Enhancing Factors of Electricity Generation in a Microbial Fuel Cell Using Geobacter sulfurreducens

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In this study, we investigated various cultural and operational factors to enhance electricity generation in a microbial fuel cell (MFC) using Geobacter sulfurreducens. The pure culture of G. sulfurreducens was cultivated using various substrates including acetate, malate, succinate, and butyrate, with fumarate as an electron acceptor. Cell growth was observed only in acetate-fed medium, when the cell concentrations increased 4-fold for 3 days. A high acetate concentration suppressed electricity generation. As the acetate concentration was increased from 5 to 20 mM, the power density dropped from 16 to 13 mW/m², whereas the coulombic efficiency (CE) declined by about half. The immobilization of G. sulfurreducens on the anode considerably reduced the enrichment period from 15 to 7 days. Using argon gas to create an anaerobic condition in the anode chamber led to increased pH, and electricity generation subsequently dropped. When the plain carbon paper cathode was replaced by Pt-coated carbon paper (0.5 mg Pt/cm²), the CE increased greatly from 39% to 83%.

Keywords: Microbial fuel cell, Geobacter sulfurreducens, electricity generation, coulombic efficiency, substrate specificity, immobilization

In a natural environment, dissimilatory metal-reducing bacteria use ferric oxide as a terminal electron acceptor which cannot diffuse across the cell membrane and into the cell. It is known that they transfer electrons to iron oxide outside the cell via direct contact by outer membrane cytochromes or use of excreted mediators [19, 21]. On the same principle, they transfer electrons from the intracellular cell to the extracellular anode instead of iron oxide as their electron acceptor in microbial fuel cells (MFCs). These electrons flow through a circuit to the cathode, where they combine with protons and oxygen. Consequently, the extracellular electron transfer of microorganisms makes MFCs produce electricity from organic matter. This is the reason why we call metal-reducing bacteria in MFCs electrochemically active bacteria or exoelectrogens [8, 16].

In the beginning of MFC studies, exogenous electron mediators were considered essential for efficient electricity production [2]. These electron-shuttling mediators accept electrons within a cell or on the cell surface and release them at the anode. Unfortunately, the toxicity and instability of synthetic mediators limit their applications in MFCs. However, Kim et al. [13] first demonstrated electricity production by a bacterium in the absence of an exogenous mediator. The mediatorless MFC is very attractive because it is operationally stable and yields high coulombic efficiency. Metal-reducing bacteria of Shewanella, Geobacter, and Rhodoferax species have been reported to directly transfer electrons to the electrode in mediatorless MFCs [5, 7, 15, 18]. Among many organisms discovered in MFCs, Geobacter species are well-known representatives of exoelectrogens that can generate electricity from organic matter [9, 14]. The complete genome of G. sulfurreducens was recently sequenced to provide the physiological properties of Geobacter species [17]. Bond and Lovley [5] demonstrated that G. sulfurreducens can completely oxidize acetate by using an electrode as the sole electron acceptor. They achieved the high electron recovery of 95% with power density of 49 mW/m² using a two-chamber MFC. This result suggests that G. sulfurreducens can directly attach to electrodes and transfer electrons via c-type cytochromes at the outer membrane. Furthermore, a conductive pilus termed a nanowire was discovered in G. sulfurreducens [22]. It provides a new insight into extracellular electron transfer via microbial nanowires.

Since there is no need to add an artificial mediator, mediatorless MFCs are advantageous in various applications such as biosensors, wastewater treatment, remote power sources, and bioremediation [10]. To further develop MFC technologies, it is necessary to better understand the

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physiological characteristics of microbial communities in MFCs. Although microbial diversity and its electron transfer mechanisms in MFCs have been broadly studied, there is still a lack of information about the optimal growth conditions of pure exoelectrogen cultures. In addition, the mixed cultures are generally complex and make it difficult to elucidate the response of an MFC. This study, therefore, focused on the physiological characteristics of the representative exoelectrogen G. sulfurreducens under various growth and operational conditions. The object of this study was to investigate the effect of substrate specificity, substrate concentration, cell immobilization, anodic pH, and electrode modification with Pt catalyst on the electrochemical activity of G. sulfurreducens.

**Materials and Methods**

**Microorganisms and Culture Conditions**

G. sulfurreducens (ATCC 51573) was obtained from the American Type Culture Collection (ATCC). The growth medium contained the following (per liter of deionized water): 0.1 g KCl, 1.5 g NHCl, 0.6 g NaHPO₄, 2.5 g NaHCO₃, 10 ml of Wolfe’s mineral solution [12], and 10 ml of Wolfe’s vitamin solution [12]. The sterilized medium was controlled to adjust the final pH to 6.8 for the optimal condition for microbial growth [14]. Batch cultivation was performed using inoculation of 10% in a 150 ml serum bottle, into which 20 mM acetate and 50 mM fumarate were added as an electron donor and acceptor, respectively. After inoculation, the bottle was flushed with a N₂-CO₂ gas (80:20) to remove oxygen, and it was kept at 30°C. The color of the culture medium changed from yellow to orange when acetate was oxidized by G. sulfurreducens. The inoculums were transferred two times for each 72 h anaerobically. Before adding it to the anode chamber of MFC, the culture of G. sulfurreducens, which was harvested during the late-exponential phase, was washed twice with the growth medium lacking acetate and fumarate. The optical density of inoculums was 0.1 at 660 nm.

To investigate the substrate specificity of G. sulfurreducens, the culture was centrifuged twice with the growth medium lacking acetate and was then inoculated into a 50 ml serum bottle (10% inoculation). The optical density at 660 nm of the initial inoculums was adjusted to 0.07, while the initial pH was 7. Four different organic acids of succinate, butyrate, malate, and acetate of 20 mM were used as electron donors in the serum bottles. The experiments were carried out in duplicate, and the values were averaged.

To evaluate the effect of bacterial immobilization on electricity production, G. sulfurreducens was inoculated into the MFCs using carbon paper and carbon felt as the anodes. The mixture of inoculums and growth medium was continuously stirred using a magnetic bar, and was then replaced by fresh medium every 72 h for 10 days. After immobilization of G. sulfurreducens, the carbon paper and carbon felt were directly used as an anode in the MFC experiments.

**Preparation and Construction of MFC**

Two-chambered MFCs were constructed as reported in a previous study [2]. Two acrylic rectangular chambers (total volume: 25 ml each; working volume: 20 ml each) served as anode and cathode chambers and were separated by a proton exchange membrane (12 cm², Nafion 117; DuPont Co., USA) held in place by rubber gaskets (Fig. 1). The chambers were fitted with ports for fixing the electrodes, adding solution components, passing gases, and sampling.

Prior to use, all components of the MFCs were pretreated to prevent possible metal and organic contamination. The acrylic plates were immersed in 0.4% (w/v) NaClO and washed carefully in Milli-Q water. The rubber gaskets and Teflon tubes were sterilized using 70% ethanol. The Nafion membrane was pretreated, rendered in the H⁺ form, by soaking it in 0.5 M H₂SO₄ for 30 min, after which it was stored in Milli-Q water. The electrodes were soaked in 1 N HCl for 30 min and in 1 N NaOH for 30 min and stored in Milli-Q water before use.

Each chamber contained a plain carbon paper electrode (11.5 cm², without wet proofing, TGPH-090; E-Tek, USA) as the anode and cathode, if not otherwise specified. The anode and cathode were spaced 1.5 cm apart. Both electrodes were attached to platinum wires with silver paste (Dotite, Fujikura Kansei Co., Japan) and epoxy (Devcon, ITW Performance Polymers Co., USA) to connect them through the external wire. The resistance of the carbon paper connecting the platinum wire was between 0.4 and 0.7.

**Operation of MFC**

The anode chamber of each MFC was filled with the G. sulfurreducens culture and the same medium that was used for cell growth, except for fumarate. The G. sulfurreducens culture, which was harvested during the late-exponential phase, was washed twice with the growth medium lacking acetate and fumarate, before use. If not otherwise specified, 5 mM acetate was used as the electron donor and carbon source. The cathode chamber of each MFC was filled with the solutions containing (per liter of deionized water) 0.1 g KCl, 0.6 g NaHPO₄, 2.9 g NaCl, and 4.8 g Tris-HCl. The cathode chamber was continuously aerated to supply oxygen as an electron acceptor, while the anode chamber was continuously aerated with argon (Ar) gas to prevent oxygen diffusing from the cathode to the anode and to mix the anolyte. The gases were filtered through 0.2 µm-pore-size membranes positioned in front of the MFC, and their flow rates were maintained at 7 ml/min. All of the stock solutions were flushed carefully with a N₂-CO₂ gas (80:20) before use. MFC experiments were carried out at 30°C in a temperature-controlled room.

![Fig. 1. Photograph of a two-chambered MFC.](image-url)
Analysis and Calculation
The voltage over the external resistance was recorded by a digital multimeter (PC20; Sanwa Co., Japan) interfaced to a personal computer. The current I in amperes (A) was calculated using Ohm’s law, I = V/R, where V is the measured voltage in volts (V), and R is the known value of the external load in ohms (Ω). The external resistance used in the experiment was 500 Ω, if not otherwise specified. The recovery of electrons, that is, the coulombic efficiency (CE), is calculated as

\[
CE = \frac{M \int I \, dt}{B \cdot v_{in} \cdot \Delta \varepsilon}
\]

where Δε is the substrate concentration change over the batch cycle over a time (t), M is the molecular weight of the substrate, F is Faraday’s constant, b is the moles of electrons defined for the substrate based on a half reaction, and v_{in} is the volume of liquid in the anode chamber [16].

Organic acids such as acetate, malate, succinate, and butyrate were analyzed using a high-performance liquid chromatograph (Model VP; Shimadzu Co., Japan) equipped with a sulfonated divinyl benzene-styrene copolymer column (300 mm × 7.8 mm, Aminex HPX-87H; BioRad, USA) and a photometric detector (216 nm). An aqueous solution of 10 mM H₂SO₄ was used as an eluting solution at 0.6 ml/min, and the column temperature was maintained at 30°C. The cell growth was determined by measuring the OD at 660 nm in a 3 ml cuvette with a 1 cm light path.

RESULTS AND DISCUSSION
Substrate Specificity of G. sulfurreducens
Pure cultures of G. sulfurreducens were cultivated using various substrates including acetate, malate, succinate, and butyrate to investigate their substrate specificity. Before use, the cultures were centrifuged twice with a growth medium lacking organic acid and were then inoculated into a serum bottle using fumarate as an electron acceptor. Fig. 2 depicts the pH variation and the cell concentrations, which were measured as the optical density at 660 nm (OD₆₆₀). During 7 days, the initial pH decreased slightly from 7 to 6.6, regardless of the substrate type. However, the growth of G. sulfurreducens was observed only when acetate was added. The cell concentration (OD₆₆₀) in the bottle into which acetate was added increased significantly from 0.1 to 0.4 for 3 days and became stable by 7 days. In contrast, the cell concentrations decreased from 0.1 to 0.05, 0.07, and 0.06 for 7 days when malate, succinate, and butyrate were used, respectively. These results are direct evidence that the culture of G. sulfurreducens prefers acetate as the carbon and energy source for growth. This is consistent with the results of a previous study that showed the Geobacteraceae family was the predominant bacteria when acetate was used as the fuel in an MFC using marine sediment as the inoculum [4].

Effect of Acetate Concentration on Electricity Generation
The MFCs inoculated with G. sulfurreducens cultures were operated using different acetate concentrations. As shown in Fig. 3, the 5 mM acetate-fed MFC went through 8 batch cycles for 20 days, whereas the 20 mM acetate-fed MFC passed through only 3 batch cycles during the same period. When 5 mM acetate was added to an MFC, the average power density of 1.2 mW/m² at the first batch cycle was greatly increased to 15.2 mW/m² at the sixth cycle. After 15 days of operation, the power density of the 5 mM acetate-fed MFC became stable at 16.2 mW/m². In contrast, the power density of the 20 mM acetate-fed MFC increased from 1.5 to 13.4 mW/m² during only 3 batch cycles, because it took a longer time for substrate of high concentration to be degraded per batch cycle. Regardless of acetate concentration, it took about 15 days for MFCs to reach a steady state in current generation.

At the steady state, the 5 mM acetate-fed MFC achieved the CE of 46.2%, which was two times higher than that in the 20 mM acetate-fed MFC. The power generation of MFCs generally increases with substrate concentrations, but this result suggests that high substrate concentrations can inhibit power production. Sharma and Li [24] reported that high substrate concentrations limited the power generation in MFCs inoculated by mixed cultures. They observed that the cell voltage had a reverse correlation with substrate concentration when the acetate concentration was higher than 8 mM. There are two possible reasons for the lower CE at high acetate concentrations. First, the electron sink for bacterial growth might reduce the CE of MFCs [1]. A high substrate concentration facilitates the cell growth of G. sulfurreducens, and it causes much of the electrons to be consumed for biomass production instead of electricity generation. Second, an excessive amount of substrate might inhibit the electron transfer activity via...
direct contact between the enzyme in the outer membrane and the anode [24]. At high substrate concentrations, more than one substrate could bind to the active site of an enzyme and consequently block the enzyme activity for electron transfer to the anode.

**Immobilization of G. sulfurreducens for Effective MFC Operation**

It is known that *G. sulfurreducens* can directly transfer electrons to the anode via bacterial surface redox active proteins or conductive pili. Accordingly, biofilm formation on the anode is important to allow direct electron transfer to be successfully conducted. However, it usually takes several weeks or even several months to establish a stable biofilm from a pure or mixed culture [3]. In this work, therefore, the cultures of *G. sulfurreducens* were immobilized onto the anode to reduce the enrichment period and enhance electricity generation. Fig. 4 shows the effect of immobilization of *G. sulfurreducens* on carbon paper and carbon felt on electricity generation. When the suspended culture of *G. sulfurreducens* and 20 mM acetate were injected into an MFC, it took more than 15 days to reach a steady condition. However, the MFC using a *G. sulfurreducens*-immobilized anode became stable in 6–8 days. Compared with the use of carbon paper, the immobilization of *G. sulfurreducens* on the carbon felt was favorable to high power production at the beginning of MFC operation. In a report by Cha *et al.* [6], it was shown that the MFCs using carbon felt anode produced higher power density than those using carbon cloth. They suggested that the high porosity of carbon felt can make bacteria be easily attached to the electrode and facilitate more rapid substrate diffusion to the biofilm.

**Effect of Argon Gas Purging on Anolyte pH and Electricity Generation**

In this study, the anode chamber was continuously aerated with argon gas to prevent oxygen diffusing from the cathode to the anode and to mix the anolyte. The flow of argon gas in the anode chamber resulted in the liberation of carbon dioxide into the atmosphere. It made a change in the equilibrium condition for carbon dioxide between liquid and gas phases. Consequently, the anodic pH increased to 9.5 after 27 days of operation, and the electricity generation stopped, as shown in Fig. 5. This result shows that current generation by *G. sulfurreducens* is severely inhibited at pH levels over 9. When 1 M HCl was added to the anode chamber to adjust the pH level, electricity was stably produced over 35 days.

The effect of anodic pH on current generation has been investigated in several studies. However, the optimal pH for maximizing the power output varied according to the type of microorganisms and reactor configuration. Gil *et al.* [11] determined the best performance at pH 7 using a two-chambered MFC, which used wastewater as the electron donor and sludge as the bacterial source. In contrast, the acidophilic condition of pH 6 in the anodic chamber showed the highest power density, where the anaerobic mixed consortia from the biofilm reactor producing fermentative
H$_2$ was used as the inoculum [25]. Zhuang et al. [26] reported that a tubular MFC produced the maximum power density with an anodic pH of 10 and a cathodic pH of 2. They suggested that the suppression of methanogenesis with highly alkaline anolyte (pH 10) contributed to better coulombic efficiency. Similar to our study, Nimje et al. [20] evaluated the effect of anodic pH on current generation using a pure culture of *Enterobacter cloacae*. When the anodic pH was increased from 6.5 to 9.5, the maximum power density decreased from 36 to 6.5 mW/m$^2$ with an increase of internal resistance. These results, therefore, indicate that appropriate pH control, considering the microorganism types and reactor configurations, is a critical factor to improve the performance of MFCs.

**Enhancing Electricity Production Using a Pt-Containing Carbon Cathode**

The slow rate of oxygen reduction on the surface of the carbon electrode leads to a high cathodic overpotential, which limits the performance of MFCs. Several studies have suggested strategies to reduce cathodic overpotentials by using a mediator, modifying the electrode with a catalyst and optimizing the operational conditions in the cathode chamber [23]. This study investigated the effect of a Pt-coated cathode on the electricity production of MFCs using *G. sulfurreducens*. As shown in Fig. 6, an MFC was continuously run in acetate-fed batch mode, where the plain carbon paper cathode was replaced by Pt-coated carbon paper (0.5 mg Pt/cm$^2$) after 38 days of operation. Using the Pt-coated cathode, the power density was greatly increased from 3.5 to 75 mW/m$^2$, and the CE also increased from 39% to 83%. This indicates that electricity production of MFCs using *G. sulfurreducens* is highly associated with cathodic overpotential for oxygen reduction.

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**References**


