Without aeration, the electrode potential further decreased to − 0.29 V (on Day 10) but increased to 0.31 V (on Day 10) when aeration was performed.

As expected, aeration can reduce the decline in electrode potential caused by fermentation. Interestingly, the electrode potential with aeration was high compared to that of the control electrode, suggesting that the higher performance of the electrode was due to the adsorption of LAB and iron ions on the electrode surface when aeration was conducted. Although without aeration, the electrode potential increased from -0.29 to 0.17 V (Day 10 to 22), suggesting that the fermentation of bamboo powder intensively occurs during only the first ten days. Furthermore, it was observed that the electrode potential had a high correlation (R = 0.96) with the solution ORP (Fig. 3b), indicating that the electrode potential depends on the characteristics of the solution. The electrode potential decreased when the electrode was place in a solution with a low ORP.

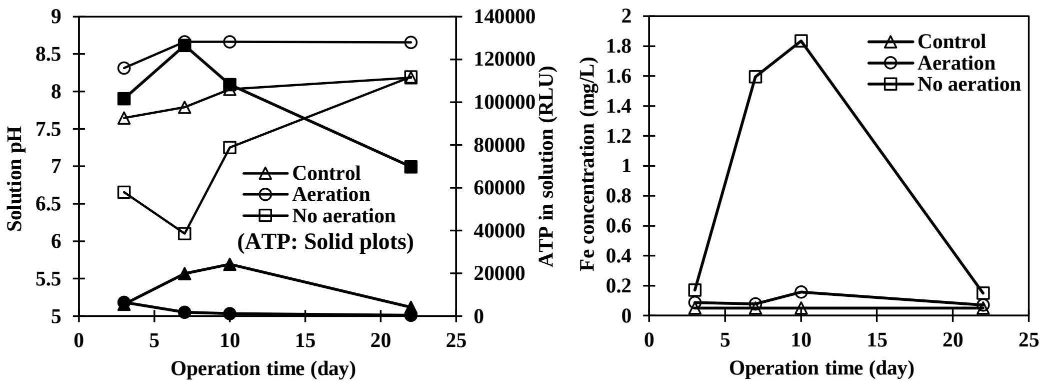



(a) Electrode potential (b) Correlation of solution ORP with electrode potential

**Fig. 3 Electrode potential under different conditions and its relationship with solution ORP**

**Effects of Dissolution of Steelmaking Slag on LAB**

Figure 4 depicts the temporal changes in the solution pH, ATP, and Fe concentration. On Day 3, the solution pH for the control case was 7.6, for the aerated case was 8.3, and for the unaerated case was 6.7 (Fig. 4a, open plots). The solution pH differed due to bamboo fermentation (acidification) and dissolution of SS (alkalization).




(a) Solution pH and ATP in solution (b) Fe concentration in solution

**Fig. 4 Temporal changes in pH, ATP, and Fe concentration**

The pH of a solution generally rises when SS is dissolved, whereas it decreases due to the release of organic acids during bamboo fermentation. With aeration, the SS dissolution was significant in changes the solution pH because the released organic acids were oxidized. This is the cause of the increase in solution pH in the aerated case. However, without aeration, the solution pH decreased to 6.1 on Day 7 due to the release of organic acids. When the fermentation was completed, the accumulated organic acids started to oxidize. In addition to the SS dissolution, this oxidation contributed to the increasing pH, as shown in Fig. 4a.

Fe concentration increased in both cases with and without aeration (Fig. 4b), indicating SS dissolution during fermentation. Remarkably, a 20-fold high in Fe concentration was observed in the unaerated case compared with that of aerated case, suggesting that fermentation without aeration can effectively facilitate the dissolution of Fe from SS.

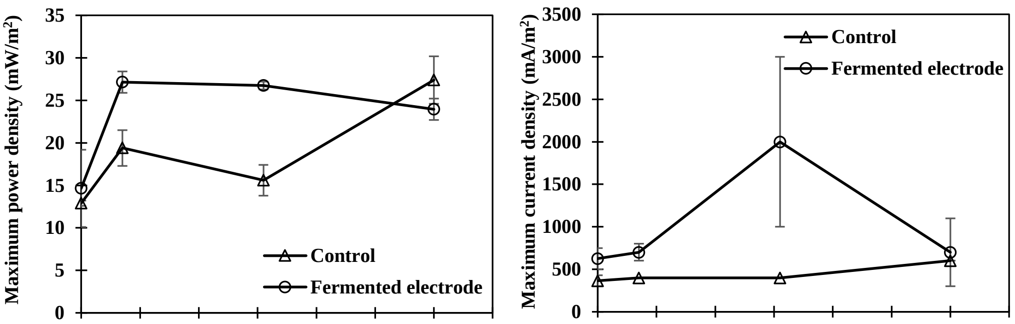
The decreasing ATP appeared when the pH was 8.0 for the control case, 8.3 for the aerated case, and 7.3 for the aerated case (Fig. 4a, solid plots). This ensured that an increase in solution pH influenced the survival rate of LAB. It is thought from this result that pH should be kept at less than 8, and preferably in an acidic state during the fermentation.

Overall, the fermentation period should be less than three days, especially if aeration is used. To increase the performance of SMFC, the electrode fermented with aeration should be used as the cathode, and the electrode fermented without aeration should be used as the anode. As shown in Fig. 2, experiments were conducted to verify our suggestion, and the experimental results are discussed in the following section.

### Long-Term Performance of the Fermented Electrode

Figures 5a and 5b show the comparison of performance (power and current density) of SMFCs when using the electrodes with and without fermentation. As expected, the maximum power density ($P_{max}$) and current density ($I_{max}$) of SMFCs that used fermented electrodes were higher than those that used control electrodes (without fermentation). When fermented electrodes were used, $P_{max}$ was 27 mW/m$^2$ (Fig. 5a) and $I_{max}$ was 2000 mA/m$^2$ (Fig. 5b) on Day 31. Compared with those that used control electrodes, a 1.7-fold increase in $P_{max}$ and a 5-fold increase in $I_{max}$ were observed. As discussed earlier (Fig. 3), the cathode is more effective when fermented with aeration, and the anode is more effective when fermented without aeration. This increased electromotive force, which increased the current density.

However, the performance was approximately the same on Day 60, suggesting that the fermented electrode is still effective within 60 days. Generally, the quality of cathode water temporally varies along with electricity generation, specifically an increase in pH (Touch et al., 2017). It is assumed that the increase in pH of cathode water influences the survival rate of LAB attached on the cathode, leading to a decrease in SMFC performance.




(a) Maximum power density (b) Maximum current density

**Fig. 5 Temporal variations of maximum power and current densities**

## CONCLUSION

This study used laboratory tests to confirm the impact of aeration on electrode potential and solution pH on the survival rate of LAB in the solution during fermentation. Although aeration was conducted during the fermentation, a 0.05 V decrease in the electrode potential was observed. However, this decrease was smaller than without aeration (a 0.11 V decrease). Interestingly, fermented electrode potential was higher than without fermentation when aeration was conducted for more than seven days. The increase in solution pH during the fermentation affected the survival rate of LAB. Based on these results, it is recommended to complete the fermentation within three days, particularly in the case of aeration. Furthermore, during the fermentation, the solution pH should be kept at or less than 8 and preferably in an acidic state. It was also found that a decrease in pH during the fermentation aided the dissolution of Fe from the SS. Using electrodes fermented with and without aeration as the cathode and anode, respectively, it was possible to improve the SMFC performance. However, the improved performance was kept within 60 days due to changes in the quality of cathode water caused by long-term electricity generation.

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