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## **Enzymatic Biofuel Cell Using Grooved Gel of Fructose between Graphene-Coated Carbon Fiber Cloth Electrodes**

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SUMMARY Improvement of output and lifetime is a problem for biofuel cells. A structure was adopted in which gelation mixed with agarose and fuel (fructose) was sandwiched by electrodes made of graphene-coated carbon fiber. The electrode surface not contacting the gel was exposed to air. In addition, grooves were added to the gel surface to further increase the oxygen supply. The power density of the fuel cell was examined in terms of the electrode area exposed to air. The output increased almost in proportion to the area of the electrode exposed to air. Optimization of the concentration of fuel, gel, and the amount of enzyme at the cathode were also examined. The maximum power density in the proposed system was approximately  $121 \,\mu$ W/cm<sup>2</sup>, an enhancement of approximately 2.5 times that in the case of using liquid fuel. For the power density after 24 h, the fuel gel was superior to the fuel liquid.

key words: biofuel cell, fuel gel with grooves, oxygen supply, graphenecoated carbon fiber cloth electrode, optimization of enzyme

#### 1. Introduction

Biofuel cells are expected to be a power source for medical and mobile devices because they can generate power easily using familiar fuel [1]–[6]. However, improving output and lifetime is a problem. It was found that the amount of oxygen dissolved in the fuel liquid was insufficient, and reaction products remaining around the cathode restricted the supply of oxygen [6], [7]. Haneda, Miyake, et al. reported that biofuel cell performance could be improved by supplying sufficient oxygen to the cathode by using a hydrogel containing fuel and exposing the cathode to air [5], [8]–[10].

This study used fuel gel to utilize enough oxygen in the air, and prevent detachment of the enzyme from the electrode immersed in fuel liquid [8]–[11]. A structure was devised in which the gelation of mixed fuel and agarose was sandwiched with anode and cathode electrodes made of graphene-coated carbon fiber cloth (GCFC). GCFC was used for the electrodes to increase the surface area and to enhance permeability of oxygen at the cathode [12], [13]. In this structure, one side of the cathode was exposed to air, and the GCFC conveyed oxygen to the opposite side of the cathode contacting the gel so that sufficient oxygen could be supplied. In addition, fine grooves on the gel surface contacting the cathode were introduced to further increase the oxygen supply. By using the fuel gel, the area

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of the electrode exposed to air could be grasped. Therefore, the relationship between the area exposed to air and the output of the fuel cell was examined. Then, optimization of the groove configuration was investigated. The power density of the proposed system was investigated in biofuel cells using fructose [3], [14], [15]. The performance of biofuel cells using ascorbic acid and glucose have been reported [4], [16], [17]. However, ascorbic acid caused reduction at the cathode (crossover) [9], [10], [12], [13] and a mediator was necessary with glucose. The reasons for using fructose were that no crossover occurred and no mediator was needed; that is, the effect of the gel-electrode structure introduced in this work could be grasped directly. The time history of output until after 24 hours was also examined as well as the gel state. The optimum concentration of fuel [8], the optimum modification amount of enzyme of Bilirubin oxidase (BOD) [4], [18]-[21], and the optimum pH [22]-[25] have been investigated. The optimum concentrations of fuel, gel and BOD were investigated in the proposed system.

#### 2. Structure, Materials, and Measurement Method

The mechanism of fuel cell power generation using fructose is shown in Fig. 1. A proton  $(H^+)$  is generated at the anode by the fructose dehydrogenase (FDH) enzyme. The proton is reduced by dissolved oxygen by the BOD enzyme at the cathode. In this work, an agarose gel mixed with fructose and McIlvain buffer solution (MBS) was sandwiched between the anode and cathode electrodes as shown in Fig. 2. Agarose was dissolved in MBS containing 100 mM fructose at a concentration of 1.5 wt%. Wu et al. reported that pH 5.0 was optimal for the BOD enzyme [25]. Therefore, MBS of pH 5.0 was used. The agarose solution was boiled, poured



Fig. 1 Components of the biofuel cell using fructose in this work.

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**Fig. 2** Structure of the fuel gel sandwiched by the GCFC electrodes. The upper figure shows the GCFC surface.

Table 1 Specifications of enzyme and fuel	gel
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	Anode	Cathode	
Enzyme	FDH* (25 µl)	BOD** (60 µl)	
Concentration	20 mg/ml	25 mg/ml	
Fuel gel	Fructose*** +	Agarose gel***	
Concentration	Fru.200 mM, Gel.1.5wt%		
рН	5.0 (MBS****)		

\* Fructose dehydrogenase, EC1.1.99.11, Toyobo Co. Ltd. \*\* Bilirubin oxidase, EC 1.3.3.5, Amano Enzyme Co.

\*\*\* Fructose, D-(-)-fructose, Nacalai Tesque Co.

Agarose gel, Agarose Basic, TAKARA Bio Co. \*\*\*\* McIlvaine buffer solution, Nacalai Tesque Co.



**Fig. 3** Shape and dimensions of fuel gel with grooves (a = 6 mm,  $b_1 = 12 \text{ mm}$ ,  $t_1 = 2 \text{ mm}$ ,  $b_2 = 1 \sim 3 \text{ mm}$  (parameter),  $b_3 = 1 \text{ mm}$ ,  $t_2 = 0.5 \text{ mm}$ ).

into a plastic case, and then cooled and gelated. The anode and cathode were modified by the FDH and BOD enzymes. Table 1 lists the specifications of them. The electrode material was carbon fiber woven fabric modified by graphene on the surface, GCFC (Incubation Alliance Co., Japan). The reason for this was to increase the electrode surface area and to enable air-breathing [12], [13]. The electrode sizes were  $5 \text{ mm} \times 12 \text{ mm} \times t0.5 \text{ mm}$  and  $10 \text{ mm} \times 12 \text{ mm} \times t2 \text{ mm}$  for the fuel gel. The electrodes were subjected to UV-Ozone treatment for 30 min to render them hydrophilic.

Figure 3 shows the grooved gel to further increase the oxygen supply to the cathode. Grooves were formed on the gel surface facing the cathode. The width of the groove  $(b_3)$  was 1 mm, the depth  $(t_2)$  was 0.5 mm, and the groove interval  $(b_2)$  was set as a parameter of 1–3 mm.

Cyclic voltammetry (CV) method was used to examine electrochemical characteristics. The electrodes were characterized by using a three electrode potentiostat (HA-1518, Hokuto Denko, reference electrode: Ag/Agcl). The measurement conditions were a scan range +0.6-0 V, initial potential holding time of 10 s, and scan speed of 10 mV/s.



**Fig. 4** Current density of cathode for different numbers of cycles in the CV method (enzyme: BOD, solution: MBS, pH 7).

Power generation of the biofuel cells was measured as follows: connecting a variable resistor (0 to 2.0 MΩ) and a voltmeter to the fuel cell device, then changing the resistance of the variable resistor and measuring the voltage at that time; the power density (*P*/*A*) was calculated from the equation:  $P/A = V^2(R \times A)$ , where *V* is the output voltage, *R* is the electrical resistance, and *A* is the area of electrode.

#### 3. Results and Discussion

### 3.1 Optimum Concentrations of Gel and Enzyme

The current density of the cathode was measured by the CV method to examine the performance of the cathode over time. The cathode electrode was immersed in the buffer solution (MBS, pH 7), and the BOD enzyme was modified on the electrode (BOD and MBS solution, concentration of BOD: 25 mg/ml, amount of solution:  $60 \mu$ l). Figure 4 shows that the current density of the cathode decreased over time. This was attributed to oxygen around the electrode being gradually consumed and lowered. Therefore, it was considered that the supply of oxygen is highly important.

The CV measurement of the anode with FDH that was immersed in the MBS (pH 5) containing different concentrations of fructose indicated that the current density was saturated when the fuel concentration was more than 100 mM. Therefore, fructose concentration was set to 200 mM in consideration of the margin for increasing the supply of oxygen. The optimum concentration of the gel was investigated. When the concentration of agarose was more than 2%, the gel became considerably hard, and when the concentration was less than 1%, the gel contained considerable moisture. Therefore, the concentration of agarose was changed in the range of 1-2%. The optimum concentration was evaluated by the output (power density) of the fuel cell. Figure 5 shows the output for different concentrations of agarose. In this measurement, the concentration of fructose in the gel was 200 mM (solution: MBS, pH 5) and the concentrations of FDH and BOD modified on the electrode were 20 mg/ml  $(25 \,\mu\text{l})$  and  $25 \,\text{mg/ml}$  (60  $\mu$ l). This result indicates that the optimum value of the gel concentration was 1.5 wt%. The reason for this optimum value was considered to be that the 1% gel had the same problem that the liquid fuel had, and 2% gel impeded migration of the protons from the anode.

The optimum value of the modification amount of



**Fig. 5** Power density of the fuel cell when the concentration of agarose gel was changed (solution: fructose 200 mM + MBS, pH 5). The error bars indicate the variations of the peak values.



**Fig. 6** Current density of cathode for different concentrations of modified BOD enzyme (solution: MBS, pH 7).



**Fig.7** Power density of different types of fuel: liquid, gel, and gel with grooves ( $b_2 = 1 \text{ mm}$ , Fig. 5) (liquid: fructose 200 mM + MBS, pH 5; gel: fructose 200 mM + MBS, pH5 + agarose gel, 1.5 wt%). The error bars indicate the variations of the peak values.

BOD at the cathode was examined. Figure 6 shows the CV diagram of the current density when the BOD concentration was changed (amount:  $25 \,\mu$ l). The cathode was immersed in MBS (pH 7) in this measurement. The figure shows that the maximum current was observed when the enzyme concentration was 50 mg/ml (amount:  $25 \,\mu$ l).

#### 3.2 Output Characteristics of Proposed Structure

The output of the fuel cells was examined by power density. Fuel and enzyme concentrations were the same as the experimental conditions in Fig. 5. Measurement was carried out for the fuel cells using fuel liquid, gel, and grooved gel ( $b_2 = 1 \text{ mm}$ , Fig. 3). Figure 7 shows the power density for the three types. The fuel liquid was measured to compare with the fuel gel. Maximum power density of the



**Fig.8** Power density of fuel-gel cells when groove interval was changed (gel: fructose 200 mM + MBS, pH 5 + agarose gel, 1.5 wt%).



**Fig.9** Maximum power densities of different types of fuel cells over time.

gel (without grooves) was 92.1  $\mu$ W/cm<sup>2</sup>, approximately 1.9 times that of the fuel liquid (49.1  $\mu$ W/cm<sup>2</sup>). This was probably due to the increase in the oxygen supply from the air. When using the grooved gel, the output was further enhanced by a factor of approximately 1.31 (120.9  $\mu$ W/cm<sup>2</sup>). This ratio roughly corresponds to the ratio of the total open area (including grooves) to the area of one side of the electrode (1.5); however, because the contact surface between the gel and the electrode was reduced, the output was lower than 1.5. In addition, although the actual area of the electrode was decreased by the grooves, the output was still improved. This indicates that the oxygen supply was more dominant than the area of the electrode. Thus, Fig. 7 suggests that the output of the fuel cell was greatly affected by the supply of oxygen from the air.

Optimization of the groove dimensions was investigated. The groove interval ( $b_2$  in Fig. 5) was varied in the range of 1–3 mm. Figure 8 shows the groove interval and the power density. Fuel and enzyme concentrations were the same as those in Figs. 5 and 7. Increasing the area of the grooves exposing to the air reduces the contact area between the gel and the electrode. Therefore, it was considered that no big difference in the three curves in Fig. 8 occurred. However, Fig. 8 shows that the groove interval 1 : 1 was slightly larger than the others. This indicated that oxygen supply was more influential than the area of the electrode. Therefore, the optimum surface configuration of the gel is considered to be many narrow grooves with small intervals.

Figure 9 shows the time histories of power density within 30 min from the start. In the case of using the gel, the power density was higher all the time for 30 min than that using the fuel liquid. Figure 10 shows the power



Fig. 10 Power density of different types of fuel cells after 24-h operation.

density 24 h from the start. The fuel gel gained a maximum power density of  $33.8 \,\mu\text{W/cm}^2$  (at  $0.320 \,\text{V}$ ), exceeding  $23.6 \,\mu\text{W/cm}^2$  (at  $0.267 \,\text{V}$ ) of the fuel liquid. The power densities of the gels with and without grooves were almost the same. The reason for this was considered to be drying of the gel, which reduced the supply of protons. Prevention of this is necessary.

#### 4. Conclusion

Output (power density) of biofuel cells was improved by using fuel (fructose) gel to increase oxygen supply. The fuel gel was sandwiched by GCFC electrodes. The output was enhanced by approximately 90% compared to that of the liquid fuel, and the output was further improved by approximately 31% by adding fine grooves on the gel surface. The output increased almost in proportion to the area of the electrode exposed to air. Even for the power density after 24-h operation, the proposed system had higher performance compared to that of the liquid fuel.

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