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Increased generation of electricity in a microbial fuel cell using *Geobacter sulfurreducens*

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Abstract−The microbial fuel cell (MFC) has attracted research attention as a biotechnology capable of converting hydrocarbon into electricity production by using metal reducing bacteria as a biocatalyst. Electricity generation using a microbial fuel cell (MFC) was investigated with acetate as the fuel and *Geobacter sulfurreducens* as the biocatalyst on the anode electrode. Stable current production of 0.20-0.24 mA was obtained at 30-32 °C. The maximum power density of 418-470 mW/m^2, obtained at an external resistor of 1,000 Ω, was increased over 2-fold (from 418 to 866 mW/m^2) as the Pt loading on the cathode electrode was increased from 0.5 to 3.0 mg Pt/cm^2. The optimal batch mode temperature was between 30 and 32 °C with a maximum power density of 418-470 mW/m^2. The optimal temperature and Pt loading for MFC were determined in this study. Our results demonstrate that the cathode reaction related through the Pt loading on the cathode electrode is a bottleneck for the MFC's performance.

Key words: Microbial Fuel Cell, *Geobacter sulfurreducens*, Electricity Generation

INTRODUCTION

The microbial fuel cell (MFC) has attracted research attention as a biotechnology capable of converting hydrocarbon into electricity production by using metal reducing bacteria as a biocatalyst. MFC has also been applied as a wastewater treatment method where organic pollutants can be removed with CO_2 and H_2O [1-5]. Most previous studies have focused on a mediator-MFC using potassium ferricyanide [6], cobalt sepulchrate, anthraquinone [7], thionine [8], and azure A [9] as the mediator in an anode chamber. However, these mediators have had limited practical applications due to their high cost and toxicity to bacteria.

Metal-reducing bacteria capable of transferring electrons directly to the electrode can be applied to an MFC without mediators. Studies on *Geobacter sulfurreducens* [4], *Geobacter metallireducen* [2], *Clostridium butyricum* [11], *Rhodoferax* ferrireducens [1], and *Shewanella putrefaciens* [12] demonstrated that those stable biofilms formed on the anode electrode transferred electrons to the electrode. Recently, it was found that a mixed microbial community, consisting primarily of *Alcaligenes faecalis*, *Enterococcus gallinarum*, and *Pseudomonas aeruginosa*, could produce electrical power in an MFC by using mediators produced by a bacterial community [13]. In an MFC, electricity is usually generated by using a variety of fuels such as glucose, lactate, pyruvate, fumarate, benzoate, acetate, hydrogen, and wastewater [1,12-17] In general, wastewaters have been reported to produce lower power density than systems using pure chemicals [18].

Many studies have focused on improving electricity production with several different techniques such as controlling the self-growth of microbial cells [19], screening of more electrochemically active microbes [12], coupling with direct hydrogen oxidation [20], designing various new electrode materials [1,2,4,21,22], selecting suitable proton-conducting materials [2,23], and controlling pH in the anode or cathode chamber [24,25]. In less than a decade, power density has increased significantly from several dozen to 1,540 mW/m^2 with O_2/Pt as the cathode electrode. Although the highest power density of 4,310 mW/m^2 was reported with ferricyanide/graphite as the cathode electrode, ferricyanide has not been considered as a promising electrode in research on MFC due to its toxicity [1-4,13]. Although the power density has been considerably improved by several orders of magnitude in some innovative studies without membrane, coulombic efficiency and energy recovery decreased considerably due to the lack of membranes in its system [3,5,17,26]. Therefore, the main issues in an MFC include high coulombic efficiency and energy recovery, as well as power density. Bond et al. studied the temperature effect of MFC containing *Geobacter sulfurreducens* inoculation with chemical as electron acceptor. However, the data were not shown clearly in their study. In addition, their study did not give us any information for the effect of temperature in MFC [27].

In this study, *Geobacter sulfurreducens* is used to investigate the effect of temperature and Pt loading on the cathode electrode in an MFC. The study results demonstrate that the cathode reaction related through the Pt loading on the cathode electrode is a bottleneck for the MFC's performance. Using different Pt loadings of 0, 0.5, and 3 mg/cm^2 on carbon paper with a cathode/anode surface area of 4 : 1, a power density enhanced many times higher than that reported in other studies was achieved by using batch mode MFC with a Nafion membrane.

EXPERIMENTAL

1. Culture and Growth Medium

Geobacter sulfurreducens, ATCC-51573, was grown in an anaerobic flask of 50ml with the following composition [28]:

NH_4Cl: 1.5 g/L, NaH_2PO_4: 0.6 g/L, KCl: 0.1 g/L, $NaHCO_3$: 2.5 g/L, Wolfe's mineral solution of 50 μl and Wolfe's vitamin solution of 50 μ, Sodium acetate: 0.82 g/L

The medium was continuously flushed under a mixture of 80% N_2

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and 20% CO_2 to remove oxygen until a pH value of 6.8 was reached, and then autoclaved at 121 °C for 15 minutes. Finally, sodium fumarate was added to the medium to obtain a solution of 8.0 g/*l* using a sterilized-filter with a 0.45 mm diameter [28]. Cultures were maintained by serial transfer of 10% inoculum into bottles containing 20% CO_2 and 80% N_2 at 30 °C in a shaking incubator at 54 rpm.

2. Construction of the Microbial Fuel Cell (MFC)

The MFC was composed of similarly sized anode and cathode chambers with 30 ml medium and 20 ml headspace of plastic. Each chamber had two ports, sealed with rubber stoppers, on the top for sample collection and gas supply. The cation exchange membrane (Nafion 117, Dupont Co., USA) separating the two chambers was physically clamped at the flattened ends of the two plastic tubes (diameter of 2.0 cm) fitted in the chambers (Fig. 1).

Plane carbon paper (without wet proofing, E-tek Co., USA) with a diameter of 1.3 cm (surface area of 1.32 cm^2) was used as the anode electrode [5]. The cathode was prepared by applying a mixture of Pt/C catalysts (10% Pt; E-tek Co., USA) and 5% Nafion solution (7 μl of Nafion solution/mg of Pt/C catalyst) onto one side of the carbon paper with a dimension of 2.0 cm×2.6 cm (5.2 cm^2), producing a final Pt loading of 0.5 mg/cm^2, as shown in Fig. 2 [3]. However, in some cases, various Pt loadings were applied to optimize the Pt loading.

The coated cathode was dried for a minimum of 1 day at room temperature before application. Some initial tests with various Pt loadings demonstrated an optimum loading of 3 mg/cm^2. Copper wire was attached to the electrodes, and all exposed metal surfaces were sealed with nonconductive epoxy resin. The effect of different external resistances on the MFC's properties was also investigated in this study.

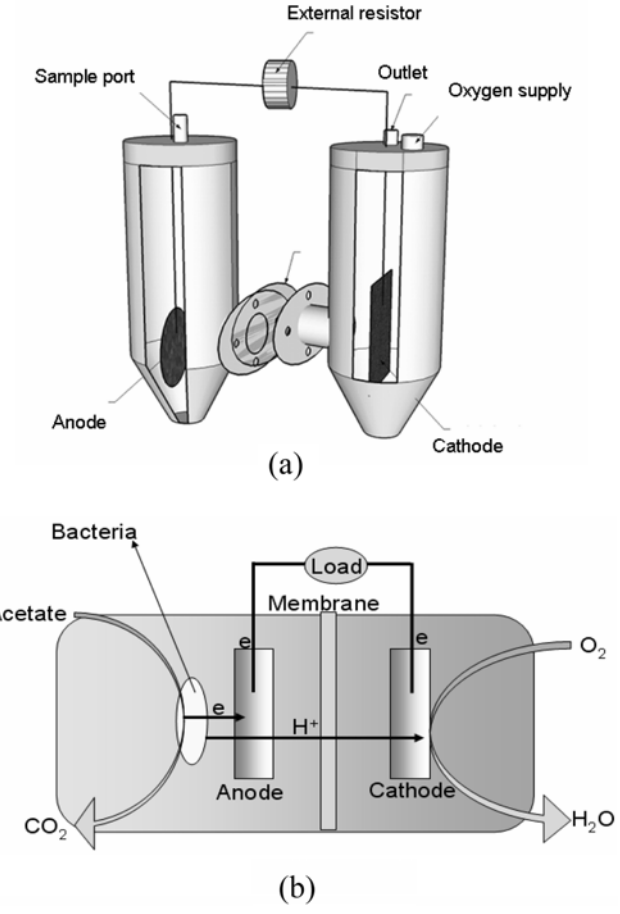

Fig. 1. Construction of a microbial fuel cell (MFC) (a) and a mechanism of mediator-less MFC (b).

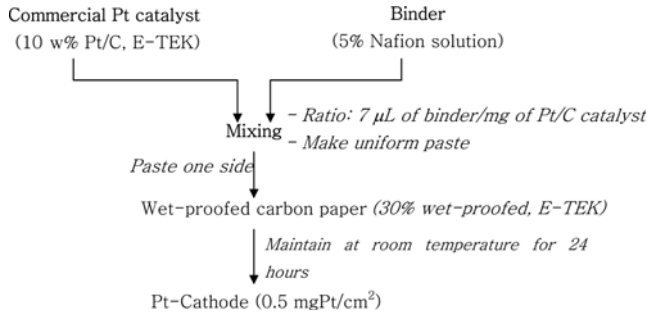

Fig. 2. Synthesis procedure for the cathode electrode.

3. Microbial Fuel Cell (MFC) Operation

The electrodes and chambers were sterilized and flushed with anaerobic gas (20% CO_2 and 80% N_2) in a glove box, after which the electrodes were assembled into the chambers. Cells collected from culture medium were centrifuged at 3,000 rpm for 10 minutes under anaerobic condition at 5 °C to remove any remaining culture solution. After the cells were dispersed into the anode chamber, a gas mixture of 80% N_2 and 20% CO_2 was supplied into the 20 ml headspace of the anode chamber to eliminate any remaining oxygen. The 30 ml of medium solution in the anode chamber was comprised as follows [28]:

KCl: 0.1 g/L, NaH_2PO_4: 0.6 g/L, NaCl: 2.9 g/L, $NaHCO_3$: 2 g/L, Wolfe's mineral solution of 30 μl and Wolfe's vitamin solution of 30 μl, Acetate 1 mM-30 mM

After the fumarate that was initially used as an electron acceptor was removed, NaCl was added to minimize differences in osmolarity between the fumarate (culture medium) and electrode (anode medium) media [4]. The acetate concentration varied from 30 to 1 mM in this study. In the cathode chamber, the cathode medium was filled with a similar anode medium in which $NaHCO_3$ was replaced with 30 mM Tris-HCl solution [4,19]. In serial tests, tris-HCl solution at various concentrations was used to study the effect of pH on the MFC's performance. The cathode chamber was continuously provided with air through a 0.45 μm pore-size filter.

The MFC was operated at 30 °C and 54 rpm in a shaking incubator for all experiments, except for the case study on the effect of temperature variation from 26 °C to 35 °C. Each temperature was kept constant for more than 5 hours before the temperature and data changes were recorded after a stable voltage was obtained.

3-1. Analysis of Acetate Concentration

The samples collected from the culture flask or anode chamber were filtered with a 0.2 μm-pore sized filter and analyzed by a gas chromatograph (GC; Hewlett Packard 5890, USA) equipped with a flame ionization detector, and a column of 30 m×0.53 mm×1.0 μm (HP-Innowax-Crosslinked polyethylene glycol, Hewlett Packard, USA).

3-2. Analysis of Hydrogen Concentration

The gas samples collected from the headspace of the chambers were analyzed by a GC (Hewlett Packard 5890, USA) equipped with a thermal conductivity detector, and a 2-m stainless column packed with carboxen 1000, 50/80 mesh (Supelco, Sigma-Aldrich Co., USA).

3-3. Monitoring Voltage

The voltages were digitally recorded in a computer connected to a multimeter (WMP6-1000, Won Atech, Korea).

3-4. Morphology of Biofilm on the Anode Electrode

The biofilm morphology was observed by an environmental scanning electron microscope (ESEM; XL30 ESEM, FEI, USA). The anode electrode was removed from the anode chamber, rinsed with sterile medium, and immersed in a sterile growth medium. Small pieces of the electrodes (0.2×0.2 cm^2) were cut to take the ESEM images. The samples were fixed with 2.5% glutaraldehyde with 0.1 M phosphate buffer at pH 7.2-7.4 for 24-48 hours, washed with 0.1 M phosphate buffer for 15 minutes, and then washed with distilled water for 5 minutes. Each washing step was repeated 4 times. Finally, the samples were dehydrated at various ethanol concentrations, and then dried under N_2 gas for about 10 minutes.

RESULTS AND DISCUSSION

1. Current Generation in Microbial Fuel Cells (MFC)

Fig. 3 shows current generation by *Geobacter sulfurreducens* at 30 ºC in an MFC with 2.5 mM acetate as an electron donor. The current increased with time to a maximum of 0.19 mA at about 80 hours, and then decreased rapidly to about 0.05 mA at 120 h. We believe that *Geobacter sulfurreducens* can use acetate as the electron donor and the anode electrode as the electron acceptor itself. Metal-reducing bacteria were estimated to have generated nanowires that could have transferred the electrons directly to the electrode in the deficient chemical acceptors [29].

Since a current was also generated immediately when replacing with the new anode medium (absent fumarate), the biofilm of *Geobacter sulfurreducens* was considered to have been stable on the anode electrode (Fig. 4). The current exhibited approximately the same value (0.20-0.24 mA) as the acetate concentration was varied

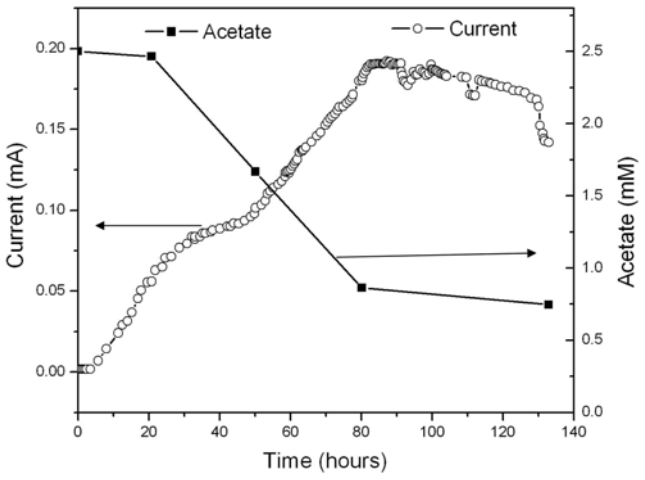

Fig. 3. Current generation by *Geobacter sulfurreducens* at 30 ºC in an MFC with 2.5 mM acetate as an electron donor.

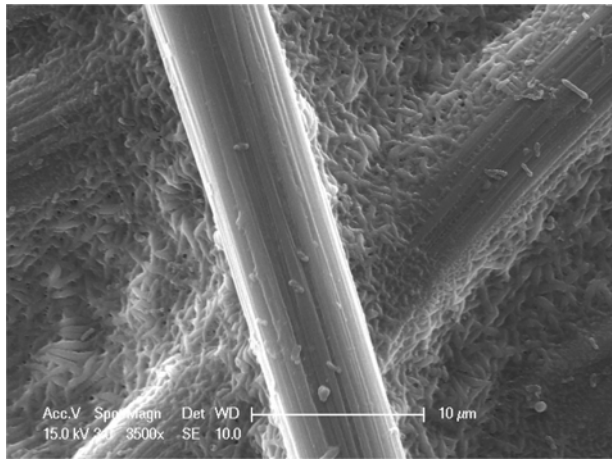

Fig. 4. Anode electrode with *Geobacter sulfurreducens* after 100 h operation in a microbial fuel cell (MFC).

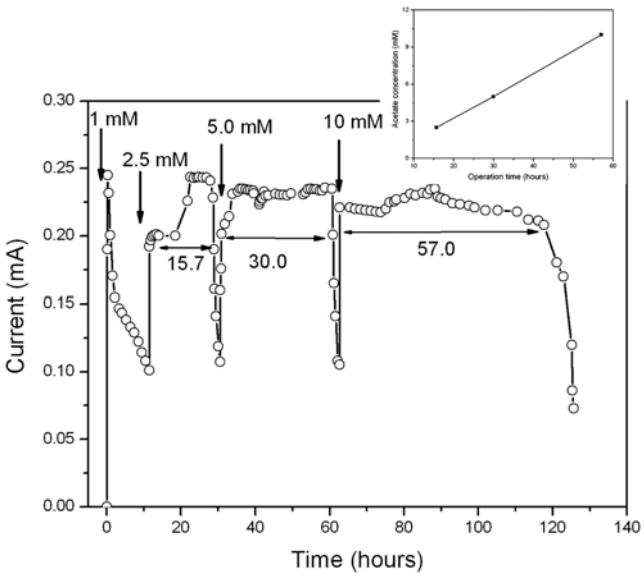

Fig. 5. Current generation with intermittent supply of acetate at various concentrations (R_{ex}=1,000 Ω and T=30 ºC). Inset figure: operation time as function of acetate concentration.

over the range from 2.5 to 10 mM (Fig. 5). However, at an acetate concentration of 1 mM, the current was about 0.25 mA for less than 1 hour, and then decreased rapidly. The phenomenon was consistent with that previously reported [5,17,30,31]. A stable current product of 1,780 mA/m^2 was obtained in this study when using a cathode electrode of 0.5 mg Pt /cm^2-coated carbon paper. This result was 27-fold higher than that reported by Bond and Lovely where *Geobacter sulfurreducens* was also used as the biocatalyst in an MFC in which glucose, instead of acetate, was used as the electron donor [4]. However, Bond and Lovely used unpolished graphite without Pt catalyst as the cathode electrode. Since the specific surface area of the carbon paper-based anode electrode is relatively high due to its porous, three-dimensional structure (78% porosity) with a pore size of 20-200 μm where bacteria can easily be accommodated, the density of cells immobilized on it was considered to have been higher

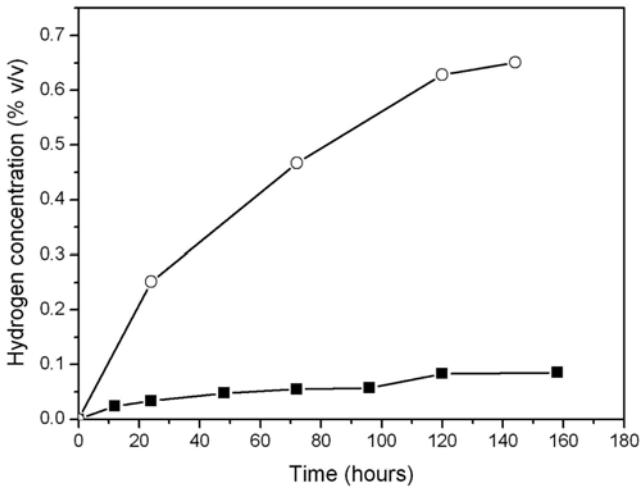

Fig. 6. Hydrogen production in headspace of flask (○) with fumarate as the electron acceptor and the anode chamber of the MFC (●) using the electrode as the electron acceptor in the absence of fumarate.

than that of unpolished graphite in which bacteria could develop only on the outer surface (Fig. 4).

2. Hydrogen Production in Pure Culture and Microbial Fuel Cell (MFC)

In this study, hydrogen was collected from the headspace of a flask using fumarate as the electron acceptor or anode chamber of the MFC. The hydrogen concentration from the flask increased with time and reached an equilibrium value of 0.65% (v/v), which is consistent with previously reported similar levels of hydrogen production by *Geobacter sulfurreducens* [32]. However, the hydrogen concentration from the anode chamber of the MFC ranged from 0.02 to 0.08% (v/v) (Fig. 6). This result was 5-fold lower than that in the case of using fumarate above.

$$CH_3COO^- + H^+ + 2H_2O \rightarrow 2CO_2 + 4H_2 \quad (1)$$

Hydrogen is generated continuously according to Eq. (1) if an equilibrium of hydrogen concentration is obtained in the anode chamber of a batch mode-MFC and acetate degradation is limited by Le Chatelier's principle. Thus, the hydrogen in the headspace of the anode chamber had to be removed to maintain the current at a high level. Other reports showed that a continuous mode always gives a higher current than a batch mode in the same MFC configuration [2,4,30,33]. We attributed the high current during performance to the removal of hydrogen and carbon dioxide gas, as well as the maintenance of a constant acetate concentration.

3. Effect of Surface Load of Pt Catalyst on the Cathode Material

Some chemicals such as $K_3[Fe(CN)_6]$ [15,30], H_2O_2 [34], and Fe^{3+} [35,36] have been suggested recently as electron acceptors in an MFC. However, oxygen is a strong candidate due to its advantages such as availability, free cost, high oxidation potential [36], sustainability, and production of nontoxic product (water). Therefore, in these experiments, the carbon paper was loaded at various Pt loadings, and O_2/Pt was used as the cathode electrode.

Fig. 7(a) shows that the open-circuit voltage, i.e., the voltage at a current of 0 mA, increased by 29.3% and 55.2% in comparison with

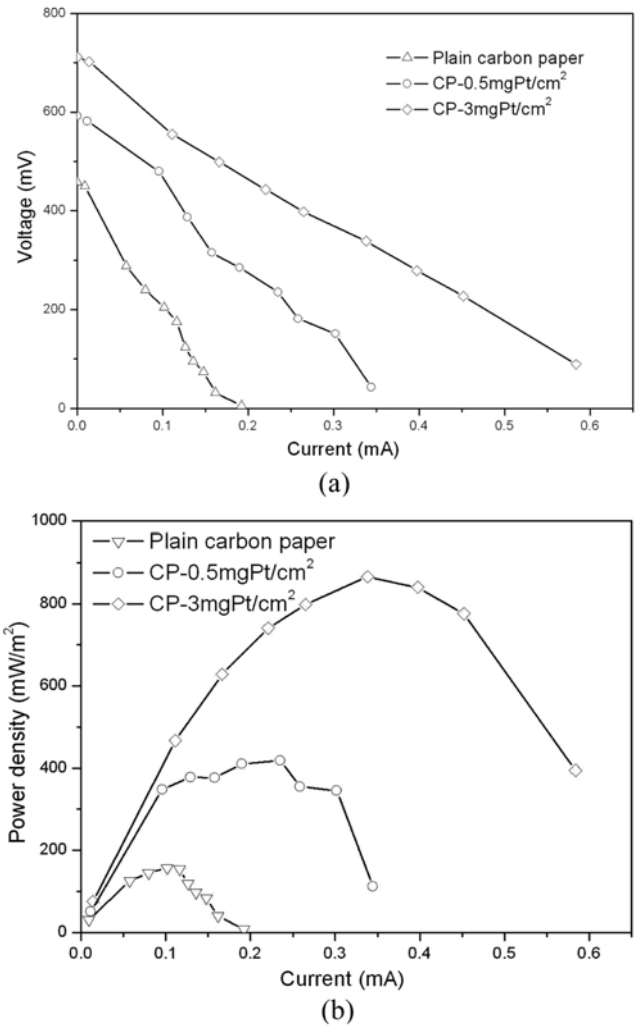

Fig. 7. Effect of Pt loading on the cathode-polarization with respect to current (T=30 °C) (a), and effect of Pt loading on the cathode-power density with respect to current (T=30 °C) (b).

that of the plain carbon paper, when the Pt loading was increased from 0.5 to 3 mg/cm^2. Similarly, the power density increased with an increasing Pt load to a maximum of 418 mW/m^2 at 0.5 mg Pt/cm^2, which was approximately 2.6-fold higher than that with the plain carbon paper (i.e., the carbon paper without Pt). In addition, the maximum power density was further enhanced two-fold when the Pt loading was increased from 0.5 to 3 mg Pt/cm^2 (Fig. 7(b)). This Pt-induced increase was attributed to the catalytic effect of Pt in considerably quickening the reaction rate at the cathode, which may be related to the increased oxygen absorption on the Pt active site or the reduced oxygen diffusion through the anode chamber.

Bond et al. reported a power density of 15.6 mW/m^2 using *Geobacter sulfurreducens* as a biocatalyst and unpolished graphite without Pt as electrodes [27]. Bond's result was 10-fold lower than the present result using plain carbon paper as electrodes. We attributed the major 4-fold difference compared to that of Bond to the ratio of cathode to anode in these experiments. In addition, since the true surface area of carbon paper is much larger than its cross sectional area, the power density calculated by using the cross sectional area increased drastically. Similarly, Ringeisen et al. reported that the

Table 1. Summarized performances of various MFCs

Our system

Pt loading on cathode (mg/cm^2)	Power density (mW/m^2)	Surface of cathode/anode	Bacteria	Anode/cathode material
0	157	4 : 1	*Geobacter sulfurreducens*	Carbon paper/O_2
0.5	418			
3	865			

Previously reported system

Pt loading on cathode (mg/cm^2)	Power density (mW/m^2)	Surface of cathode/anode	Bacteria	Amode/cathode system	Ref.
0	16.8	1 : 1	*Rhodoferrax ferrireducens*	Graphite left/O_2	1
1	40	1 : 1	*Geobacter metallireducens*	Carbon paper/ O_2	2
0	13.2, 16.56	1 : 1	*Geobacter sulfurreducens*	Graphite left/O_2	4, 27
1	24	1 : 1	*Shewanella oneidensis*	Carbon paper/ O_2	38

power density was enhanced 300-fold when the true surface area was replaced by the cross sectional area of the remaining graphite [38]. Table 1 shows summarized performances of our systems and previously reported MFCs.

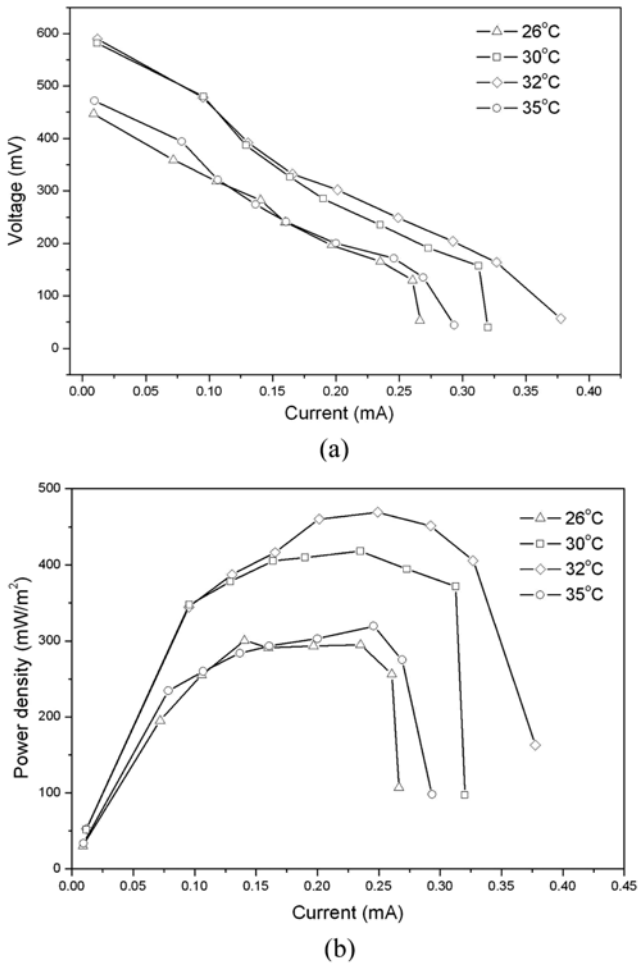

Fig. 8. Effect of temperature on MFC performance (polarization with respect to current; R_{ex}=1,000 Ω and with 0.5 mg Pt/cm^2-cathode) (a), and effect of temperature on MFC performance (power density with respect to current; R_{ex}=1,000 Ω and 0.5 mg Pt/cm^2-cathode) (b).

The above results indicate that the ratio of cathode to anode surface area and the Pt loading on the cathode should be optimized in the MFC design. On the other hand, the high cost of Pt greatly increases the MFC cost. Therefore, other researchers have previously suggested using a cathode with 0.5 mg Pt/cm^2 due to its high power density and relatively low cost [3,17,21,26], even though Shaoan obtained good results using 0.1 mg Pt/cm^2 for the MFC [39].

Recent studies have also been focused on upgrading MFC systems by exposing the cathode electrode to air either with or without a membrane [3,5,22,26]. These systems generated a higher power density than that of the liquid cathode MFC. For example, Cheng et al. reported a power density of 1,540 mW/m^2 with 0.5 mg Pt/cm^2-cathode electrode and a cathode to anode ratio of 1 : 1 [3].

4. Effect of Temperature

MFC was operated at various temperatures ranging from 26 °C to 35 °C at a fixed acetate concentration of 10 mM, and the results were analyzed through the polarization curve method. Fig. 8(a) shows that the voltage exhibited a strong linear decrease with increasing current. Therefore, the ohmic losses significantly affected all experiments. These results are consistent with those reported by Moon et al. using a continuous mode [33].

The optimum temperature of *Geobacter sulfurreducens* in an MFC was around 30 °C-32 °C, providing a power density of 418-470 mW/m^2. The bacterial activity decreased significantly (≈35%) below 26 °C and above 35 °C (Fig. 8(b)). Bond et al. reported an optimum growth temperature for *Geobacter sulfurreducens* of between 30 and 35 °C with fumarate as an electron acceptor [27]. We, therefore, assumed that there is no significant difference in the optimum temperature of *Geobacter sulfurreducens*, irrespective of either using an anode electrode in an MFC or fumarate as an electron acceptor in inoculation.

CONCLUSIONS

The current production was maintained at a stable level of 0.20-0.24 mA by using *Geobacter sulfurreducens* as the biocatalyst and acetate as the fuel in an MFC. The optimal temperature was determined to be between 30 and 32 °C under batch mode operation with a maximum power density of 418-470 mW/m^2. The power density

was strongly influenced by the Pt loading on the cathode electrode and was enhanced over 2-fold (from 418 to 866 mW/m^2) when the Pt loading was increased 6-fold (from 0.5 to 3.0 mg Pt/cm^2). The power density was maximized at an external resistance of 1,000 Ω.

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