Biomolecular modification of carbon nanowalls

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Abstract: Carbon nanowalls (CNWs) with large specific surface area were applied to the electrodes for biofuel cells (BFCs) in this study. The CNW surface was modified with glucose oxidase (GOD) by immersing CNW film in a GOD solution, and then enzyme activity was measured for GOD immobilized on CNWs by colorimetric method. As a result, it was confirmed that GOD reacts with glucose, suggesting that CNWs are effective as enzyme carrier electrodes for BFCs.

Keywords: carbon nanowalls, ICP-CVD, GOD

1. Introduction

Biofuel cell (BFC) is one of fuel cells that use enzymes or microorganisms as catalysts. The enzyme-catalyzed BFCs have many advantages such as the use of biological resources as fuel, the elimination of precious metal catalysts such as platinum, the ability to operate near body temperature, and the ease of miniaturization. By taking advantage of them, BFCs are expected to be used as a power source for implantable medical devices and portable devices using fuel that is safe for the human body. However, the power density of BFCs is not so high compared with other power storage devices. Therefore, the use of electrode materials with a larger specific surface area is required to improve the power density of BFCs [1].

Carbon nanowalls (CNWs) are composed of multilayer nanographene grown perpendicular to the substrate and have a large specific surface area, excellent electrical conductivity, and chemical stability. Therefore, the CNWs are expected to be used as electrodes for batteries and biosensors by modifying the surface with metal nanoparticles and enzymes [2].

In this study, CNWs were investigated as electrode materials for BFC. Glucose oxidase (GOD) was selected as the enzyme and the surface of CNWs was modified with GOD. The enzyme activity of GOD modified CNWs was measured by colorimetric method.

2. Experiment

The CNWs were grown on a Si substrate using inductively coupled plasma chemical vapor deposition (ICP-CVD) [3]. Figure 1 shows a diagram of the ICP-CVD system. A mixture gas of CH_4 and Ar was used, and their gas flow rates were fixed at 30 and 25 sccm, respectively. The pressure in the chamber was kept at 15 mTorr, and the temperature of the Si substrate surface was maintained at 600 °C. The ICP was generated by applying 600 W of RF power to the one-turn coil antenna at the above condition, and CNWs were grown for 30 minutes.

To apply oxygen-containing groups such as carboxyl groups to the surface of CNWs, surface treatment of CNWs with a microwave excited atmospheric pressure plasma jet (MW-APPJ) was carried out.

The surface-treated CNWs were immersed in a solution of 3.5 ml of 0.1 M phosphate buffer (pH6.5) with condensation regent (35 mg) and GOD (15 mg) for 24 hours at 2 °C. After the immersion with 24 hours, they were immersed in 0.1 M phosphate buffer for storage [4].

The colorimetric method was used to examine whether GOD immobilized on CNWs reacts with glucose as an enzyme. In this method, hydrogen peroxide (H_2O_2) produced in the oxidation of glucose by GOD reacts with phenol and 4-aminoantipyrine in the presence of POD to yield a colored product called red quinone dye, and its absorption reaches a maximum at 505 nm of wavelength, and the presence of absorbance indicates that there was a reaction between GOD and glucose. The reaction of glucose with GOD on the surface of CNWs is as follows.

$$glucose + O_2 + H_2O \xrightarrow{GOD} gluconic\ acid + H_2O_2$$
$$2H_2O_2 + 4 - aminoantipyrine + phenol \xrightarrow{POD} red\ quinone\ dye + 4H_2O$$

In this study, 10, 30, and 50 mM glucose solutions were prepared and kept at room temperature for 12 hours or more. And solution (1) was produced by dissolving 16 mg of 4-aminoantipyrine and 1.25 mg of POD in 100 ml of 0.1 M phosphate buffer and adding 2.1 mg of phenol and 2 ml of pure water. 0.5 ml of glucose solutions and 5 ml of solution (1) were mixed and maintained at 40 °C. The CNWs modified with GOD were immersed in the mixed solution for 1 hour, and then the CNWs were removed from the mixed solution and the solution was cooled [5]. The amount of generated H_2O_2 was estimated by measuring the absorbance of the reaction solution at 505 nm using a UV spectrophotometer.

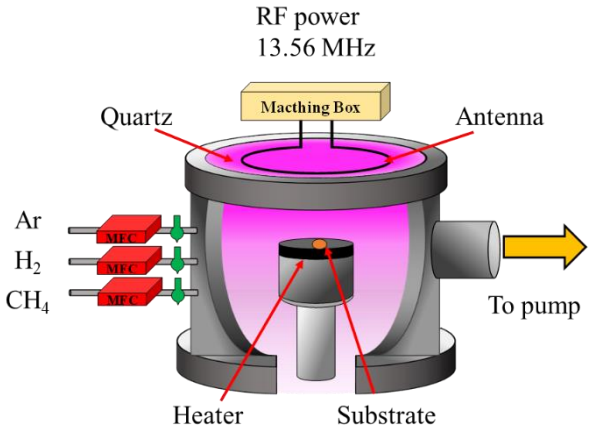

Fig. 1. Schematic of ICP-CVD setup

3. Results and discussion

The SEM images of the CNW film used in this study are shown in Figures 2(a) and (b). From the SEM images, it is confirmed that typical CNWs could be deposited. In addition, the CNWs are considered to have a large effective surface area because the wall height was 4 μm.

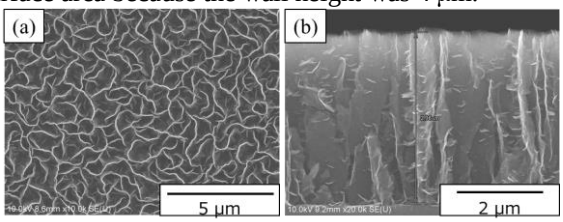

Fig. 2. (a) SEM images of top and (b) side view of CNW film

Figures 3(a) and (b) show the calibration curve to estimate the concentration of H_2O_2 in colorimetric method and the absorption spectra of solution after the enzyme reaction measured by UV spectrophotometer, respectively. The absorbance at 505 nm increases with the increase in the concentration of glucose. From the calibration curve in Fig. 3 (a), the amounts of H_2O_2 generated at glucose concentrations of 10, 30, and 50 mM could be estimated to be 2.1, 3.0, and 5.2 mM, respectively. The amount increased with the increase in glucose concentration. From the results, it was confirmed that GOD immobilized on CNWs also reacts with glucose as an enzyme.

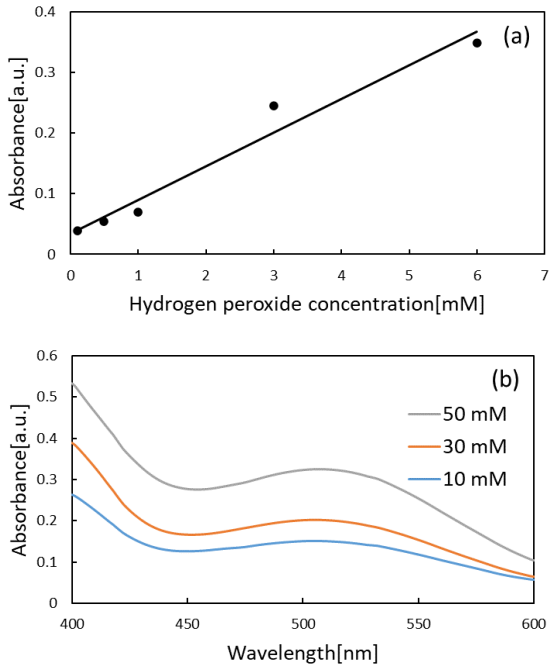

Fig. 3. (a) Calibration curve with H_2O_2 and (b) absorption spectra of solution after enzymatic reaction of GOD modified on CNWs

Figure 4 shows the results of enzyme activity measurement of GOD at a glucose concentration of 10 mM for CNWs with/without surface treatment using a MW-APPJ. The absorbance in case of the surface-treated CNWs was higher than that of the non-surface-treated CNWs. The amounts of H_2O_2 generated by the enzyme reactions estimated from the calibration curve was 2.1 mM with surface treatment and 1.2 mM without surface treatment. The surface treatment with MW-APPJ provides hydrophilic groups such as carbonyl and carboxyl groups to the surface of CNWs [6]. This suggests that the amount of GOD immobilization increased due to the dehydration condensation reaction between the carboxyl groups on the surface of the CNWs and the amino groups of the enzyme by the condensation reagent.

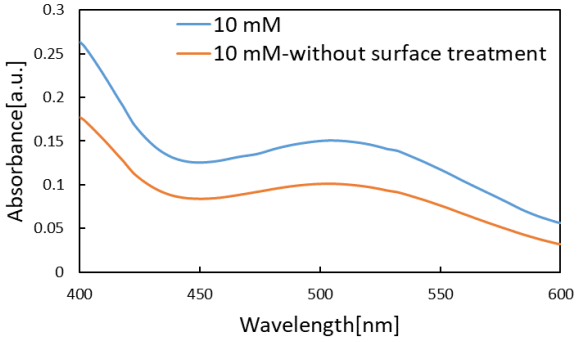

Fig. 4. Absorption spectra of solution after enzymatic reaction of GOD modified on CNWs : CNWs treated with a MW-APPJ and CNWs without surface treatment

4. Conclusions

In this study, CNWs were grown using ICP-CVD, and the surface of CNWs was modified using GOD by dehydration-condensation, and then the enzyme activity was measured. The amount of H_2O_2 generated by the enzyme reaction increased with increasing glucose concentration, suggesting that the GOD immobilized on the surface of the CNWs worked as an enzyme. The amount of H_2O_2 generated in reacting with glucose was measured for surface-treated and non-surface-treated CNWs, and the results showed that the amount of H_2O_2 generated in case of the surface-treated CNWs was higher. This result indicates that the surface treatment with MW-APPJ increased the amount of GOD immobilized on the surface of the CNWs.

5. References

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