Direct Current Generation from NADH and L-Cysteine Using Carbon Fiber: Possible Uses in Biofuel Cells

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Carbon fibers were found to directly oxidize β-nicotinamide adenine dinucleotide reduced form (NADH) and L-cysteine and to generate current without any mediator when the fibers as anode were connected to a cathode. After the carbon fibers were washed with water, ethanol, toluene, ethanol, and water in order, and dried, the fibers were heated at 700 °C for 5 min under aeration in order to eliminate any binder, for reformation of the carbon fibers. The maximum current value reached 6.84 mA after 2 min, and the value gradually decreased to 1.0 mA after 45 min, when the anode equipped with carbon fibers of 103 mg in the electrolyte composed of 1 mM NADH in 0.2 M phosphate buffer (pH 8.0) was connected with the cathode in 0.2 M NaH_2PO_4. At this time, the amount of remaining NADH was 10.8% of the initial amount, and a second addition of 41.5 mg of NADH to again reach 1.0 mM was performed. At the endpoint, the amount of remaining NADH was 4.9% of the total amount of added NADH. The current efficiency was 85% and the total quantity of electricity was 16.5 C. The carbon fibers were found to have NADH oxidation activity; the two electrons released, accompanied by NADH oxidation, are assumed to be absorbed by the fibers as anode, after the electrons flowed to the cathode. The specific surface area and the graphite content was 91.8 $m^2 g^{-1}$ and about 50%, respectively. The carbon fibers are considered to be very useful for construction of biofuel to which dehydrogenases using $NAD(P)^+$ as a cofactor could be applied.

β-Nicotinamide adenine dinucleotide (phosphate) reduced form (NAD(P)H) is one of the most important coenzymes. It has strong reducing potential. Most of the dehydrogenases in a living body are known to convert $NAD(P)^+$ to NAD(P)H as high energy material. Because NAD(P)H has a high oxidation–reduction potential of −0.32 V, it is very useful for high energy transfer in the biochemical reaction of a living cell. Accordingly, the generation of electricity has been tried by using NADH obtained as by-product in the production of gluconolactone from D-glucose.[1,2] A detailed system is shown in Figure 1. NADH is continuously regenerated from NAD^+ by the recycling dehydrogenation using glucose, and as the next step, the oxidized form of mediator is converted to the reduced form of mediator by NADH and diaphorase. As the third step, electrons are generated from the reduced form on the surface of the anode and flow to the cathode. A large amount of gluconolactone is industrially utilized as a coagulating agent for production of soybean curd. At present, gluconolactone is produced by an oxidative fermentation process using air so that current generation is impossible. Therefore, if the application of the system shown in Figure 1 is successful, power generation will be possible as the by-product from the production of gluconolactone. This type of research has been applied to development of biofuel cells. NADH produced from methanol, formaldehyde, and formic acid as the substrates by the dehydrogenases has also been used for the generation of electricity.[3] However, the high oxidation potential of NAD(P)H on the anode causes a large problem of how to take the current out; thus utilization of various kinds of mediators has been investigated for decrease of the high oxidation potential. In this case, mediators such as phenazine methosulfate or phenazine

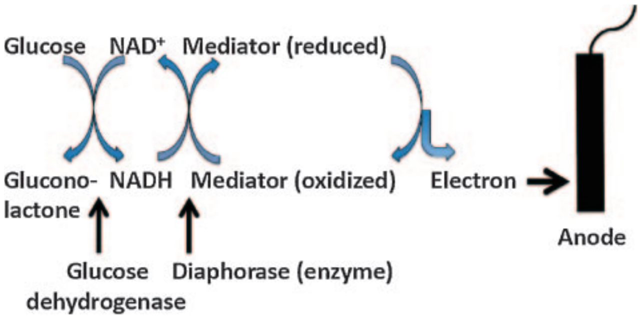

Figure 1. Coupling reactions for current generation from glucose. Glucose and NAD^+ are converted to gluconolactone and NADH by glucose dehydrogenase as the first step, NADH and a mediator of oxidized form are converted to NAD^+ and the mediator of reduced form by diaphorase as the second step, and electrons are finally released from the mediators to the anode as the third step.

ethosulfate, and diaphorase as the enzyme are used as components of the electrolyte in the anode cell.[1–6] Recently, direct electron transfer (DET) systems have been investigated for increase of efficiency of current generation. In DET systems, enzymes, cofactors as NAD(P)H, and mediators altogether were entrapped or immobilized around the anode and cathode for increase of the efficiency.[7–9] The following attempt to perform current generation from glucose have been reported as glucose/O_2 cells, in which no dehydrogenases and NAD(P)H were used. Carbon alloy containing Pt and Au catalyst was used as anode, and glucose oxidation was performed on the surface of the carbon alloy anode. The cathode was prepared by coimmobilization of laccase, of which the formal name is benzenediol: oxygen oxidoreductase, with the mediator.[10] Different glucose/O_2 cell tests were also performed by using porous carbon tubes coimmobilized with glucose oxidase with the mediator as anode. The cathode was prepared by coimmobilization of laccase with the mediator on the porous carbon tubes.[11] In other DET systems, enzymes containing heme and/or flavin inside the protein were immobilized on the anode, to which the electrons generated from the heme or flavin in the enzymes protein were directly and efficiently transferred,[12,13] however, application of this system is largely limited to a very small number of oxidoreductases.

The following carbon materials are known as electrode materials combined with enzymes: original carbon fiber,[14] felt, and paper derived from a carbon fiber,[8,15] carbon graphite and felt derived from the graphite,[3,16–20] and glassy carbon.[21] Carbon fiber is considered to be superior to the other materials from the viewpoints of the cost and surface area. We had investigated applications of an electrochemical NAD^+ recycling system involving a string-like carbon fiber to an enzyme reactor.[14] In this case, +0.6 V was loaded by a Potentiostat for performance of the electrochemical reaction without any mediator. As the next step, when the electrochemical reaction was carried out by using carbon fibers treated at 700 °C for 5 min, a new finding was obtained.

We found that NADH was oxidized on the carbon fibers as anode without any mediator, any enzymes, and any loading of potential. The current spontaneously generated on the carbon fiber electrode was detected on the zero shunt ammeter when the terminal end of anode was connected with that of the cathode equipped with Pt cladding Ti mesh. In this case, carbon fibers had their own catalytic activity for oxidation of NADH, and no mediator was necessary for the current generation. Such new characteristics of carbon fibers have not been reported to our knowledge. In this paper, those characteristics and some possibilities for applications of the carbon fibers to a biofuel cell are described.

Experimental

Carbon fibers (T 300-3k; Toray, Tokyo) were used as anode after the following treatment. A bundle of the fibers (5 cm length, about 1 g) was fixed on a glassy carbon rod by cotton thread, and the materials were washed with water, ethanol, toluene, ethanol, and water in that order. Next, after drying, the carbon fibers were heated at 700 °C for 5 min under aeration to eliminate all binders, for reformation of the carbon fibers. Pt foil-cladding Ti-mesh (No. 1001; SPF, Nagoya, Japan) was used as material for the cathode. A cationic exchange membrane (Neosepta CMX, Asahi-Kasei, Tokyo) was used as the diaphragm between the anode cell and the cathode cell. NADH of 85.4% purity, based on absorbance at 340 nm (Oriental Yeast, Osaka), was used. Special-grade L-cysteine hydrochloride monohydrate (Wako Pure Chemicals, Osaka) was used as L-cysteine.

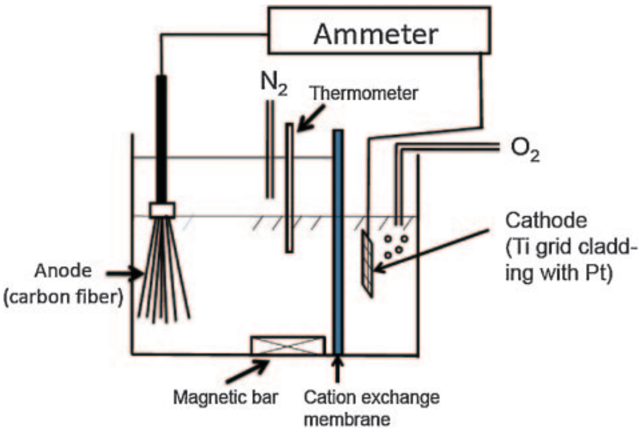

Figure 2. Scheme of the current generation cell. The anode cell and the cathode cell are divided by the cationic exchange membrane. The carbon fibers (about 0.1 g) were fixed on a glassy carbon rod with a rubber band, and the resulting construct was used as the anode. Two pieces of Pt foil-cladding Ti-mesh (30 × 60 mm^2) were put in the cathode cell after bending to make it possible to insert the mesh into the cathode cell.

The amount of NADH was determined by the absorbance at 340 nm.

The amount of L-cysteine was determined by using 6,6′-dinitro-3,3′-dithiodibenzoic acid.[22]

Specific surface area of the heat-treated carbon fibers was assayed by the Brunauer–Emmett–Teller (BET) method, in which the amounts of nitrogen adsorbed to the surface of the carbon fibers at the temperature of liquid nitrogen are measured. The specific surface areas were determined by multiplying the cross section of a nitrogen molecule by the total number of adsorbed molecules. After the carbon fibers were pretreated at 30 °C for 14 h under high vacuum conditions of 6.7×10^{-2} Pa, the surface areas were determined by Quadrosorb SI, Standard (Sysmex, Kobe, Japan).

The graphite content of the heat-treated carbon fibers was determined by measurement of Raman scattering spectra at room temperature in air with a JOBIN YBON HR300 spectrometer. The 514.5 nm excitation line of an Ar ion laser was used. The optical power through an objective lens (×100) at the sample position was about 2 mW.

The instruments and the operational conditions of the experiments were as follows. The shapes of the anode cell and cathode cell, and features of the anode and the cathode are shown in Figure 2. The sizes of the parts of both cells and the position of the anode are shown in Figure 3. The anode cell was kept under an atmosphere of nitrogen, and oxygen gas was blown into the electrolyte of the cathode cell at the flow rate of about 0.3 L min^{-1}. The temperatures of both anode cell and cathode cell were kept at 35 ± 0.5 °C. Fifty mL of 0.2 M

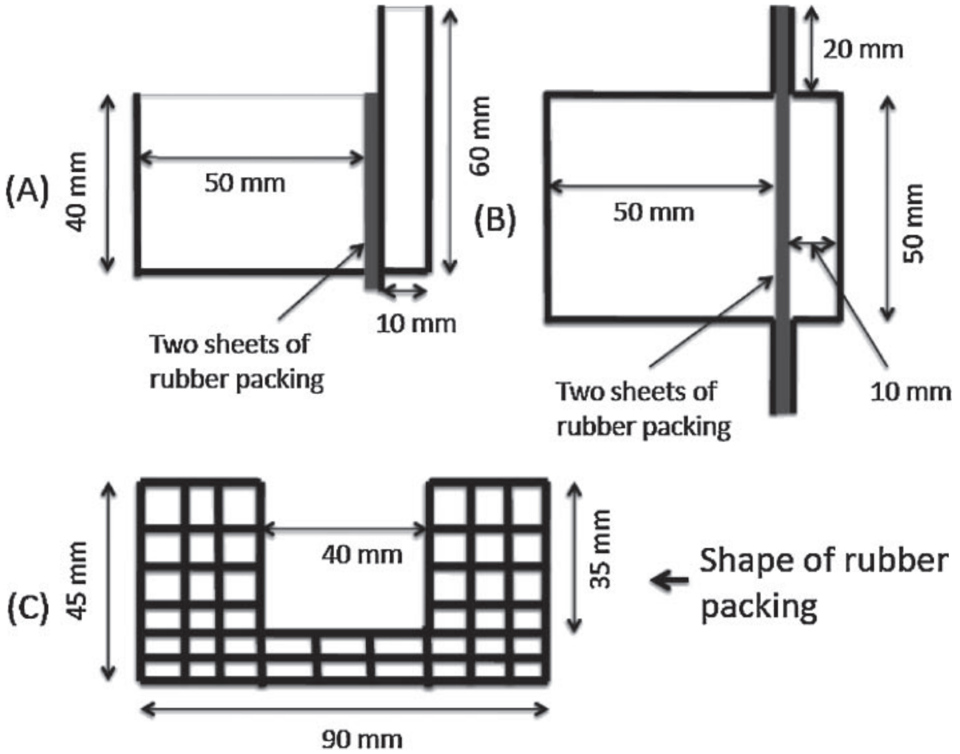

Figure 3. Sizes of the anode cell and the cathode cell, shape of the rubber packing, and position of the anode. The cationic exchange membrane was put between 2 pieces of rubber packing, and the shape of the rubber packing is shown in (C). The cationic exchange membrane was put in the open space $35 \times 40\,\text{mm}^2$ in the center of the packing. The left side cells in the above A and B are anode cells, and the right side cells are cathode cells. Those cells were prepared by using a 3 mm thick poly(vinyl chloride) board. The carbon fiber electrode was put at the upper left corner in anode cell shown as the above B, and a 2 cm magnetic bar was put at the lower right corner shown in the anode cell. The mixture was stirred at about 600 rpm.

sodium phosphate buffer (pH 8.0) (Buffer I) and 15 mL of 0.2 M NaH_2PO_4 solution was added into the anode cell and the cathode cell as the electrolytes, respectively. The terminal ends of the anode and the cathode were connected to a zero shunt ammeter (HM 104A, Hokuto Denko, Tokyo); 41.5 mg of NADH dissolved in 200 µL of Buffer I was added in the electrolyte of the anode cell to start current generation when the background current was less than 0.3 mA. In this case, the molar concentration of NADH was 1.0 mM.

In the case of L-cysteine oxidation, L-cysteine/HCl monohydrate powder of 8.8 mg was directly added into 50 mL of Buffer I. The pure L-cysteine content in special-grade L-cysteine/HCl monohydrate was 73.3%, based on determination by dividing the absorbance at 412 nm by the molecular extinction coefficient, 13600, in the assay using 6,6′-dinitro-3,3′-dithiodibenzoic acid. Eight and eight tenth grams of the above powder contained 6.45 g of pure L-cysteine, and the molar concentration in the 50 mL electrolyte was 1.06 mM.

Current efficiency was determined as follows. The total current volume (mA h) generated during oxidation of NADH was determined by weight assay; the paper clipped from a copy of a chromatogram of the oxidation current was weighed. The total background current volume was subtracted from the total current volume. The current efficiencies were calculated by dividing the total current volume stated above by the theoretical current volume calculated from the consumed amounts of NADH.

Results

Current Generation from NADH by Using Carbon Fibers. As a result of investigation of the current generation from NADH as described in Experimental, the current generation curve is shown in Figure 4. The maximum current reached 6.84 mA after 2 min, and the value gradually decreased to 1.0 mA. At this time, the amount of remaining NADH was 10.8% of the initial amount, and a second addition of 41.5 mg of NADH to again reach 1.0 mM was performed. At the endpoint, the amount of remaining NADH was 4.9% of the total amount of added NADH. The current efficiency was 85% and total quantity of electricity was 16.5 C. Decrease of the current is assumed to be mainly caused by decrease of NADH. However, when the second addition was performed, the maximum current value was considerably lower than that in the case of the first addition. This is thought to mean that inactivation of carbon fibers in the electrode occurred to some extent. The maximum current values at first use were in the range from 3.0 to 7.0 mA when 103 ± 1.0 mg of carbon fibers was used, and the values obtained were random to some extent. The maximum current values obtained in most cases were about 3.0 mA. This is assumed to be caused by the dispersed condition of the fibers on the electrode.

The stability of current-generation activity of carbon fibers as anode should be important. Accordingly, the effect of repeated use on the maximum current value was investigated.

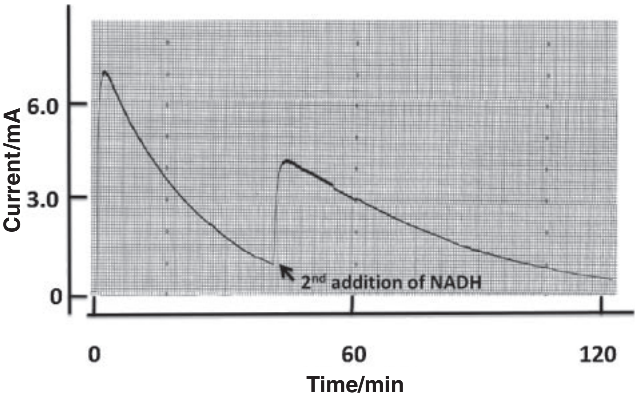

Figure 4. Current generation curve from NADH. 103 ± 1.0 mg of carbon fibers were used. The experimental conditions are described in Experimental. The background current was 0.21 mA at starting point. Second addition of 41.5 mg of NADH to again reach the 1.0 mM was performed at the arrow.

We found that 2.95 mA at the first run decreased to 1.81 mA at the fourth run, as shown in Table 1. After immersion in concd HNO_3, the maximum current values recovered. However, the maximum current value decreased to 1.84 mA at the seventh run again. Furthermore, after immersion in 12% HNO_3, the maximum current again recovered to 2.37 mA at the eighth run. Therefore, the carbon fiber electrode was immersed in 12% HNO_3 for at least 2 h every time for reactivation after use. The amount of the carbon fibers on the electrode was 97 mg at the point of first use, and the amount decreased to 87 mg at the point of eighth use due to peeling off of very small fibers.

Data of the current efficiencies were in the range from 70% to 90%, and those values are a little lower than 100%. This is assumed to be caused by the following. Carbon fiber itself has catalytic activity, as described later, by which NADH is converted to NAD^+, H^+, and two electrons on the surface of the fibers. In this case, most of the electrons generated flow to the anode however a part of the electrons might disappear through reactions with oxidative agents such as the remaining oxygen in the electrolyte.

Next, the following is thought to be the reason for the recovery by HNO_3 subsequent to decrease of the activity of carbon fibers. Carbon fibers are well known to have various O_2-containing groups such as carbonyl, hydroxy, carboxy, epoxy, and double bonds on the surface.[23,24] Thus, the above groups involved in the catalytic activity might be reduced by NADH, which is a strong reducing agent, and the activity of carbon fibers might decrease by reduction of the active groups. On the other hand, carboxylic acid, ester, lactone, enol, and quinone structures are reported to be formed on the surface of carbon fibers by HNO_3.[25] Therefore, the modified surface structure of carbon fibers by NADH is assumed to be regenerated by treatment with HNO_3.

When 86 ± 1.0 mg of the carbon fiber was used, the effect of concentration of NADH was as follows. The maximum current values were 1.54, 2.37, and 2.92 mA at the concentrations of 0.5, 1.0, and 2.0 mM, respectively. The current values increased, accompanied by an increase of concentration of NADH.

Table 1. Stability of the Carbon Fiber Electrode[a)]

Times of use	Maximum current value/mA (Total quantity of electricity/C)[b)]	Current density[c)] /nA h cm^{-2}	Remaining amount of NADH /%	Current efficiency /%
1	2.95 (7.02)	22	12.7	84
2	2.51 (6.48)	—	20.9	85
3	2.02 (6.34)	—	24.3	87
4	1.81 (5.54)	—	29.4	82
After treatment with concd HNO_3 for 2 h				
5	2.38 (6.73)	—	9.5	77
After treatment with concd HNO_3 for 14 h				
6	2.51 (6.84)	—	13.3	82
7	1.84 (5.72)	—	17.4	72
After treatment with 12% HNO_3 for 2 h				
8	2.37 (7.09)	24	16.2	88

a) The amount of carbon fibers on the electrode was 97 mg at the point of 1st use, and decreased to 87 mg at the point of 8th use due to peeling off of very small fibers. The experimental conditions are described in Experimental. b) The quantity of electricity (C) was calculated by multiplying the total current volume (mA h) by 3.6 mA h. c) Current density was calculated by dividing the total current volume (mA h) by the surface area of carbons fibers of which the weight was definitely determined.

The catalytic activity of the carbon fibers was investigated for elucidation of the mechanism of NADH oxidation. Absorbance at 340 nm from 1 mM NADH decreased to 27% of the initial after 2 h when the carbon fiber electrode was hung in the reaction mixture as described in Figure 5. On the other hand, the absorbance was completely constant without the carbon fiber electrode. NADH was completely stable at pH 8.0. In this case, the 2 electrons generated from NADH on the surface of the carbon fibers by the catalytic activity as described above might also disappear due to reactions with oxidative agents such as oxygen dissolved in the buffer.

On the other hand, current generation was measured by using two pieces of Pt mesh (20 × 30 mm), 2 pieces of Pt cladded Ti mesh (30 × 60 mm), and 7 pieces of glassy carbon rod (ϕ0.3 × 25 mm) as anode instead of carbon fibers. The instruments and the operational conditions were the same as those mentioned above. As a result, current generation was not found in the above three cases, and the current values were almost the same as the background value. Therefore, current generation is specific for carbon fibers, and the generation is assumed to be caused by the catalytic activity.

Measurement of Open Circuit Voltage between Anode and Cathode, and the Anodic and the Cathodic Overpotentials. The terminal end of the anode and the terminal end of the cathode were connected to the corresponding terminal ends of the electrometer, respectively, after the background current between anode and cathode reached less than 0.3 mA. The behavior of the voltages is shown in Figure 6. At first, the voltage increased to + 0.215 V without NADH; after addition

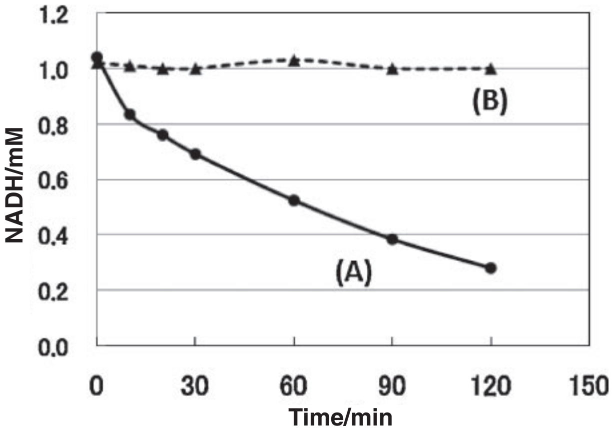

Figure 5. NADH oxidation activity of the carbon fibers. (A) A 103 ± 1.0 mg carbon fiber electrode was hung in 50 mL of 0.2 M sodium phosphate buffer (pH 8.0) in a 50 mL beaker at 35 ± 0.5 °C, and 41.5 mg of NADH was added to reach a concentration of 1.0 mM. The NADH solution was stirred with a magnetic bar. The absorbance was assayed at regular intervals. (B) A control experiment was carried out without any carbon fiber electrode.

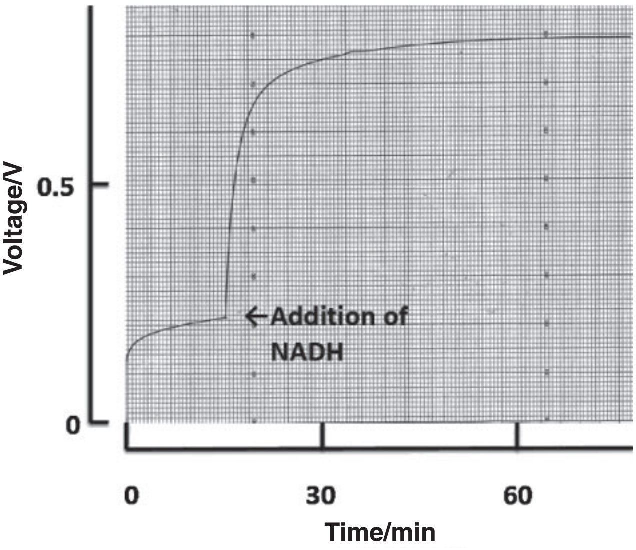

Figure 6. Open circuit voltage between anode and cathode. The same instruments for current generation and the same operational conditions as described in Experimental, and electrometer (HE-104A, Hokuto Denko) were used. A 103 ± 1.0 mg carbon fiber electrode was used.

of 1 mM NADH, the voltage gradually increased and reached + 0.794 V after more than 75 min.

Theoretical standard electromotive force at 35 °C is calculated as follows. Potential difference of intermediate oxidation reduction potential of aqueous solution at pH 7.0 vs. SHE is −0.427 V. Intermediate oxidation reduction potential of $NAD^+ + H^+ + 2e^- \rightleftharpoons NADH$ in the anode cell is calculated to be −0.324 V by using ΔG,[26] so that the oxidation reduction potential vs. SHE is +0.103 V. On the other hand, oxidation reduction potential vs. SHE of $1/2O_2 + 2H^+ + 2e^- \rightleftharpoons H_2O$ in the cathode cell is calculated to be +1.218 V at 35 °C. Theoretical standard electromotive force at 35 °C is calculated to be +1.115 by the following equation. Theoretical standard electromotive force $= E_{\text{the right side}} - E_{\text{the left side}} = +1.218\,\text{V} - (+0.103\,\text{V}) = +1.115\,\text{V}$.

The anodic and the cathodic overpotentials were measured by current interruption at 35 °C and in 1 mM NADH by using 300 ± 6.0 mg of carbon fibers. When the terminal voltage and output current was +0.294 V and 2.94 mA, respectively, the change of the anodic voltage by the current interruption was recorded on a storage digital oscilloscope (Kikusui Denshi Kogyo, 5521). On the other hand, the voltage between the anode and the reference electrode was −0.191 V in the anode cell at 35 °C, 1 mM NADH, and disconnection of the cathode. The anodic overpotential was +0.236 V and the Ohmic loss 22 mV, based on these data.

The cathodic overpotential was determined under the same conditions as the above. When the terminal voltage and output current was +0.294 V and 2.94 mA, respectively, the change of the cathodic voltage by the current interruption was also recorded on the storage digital oscilloscope. On the other hand, the voltage between the cathode and the reference electrode was +0.522 V in the cathode cell at 35 °C, 1 mM NADH, and disconnection of the anode. The cathodic overpotential is calculated to be −0.262 V, based on these data. The electromotive force at 35 °C of this fuel cell is calculated to be +0.595 V as follows. The electromotive force of the fuel cell = theoretical standard electromotive force at 35 °C $[+1.115\,\text{V}] - (+0.236\,\text{V}) - |0.262\,\text{V}| - (+0.022\,\text{V}) = +0.595\,\text{V}$.

Measurement of Cyclic Voltammogram. When the cyclic voltammogram at 1 mM NADH shown in (A) of Figure 7 was compared with that without NADH shown in (A′) of Figure 7, the current over the whole range from 0 to +1.0 V in (A) is more than that in (A′). This means that the current generation is not affected by loading of voltage, and the current spontaneously generates from NADH on the surface of the carbon fiber electrode. On the other hand, a small amount of current generation is also found in the case of (A′) without NADH. This means that current is generated by the effect of some unknown catalytic activity on the surfaces of carbon fibers in the buffer. Furthermore, the final ampere value did not reach 0 A at final voltage of 0 V, in all cyclic voltammograms of Figure 7. The final ampere value usually reaches approximately 0 A at final voltage of 0 V. This gap is also assumed to be caused by contaminated oxides or reducing agents generated by the effect of the unknown catalytic activity. Next, the effect of the difference of the scanning speeds of 0.1, 1.0, and 5.0 V min^{-1} was investigated. The results are also shown in Figure 7. The current generation was largely affected and increased, accompanied by increase of the scanning speeds. Such rapid change of voltage might accelerate the catalytic activity, although the mechanism is unknown.

Relationship between the Current and the Voltage in the Current Generation Cell, Namely the Biofuel Cell. The relationships between the current and the voltage were measured to investigate the electrical resistance of the current generation cell. The results are shown in Figure 8. The very large decrease of the voltage curve (A) in the range of the current of less than 0.5 mA means there is a large overpotential for NADH oxidation, so that the reaction speed for the current generation is considered to be low. The comparatively smaller

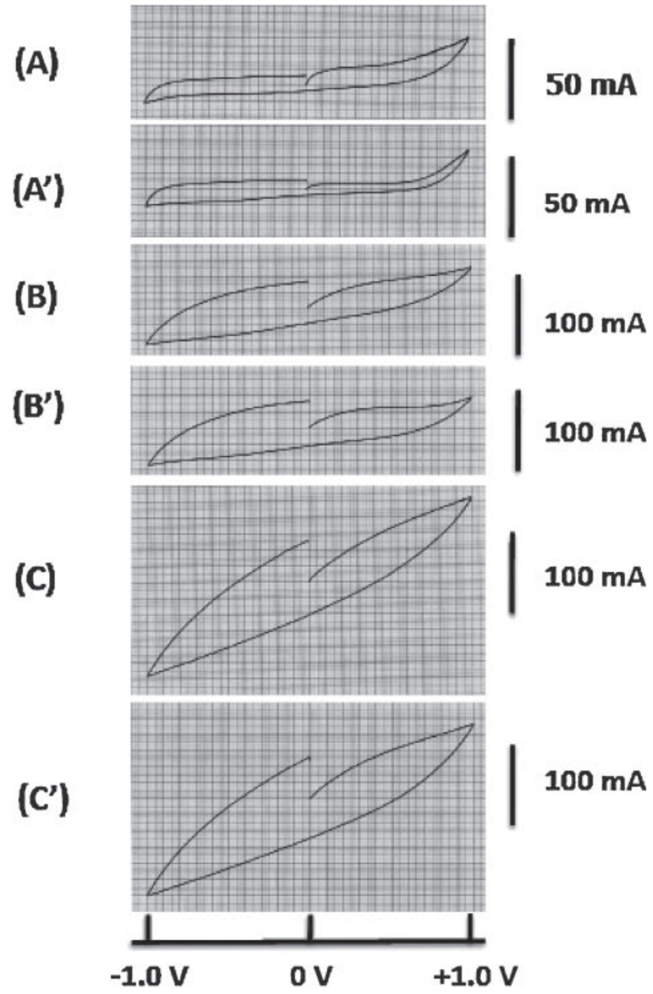

Figure 7. Cyclic voltammograms of the carbon fibers. The same instruments for current generation and the same operational conditions as described in Experimental were used to determine cyclic voltammograms. In the cyclic voltammogram, the corresponding oxidizing and reducing current values are shown on the y axis, accompanied by scanning of loading potential from 0 V to $+1.0$ V, back to 0 V, to -1.0 V, and back to 0 V in the x axis. An Ag/AgCl reference electrode was put in the electrolyte of the anode cell. The connection terminals of the reference electrode, the anode and the cathode were connected to the corresponding terminal ends of a Potentiostat (HAB-151, Hokuto Denko). Scanning was carried out at scanning speeds of 0.1, 1.0, and 5.0 V min^{-1}. Cyclic voltammograms were recorded on an XY recorder (F-35 CN; Riken Denshi, Tokyo). A 103 ± 1.0 mg carbon fiber electrode was used. (A) 1 mM NADH, scanning speed, 0.1 V min^{-1}; (A′) without NADH, scanning speed, 0.1 V min^{-1}; (B) 1 mM NADH, scanning speed, 1.0 V min^{-1}; (B′) without NADH, scanning speed, 1.0 V min^{-1}; (C) 1 mM NADH, scanning speed, 5.0 V min^{-1}; (C′) without NADH, scanning speed, 5.0 V min^{-1}.

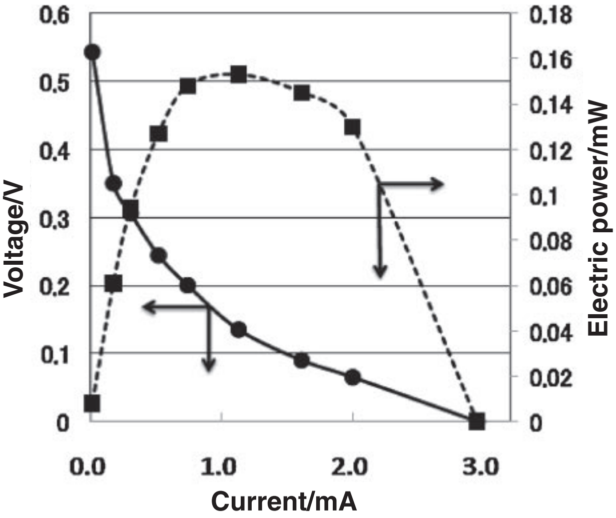

Figure 8. Relationship between the current and the voltage in the current generation cell. The relationship between current and voltage of the current generation cell, namely a biofuel cell, were measured by using resistors of 30, 56, 120, 270, 470, 1000, 2000, and 39000 Ω. The same instruments for current generation and the same operational conditions as described in Experimental were used. A 103 ± 1.0 mg carbon fiber electrode was used.

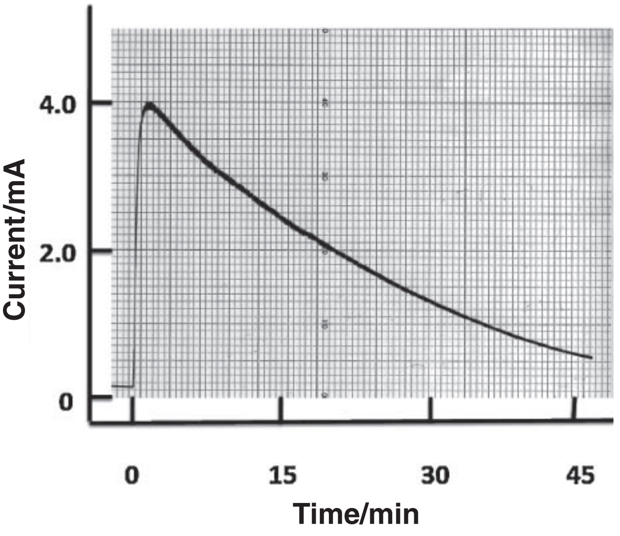

Figure 9. Current generation curve from L-cysteine. The experimental conditions are described in Experimental. A 103 ± 1.0 mg carbon fiber electrode was used.

decrease of voltage in the range of the current of more than 1.0 mA means that the electrical resistances of the electrolyte and cationic exchange membrane are not so large. The electric power obtained from the current and the voltage is shown in the broken line of Figure 8. The maximum electric power was 0.152 mW.

Current Generation from L-Cysteine. The current generation curve obtained from L-cysteine is shown in Figure 9. The maximum current was 3.80 mA, the total electric power was 5.08 C, and the remaining amount of L-cysteine was 2.0% of the initial. In the case of L-cysteine oxidation, the moment that one molecule releases both one electron and one proton, the two molecules are converted to 1 molecule of L-cystine. The current efficiency obtained was 100.6%, which was considered to show efficient current generation.

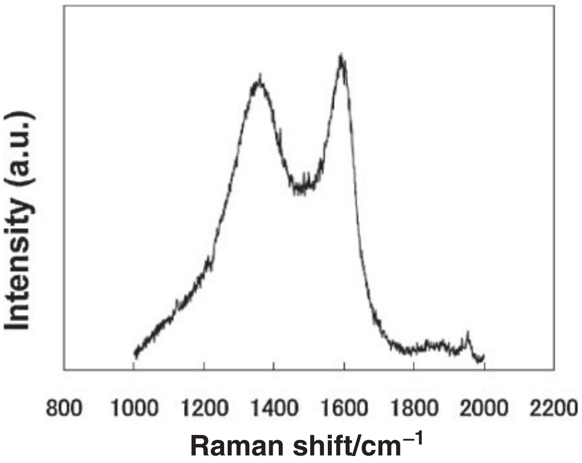

Figure 10. Laser Raman scattering spectrum of the carbon fibers. The experimental conditions are described in Experimental.

Measurement of Specific Surface Area and Graphite Content of the Heat-Treated Carbon Fibers. As a result of the measurement of the surface area as described in Experimental, the surface area was found to be 91.8 m^2 g^{-1}, which is considered to be a reasonable result, compared with the other datum, 21.7 m^2 g^{-1}.[14] The current density is calculated to be 22 nA h (nanoampere•hours) cm^{-2} in the case of NADH oxidation, when 103 mg of carbon fibers were used. The value is extremely low, so that the NADH oxidation activity on the surface of the carbon fibers is thought to be very low.

On the other hand, carbon fibers prepared by treatment at a very high temperature of 2000 to 3000 °C are known to have graphite structure. Very recently, graphite nanofiber composed of graphene sheets was reported to have catalytic activity by which ethanol was converted to acetaldehyde and ethyl acetate in the presence of oxygen.[27] Other catalytic syntheses using graphene have also been reported.[28,29] Therefore, since the catalytic activity might be caused by the graphite structure of carbon fibers, the graphite content was determined by measurement of the Raman scattering spectrum. The content was found to be about 50%, based on the data in Figure 10. Namely, the graphite content is shown by the ratio of I_{1360}/I_{1589}. However, it is not clear at present whether our new finding is truly caused by the graphite structure or not.

Discussion

Most dehydrogenases in a living body use NAD(P)$^+$ as cofactor, so that biofuel cells for which dehydrogenases using NAD(P)$^+$ as a cofactor are applied should be widely acceptable.

A high-power glucose/oxygen biofuel cell has been reported as the most advanced biofuel cell.[9] A carbon fiber sheet was used as the anode, and glucose dehydrogenase, NAD$^+$, VK$_3$ as the mediator, and diaphorase altogether were immobilized on the anode. A carbon fiber sheet was also used as the cathode, and bilirubin oxidase and Fe(CN)$_6$$^{4-/3-}$ as the mediator were immobilized on the cathode. The biofuel cell generated a power of over 100 mW. In this case, 2 kinds of mediators: diaphorase and bilirubin oxidase were used besides NAD$^+$. In the case of our current generation cell without any mediator, power is calculated to be 0.152 mW from Figure 8, when 103 mg of carbon fibers were used. The power of the latter is much lower than that of the former at present however the power of the latter can be raised by increase of the amount of carbon fibers used as anode and an innovative arrangement. Thus, improvements of the generation cell and the operating conditions of current generation cell can be expected in future.

In the preliminary experiment, carbon fibers were applied as anode in the NAD$^+$ recycling system combined with gluconolactone production from glucose by using glucose dehydrogenase, and the activity was fully maintained for 10 h without any adsorption of the enzyme on the surface of carbon fibers. Therefore, the application of carbon fibers for electrode materials should be widely investigated in electrochemistry and enzyme engineering in the future.

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