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Oxygen reduction activity of carbon fibers cathode, and the application for current generation from the NAD^+ and NADH recycling reaction

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Carbon fibers treated at 700 °C for 10 min were found to have O_2 reduction activity when being used as a cathode. The special type of partition combined with both cationic and anionic exchange membranes was applied between anode cell and cathode cell in order to use a highly acidic solution such as 0.5 M H_2SO_4 as an electrolyte of the cathode cell for increasing the efficiency of O_2 reduction activity. The current generation from NAD^+ and NADH recycling system combined with D-gluconolactone production from 500 mg of D-glucose was performed by applying only carbon fibers for both anode and cathode. The total current volume obtained was 81.4 mAh during the reaction for 10 h, and the current efficiency was 93%. One gram of carbon fibers was pressed with Nafion paste on a piece of carbon paper (area : 50 mm×50 mm) with heating to prepare the cathode, and this construct was combined with conventional fuel cell. The power density was 3.6 mW/cm², and the total power volume was calculated to be 90 mW per 1 g of carbon fibers.

1. Introduction : Powdered carbon material, graphite material, carbon fiber material, glassy carbon, and carbon nanofibers are well known as to be electrode materials. In industry, acetylene black, carbon black, graphite powder, graphite rod, and pitch and PAN-based carbon fibers were applied for constructions of primary, secondary, and storage batteries, and fuel cells [1]. Carbon fibers (CF) [2], felt and paper derived from CF [3, 4], graphite carbon, and felt derived from the graphite [5, 6, 7, 8, 9, 10], and glassy carbon [11] were frequently applied for construction of biofuel cell. Researches on biofuel cells combined with dehydrogenases, β -Nicotinamide adenine dinucleotide (phosphate) reduced form (NAD(P)H) as a cofactor, mediators, and diaphorase enzyme are well known [12, 13 - 16]. The detailed reaction system is shown in Figure (1A). NADH is continuously regenerated from NAD^+ by the recycling dehydrogenation using D-glucose. 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 high oxidation potential.

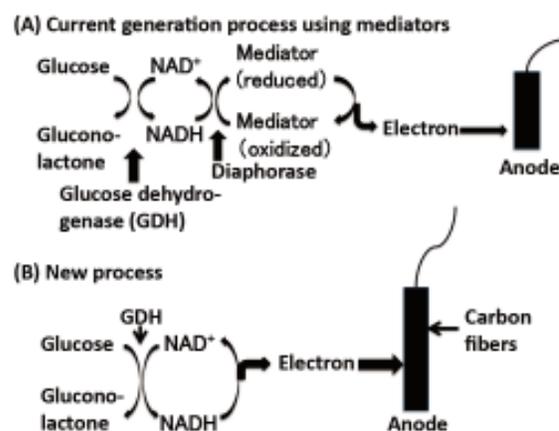


Figure (1) : Current generation from NAD^+ and NADH recycling system combined with D-gluconolactone production from D-glucose.

Recently, new direct electron transfer (DET) systems besides the above biofuel cell systems have been investigated to increase the efficiency of current generation, and those were reviewed in our previous paper [2].

On the other hand, we had investigated applications of an electrochemical NAD^+ recycling system involving a string-like carbon fiber electrode (CFE) to an enzyme reactor [17]. 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 electro-chemical reaction was carried out by using carbon fibers (CF) treated at 700 °C for 5 min, CF were found to have their own catalytic activity for oxidation of NADH, and no mediator was necessary for the current generation [2], as shown in Figure (1B).

Afterward, CFE was newly found to be applicable as a cathode for O_2 reduction activity. In other words, the current was instantaneously detected on the zero shunt ammeter when the terminal end of CF anode was connected with that of CF cathode. CF has been widely used as supports for metal catalysis such as iron, nickel, platinum and rhodium in various reactions [18, 19]. Carbon nanofibers containing elemental Fe, which were prepared by pyrolysis of iron(II)phthalocyanine and by carbonization of the mixtures of ferrocene and poly(furfuryl alcohol), were shown to have O_2 reduction activity by electrochemical methods [20, 21]. However, the catalytic effects of CF themselves had been reported by only a few papers. Graphite nanofiber composed of graphene sheets had the catalytic activity by which ethanol was converted to acetaldehyde and ethylacetate in the presence of oxygen [22], and activated CF had catalytic oxidation activity of H_2S [23].

Next, in biofuel cells, the pH of the electrolyte in the anode cell generally has to be kept around neutral condition for good performance of the enzyme reaction, so that a highly acidic electrolyte such as 0.5 M H_2SO_4 cannot be used in a cathode cell due to rapid penetration of H^+ ions from cathode cell to anode cell. Actually, when 0.5 M H_2SO_4 is used, a rapid decrease of pH resulted. Accordingly, we partitioned between anode and cathode cells by using sandwiched construct combined with cationic and anionic exchange membranes as shown in Figure (2), to prevent from the H^+ penetration. As a result, the H^+ penetration was found to be very largely depressed by using the partition, so that use of 0.5 M H_2SO_4 as the electrolyte in the cathode cell was possible.

In this paper, these new findings are described.

2. Experimental :

2.1 Preparation of CF anode, CF cathode, and Pt-mesh cathode : CF (T 300-3k; Toray, Tokyo) were used as anode and cathode after the following treatments. A bundle of the fibers (5 cm length, about 2 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 CF were heated at 700 °C for 10 min under aeration to eliminate all binders for reformation of the CF. After use, the CF were renewed by treatment with 12 % HNO_3 . Two pieces of Pt foil-cladding Ti-mesh (PFCT-mesh) (No. 1001; SPF, Nagoya) were used after Pt plating and bending to make it possible to insert the mesh into the cathode cell. Platinum plating on PFCT-mesh was performed as follows. Two pieces of PFCT-mesh (30 mm × 60 mm) were dipped in an electrolyte in which 0.5 g of $\text{H}_2\text{PtCl}_6 \cdot n\text{H}_2\text{O}$ (Pt content, 38.17 %; Kojima Kagaku Yakuhi, Sayama, Saitama) and 37 mg of $(\text{CH}_3\text{COO})_2\text{Pb} \cdot 3\text{H}_2\text{O}$ had been dissolved. Two hundred mA was loaded on the PFCT-mesh as cathode for the plating at room temperature for 30 min by a power supply (Model 3150a, ANATECH, Tokyo). The increased weight of PFCT-mesh was 148 mg after the plating.

2.2 Assays of amounts of NAD^+ , D-glucose, and D-glucono-1, 5-lactone and glucose dehydrogenase activity : The amount of active NAD^+ remaining after current generation was determined by a method using alcohol dehydrogenase and semicarbazide [24]. The amount of D-glucose remaining was determined with a colorimetric glucose kit, based on glucose oxidase (Glucose C-II Test; Wako Pure Chemical, Tokyo). The amount of D-glucono-1,5-lactone produced was determined by HPLC (SCL 10A, LC-10AS, and SPD 6S; Shimadzu, Kyoto) on an ion exchange resin column of TSKgel SCX⁺(Tosoh, Tokyo) as described in the previous paper [17]. The assay of glucose dehydrogenase (GDH) activity was performed at 37 °C in 100 mM sodium phosphate buffer (pH 7.0) containing 1.0 mM NAD^+ and 30 mM glucose; the initial velocity of the increase in absorbance at 340 nm was determined. One unit of enzyme activity was defined as the catalytic rate of conversion of one μmole of substrate per min so that 20 units means production of 20 μmoles of NADH per min, namely 1200 μmoles per hour. One and two tenths mmoles of NADH per hour is theoretically calculated to mean that the current generation volume is 64.3 mA per hour. Therefore, in this experiment, 20 units is assumed to be enough GDH activity. Furthermore, K_m for D-glucose and K_m

for NAD^+ of the GDH were 23.5 mM and 0.275 mM, respectively.

2.3 Current generation cell and the operation conditions : Positions and features of CF anode and CF cathode are shown in Figure (2). The sizes of the parts of both cells and the shapes of the rubber packing and the spacer are shown in Figure (3).

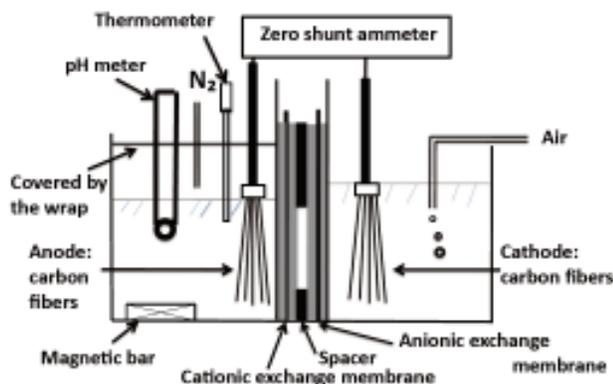


Figure (2) : Scheme of the current generation cell.

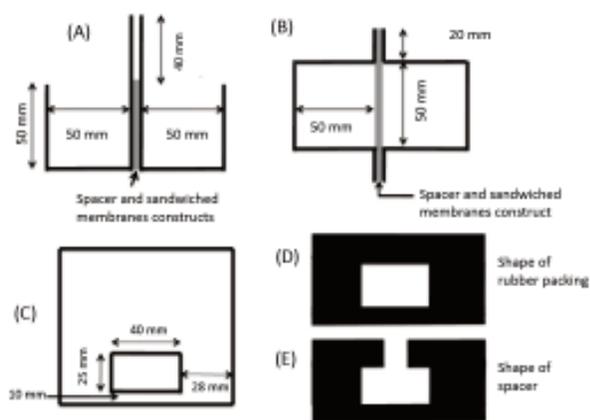


Figure (3) : Sizes of the anode cell and the cathode cell, the shape of the rubber packing and the spacer. (A), side view. (B), plan view. (C), front view from rubber packing side. The shape of the rubber packing having the open space is shown in (D). The shape of spacer is shown in (E). Those cells and the spacer were prepared by using a 3 mm thick Poly(vinylchloride) board. A 2 cm magnetic bar was put in the anode cell for stirring. The mixture was stirred at about 600 r. p. m.

The anode cell and the cathode cell are divided by a partition equipped with cationic exchange membrane (Neosepta CMX, Asahi-Kasei, Tokyo) -rubber packing constructs on the anode side of spacer and anionic exchange membrane (Neosepta AHA, Asahi-Kasei) -rubber packing constructs on the cathode side,

as shown in the drawing. The cationic and the anionic exchange membranes were sandwiched by rubber packing (thickness 3 mm), respectively. The open part in the spacer was filled up by 0.5 M $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$ buffer (pH 8.0). CF (300 ± 6.0 mg) and CF (800 ± 16 mg) were fixed on a glassy carbon rod with a rubber band, and the resulting constructs were used as the anode and the cathode, respectively. The partition was used to largely decrease each other's transfers of H^+ ions between the two cell, so that 0.5 M H_2SO_4 as the electrolyte was able to be used as an electrolyte of the cathode cell. 60 mL of 0.5 M sodium phosphate buffer (pH 8.0) containing 41.2 mg of NAD^+ , to make 1.0 mM NAD^+ , and 500 mg of glucose was added into the anode cell as the electrolyte. 70 mL of 0.5 M H_2SO_4 was added into the cathode cell. The terminal ends of the anode and the cathode were connected to a zero shunt ammeter (HM 104A, Hokuto Denko, Tokyo). 20 units of GDH were added to the electrolyte of anode cell when the background current reached as low a value as possible. The pH in the anode cell was kept in the range from pH 7.90 to pH 8.05 by 1.0 N NaOH. The anode cell was kept under an atmosphere of nitrogen, and air was blown into the electrolyte of the cathode cell at the flow rate of about 4 L/min. The temperature of the anode cell was kept at 35 ± 0.5 °C. In the current generation, about 2 ml of Milli-Q water was added into the electrolyte in cathode cell at regular intervals to keep the water level constant. Current efficiency was determined by a weight assay of the clipped paper, as described in the previous paper [8]. The total background current volume was subtracted from the total current volume.

2.4 Determination of oxygen reduction activity of carbon fibers : Oxygen reduction activity of carbon fibers used as cathode was measured as follows. The scheme of conventional fuel cell for the measurement is shown in the left side of Figure (4). Complex of anode, Nafion-117 membrane (thickness, 175 μm), and complex of cathode were sandwiched by electron collectors as shown in the right side of Figure (4). The carbon paper area of anode and cathode was 50 mm \times 50 mm. One gram of carbon fibers was pressed with Nafion paste on the area with heating to prepare the cathode. On the other hand, 12.5 mg of Pt powder (Tanaka Kikinokoku, Tokyo, Japan), of which the particle size and specific surface area were a few nanometers and 120.4 m^2/g , was also pressed with Nafion paste on the carbon paper with heating to prepare the anode or the cathode. Operational conditions of the fuel cell were regulated by a Fuel Cell Evaluation System (GTF-MSG-01-KS, Toyoh Technica, Tokyo).

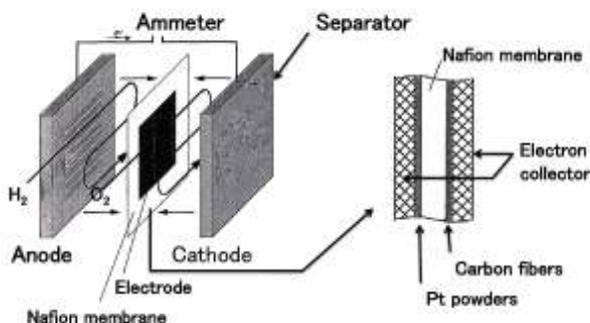


Figure (4) : Scheme of fuel cell for measurement of O_2 reduction activity of carbon fibers. The cell temperature was $80^\circ C$. Both flow rates of H_2 and O_2 were 100 mL/min . cell.

3. Results and Discussion :

3.1 Current generation cell using carbon fibers as both anode and cathode :

CF was already reported in the previous paper to have the catalytic activity for oxidation of NADH and L-cysteine [2]. When the functions of CFE were newly investigated, CFE was found to have O_2 reduction activity as a new function of the cathode. Thus, the current generation for 10 h was carried out by using CFE as both anode and cathode. In this case, current generation from NAD^+ and NADH recycling reaction combined with D-gluconolactone production from D-glucose was performed. The results are shown in Figure (5).

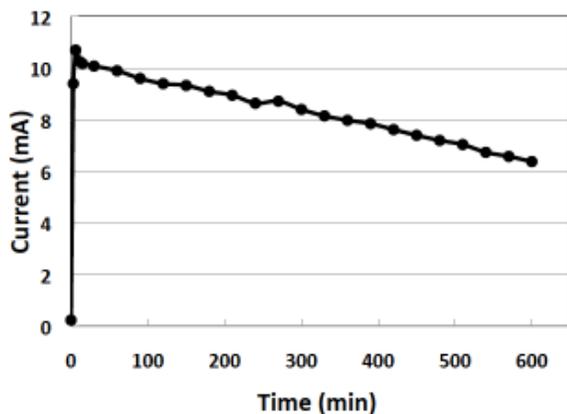


Figure (5) : Current generation from D-gluconolactone production cell

The current value increased from the background value of 0.24 mA to about 10.2 mA after 12 min, and the value was stable at 9.9 mA after 1 h. Afterwards, the line of the current generation showed a tendency of gradual decreasing; the value at 10 h was 6.4 mA . The data after current generation for 10 h are shown as follows. The remaining amount of NAD^+ was 71 %

of the initial activity. The remaining GDH activity was 55 % of the initial activity. The consumed amount of D-glucose was 294 mg out of the original 500 mg . The produced amount of D-gluconolactone was 290 mg . The total current volume obtained was 81.4 mAh , and the current efficiency was 93 %. NAD^+ and GDH was not so stable for the above 10 h recycling reactions.

In the partition, cations are rejected by anionic exchange membrane, and anions are rejected by cationic exchange membrane. Therefore, flows of cations and anions between anode cell and cathode cell are assumed to be completely blocked, and the current also to stops. Truly, when the buffer in the opening space of the spacer was removed out, the current completely stopped. However, in our experiment, although flows of cations and anions were considered to be blocked by two kinds of membrane, the current flowed. In the process of current generation for 10 h using the partition, 7.5 ml of 1.0 M NaOH was added into the electrolyte in anode cell to keep pH 8.0. Three and twenty-six hundreds ml out of the 7.5 ml is assumed to be needed for neutralization of both amount (1.63 mmole) of H^+ generated from the dehydrogenation of D-glucose and amount of D-gluconic acid converted from 290 mg (1.63 mmole) of D-gluconolactone produced. The remaining volume, 4.24 ml of 1.0 M NaOH , is assumed to be needed from neutralization of amount of H^+ transferred through the partition from the cathode cell. Thus, H^+ transfer from the cathode cell is considered to occur to some extent, and not to be completely rejected by the anionic exchange membrane.

On the other hand, a control test was performed as follows. Only cationic exchange membrane sandwiched by 2 pieces of the rubber packing was used as the diaphragm, and 60 ml of $0.5\text{ M Na phosphate buffer}$ (pH 8.0) was added into the anode cell except for D-glucose, NAD^+ , and GDH, and 70 ml of $0.5\text{ M H}_2\text{SO}_4$ was added into the cathode cell, respectively, and then 1.0 M NaOH was added into the electrolyte in the anode cell to keep pH 8.0 under the conditions of stirring in the anode cell and air-bubbling into the cathode cell at $35^\circ C$. As a result, addition of 29.3 ml of 1.0 M NaOH was necessary in the operation for 10 h to keep pH 8.0. The added volume of 1.0 M NaOH in a control test was much more than that in the case of application of the partition. Therefore, the effect of our partition on decrease of H^+ transfer rate is clear.

Next, we examined the cause of the gradual decrease of the current value in Figure (5). The relationship

between concentration of NAD^+ and the current generation was first investigated, and the results are shown in Table (1). The current generation was recognized to be affected by the concentration. Therefore, when 71 % of the initial amount of NAD^+ , namely 0.71 mM of NAD^+ , remained in the reaction mixture after the continuous current generation for 10 h, the current value was assumed to decrease to 85.5 % of the initial value.

Table (1) : Effect of concentration of NAD^+ on the current generation.

Sr. No.	Concentration of NAD^+ (mM)	Current Value (mA)	The Relative Value (%)
1	0.5	6.43	63
2	0.6	8.13	80
3	0.7	8.70	85
4	0.8	9.23	90
5	0.9	9.73	95
6	1.0	10.21	100

- The Current values in the table do not include the background value 0.47 mA.

However, the current value after the continuous current generation for 10 h decreased to 63 % of the initial value, and the decreasing rate was larger than that caused by the decrease of concentration of NAD^+ . Thus, the remaining difference, 22.5 %, of the decreasing rates is considered to be caused by adsorption of some substances on the surface of CF or conversion of some chemical groups involving in the catalytic activities on the surface. However, the activity is reproduced by immersing in 12 % HNO_3 .

Decrease of the activity of NAD^+ might be caused by degradation of glucoside linkage between the base and D-ribose moiety, because this linkage is known not to be so strong. As one more possibility, some side reactions might occur due to NAD radicals that were obtained with the progress of two electron oxidation of NADH on the surface of CF, resulting in the deactivation.

GDH is well known to be very unstable in distilled water, and high molar concentration, 2 or 3 M, of NaCl is necessary for its stabilization. Therefore, the 0.5 M Na phosphate buffer (pH 8.0) used as an electrolyte in anode cell might be too weak for increasing the stabilization.

As a control test, the experiment for current generation using 1 piece of CFE (300±6 mg) as anode and 2 pieces of PFCT-mesh plated by Pt as cathode was performed. In this case, the partition combined with both cationic and anionic exchange membranes

was used, and 0.5 M H_2SO_4 was applied as an electrolyte of cathode cell. As a result, the current value increased from background current value of 1.3 mA to about 17.5 mA after 17 min. Thus, some effect of PFCT- mesh plated by Pt was recognized. However, the extent of the effect is only about 1.6 times higher than the case of CFE (800 mg) as cathode. Therefore, application of CF for cathode material is assumed to be possible.

3.2 The relationships between the current and the voltage of the current generation cell :

The relationships between the current and the voltage were measured to determine the maximum electric power of the current generation cell. The resistors shown in Figure (6) were put between the anode equipped with 2 pieces of CFE (300 mg) and the ammeter, and the current values were measured.

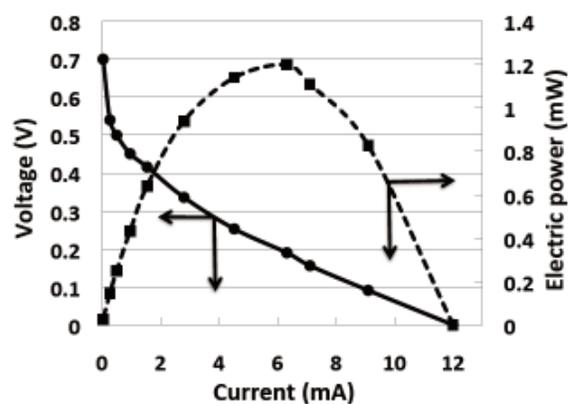


Figure (6) : Relationship between the current and the voltage in the current generation cell

The results are shown in Figure (6). The large decrease of the voltage curve (solid line) below less than 0.5 mA of current value means there is a large overpotential for NADH oxidation, and the comparatively smaller decrease of voltage over more than 5.0 mA of current value means that the electrical resistance values of the electrolyte and the partition are not so large. The electric power obtained from the current and the voltage is shown in the broken line of Figure (6). When a 30 Ω resistor was used, the current value was 6.32 mA, so that the voltage is calculated to be 0.19 V. The maximum electric power value of this cell is calculated to be 1.2 mW.

The resistors of 10, 22, 30, 56, 120, 270, 470, 1 k, 2 k, and 20 k Ω were used. Two pieces of CFE (300 mg) were tightly tied by a rubber band, and the resulting construct, having carbon fibers of 600±12 mg in total, was used as anode.

3.3 Measurement of O₂ reduction activity of CF by conventional fuel cell : A fuel cell was constructed as shown in Figure (4), and the activity was measured according to Section (2.4). The results are shown in Figure (7). When the cell voltage was 0.2 V, the current density was 18 mA/cm². Thus, the power density was 3.6 mW/cm², and the total power volume is calculated to be 90 mW per 1 g of CF, based on 25 cm² of the cathode area. As a control test, when the Pt powder (size, a few nanometer) of 12.5 mg was used as the cathode, the power output was determined according to Section (2.4). As a result, the maximum power density was 280 mW/cm².

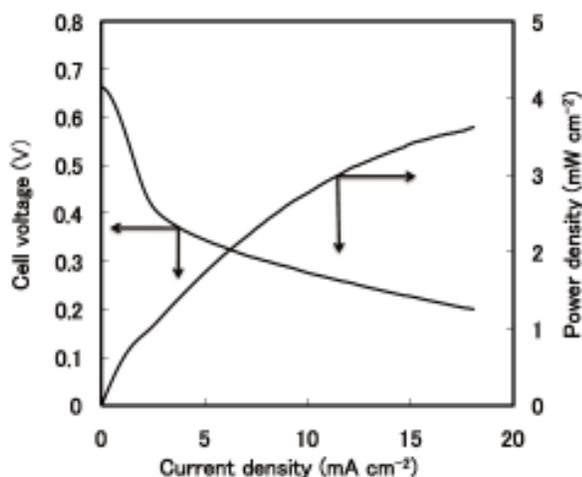


Figure (7) : O₂ reduction activity of carbon fibers.

The total power volume of the cell is calculated to 7.0 W per 12.5 mg of the Pt powder, based on 25 cm² of the cathode area. The power volume obtained from 1 g of the Pt powder is calculated to roughly 6220 times higher than that obtained by CF at present. The much lower O₂ reduction activity of CF is assumed to be caused by decrease of the surface area due to overlapping of the fibers each other. On the other hand, when H₂ oxidation activity of CF was tested as anode by using the conventional fuel cell, no significant H₂ oxidation activity of CF was found.

4. Conclusion :

(1) Carbon fibers treated at 700 °C for 10 min had O₂ reduction activity as a new function when being used as a cathode.

(2) Carbon fiber cathode was shown to be applicable for the current generation from NAD⁺ and NADH recycling system combined with D-gluconolactone production from D-glucose.

(3) The power density of 1.0 gram of carbon fibers as the cathode (area : 50 mm × 50 mm) was 3.6 mW/cm²,

when combining with conventional fuel cell, and the total power volume was calculated to be 90 mW per 1 g of carbon fibers.

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