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Enhancing catalytic activity of bioanode for glucose biofuel cell by compressing enzyme, mediator and carbon support through centrifugation

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Dedication ((optional))

Abstract: In this study, we demonstrate the fabrication of a graphene-based bioanode utilizing ferrocenemethanol as mediator and glucose oxidase as enzyme catalyst through centrifugation. The enhanced catalytic activity for glucose oxidation was achieved via centrifuge compressing respective components within the mixture. The glucose/O₂ biofuel cell (GBFC) was assembled by having the bioanode integrated with Pt wire as cathode. The GBFC produced maximum power density and open circuit voltage of ~70 $\mu\text{W cm}^{-2}$ and 0.38 V.

The study of enzymatic biofuel cells (EBCs) has been of increasing importance as potential power sources for decades, particularly after the introduction of implantable devices.^[1] EBCs involve the chemical redox reactions of biofuels catalysed by specific enzymes, converting chemical energy to electrical energy in the process. The enzyme-catalysed power generation process is clean, efficient in mild operating condition and selective due to the nature of enzymes employed for the reactions.^[2] Such properties of enzymes show promising benefits in replacing the conventional platinum-based catalysts which are expensive and have poor specificity in catalysing reactions. Considering the application for implantable biofuel, glucose biofuel cells (GBFCs) are of greatest interest since glucose and oxygen (as biofuels) is readily present in most physiological fluids. Both metallic and enzymatic catalysts had been studied intensively when assembling GBFCs. Mesoporous metallic catalysts were employed for glucose oxidation considering their tunable properties by easily changing their structures (size, shape and composition).^[3] In regards to enzymatic catalyst for glucose oxidation, glucose oxidase (GOx) was frequently utilized due to its high catalytic activity, selectivity and stability.^[4] The immobilization of enzymes is of high importance in establishing good electron transfer between the redox active species to the electrode surface due to the fact that redox active sites are buried deep in the enzymes' protein structure. The efficiency of the catalysed reaction is greatly attributed to the ease of electron transfer, thus effective immobilization of enzymes is crucial in electrode fabrication. The electron transfer process can be classified as direct electron transfer (DET) and mediated electron transfer (MET) respectively.

In the approach of DET,^[5] electrical wiring was established

from the redox active centers of enzyme to the electrode surface directly, allowing electrons to flow through. The successful assembly of electrical wiring is usually achieved by the usage of carbon materials like carbon nanotubes (CNTs) and graphene which have high electrical conductivity and surface area. Cosnier and coworkers demonstrated the approach for bioelectrode fabrication through mechanically compressing the mixture of enzyme and CNTs.^[6] Through mechanical compression, the sludge-like mixture containing the enzymes and CNTs can achieve closer contact and subsequently increase the probability of establishing electrical connectivity. Electron transfer between active centers and electrode surface can also be achieved through redox mediators shuttling the electrons generated.^[7] MET by osmium-based redox hydrogels bioelectrodes were demonstrated by Mano and co-workers, giving high power generation in GBFCs.^[8] Mechanical compression of enzyme, mediator and graphite particles was also established to fabricate MET-based bioelectrodes.^[9] The MET process was revealed to produce higher catalytic activity than DET due to its efficiency in shuttling electrons but the operating cell voltage (OCV) will be slightly compromised and there is also concern for immobilizing the mediators with minimal leakage.^[9b]

In this study, we report the fabrication of a graphene-based bioanode utilizing ferrocenemethanol as mediator and glucose oxidase as enzyme catalyst through centrifugation of the mixture solution. The mixing of components was conducted in higher volume of solution to ensure greater mobility of molecules within the mixture, facilitating uniform packing and interactions of the different components. The centrifuge compression method allowed better interaction of enzyme, redox mediator and graphene-chitosan (Graphene-CS) support under the applied centrifugal force. The immobilisation of enzymes and redox mediators within the Graphene-CS matrix during centrifuge will enable higher degree of connectivity between respective components within the matrix before constructing the spherical bioanode. The fabricated bioanodes were tested electrochemically in the presence of glucose and a GBFC constructed with the bioanode and Pt wire (as cathode) was also evaluated for its power generation and stability.

Mediated electron transfers between the respective components (enzyme, redox mediators and Graphene-CS matrix) were observed within the synthesized bioanodes (Figure 1a). The polarization curve shows an anodic peak in the range of 0.24 – 0.27 V for the centrifuged bioanodes attributed to the redox oxidation peak for Fc-MeOH.^[7, 10] This proves that Fc-MeOH molecules were electrically connected to the graphene-chitosan matrix. It is noted that in the absence of redox mediator, the centrifuged bioanodes did not exhibit significant catalytic current toward glucose oxidation (see Figure S1 in Supporting

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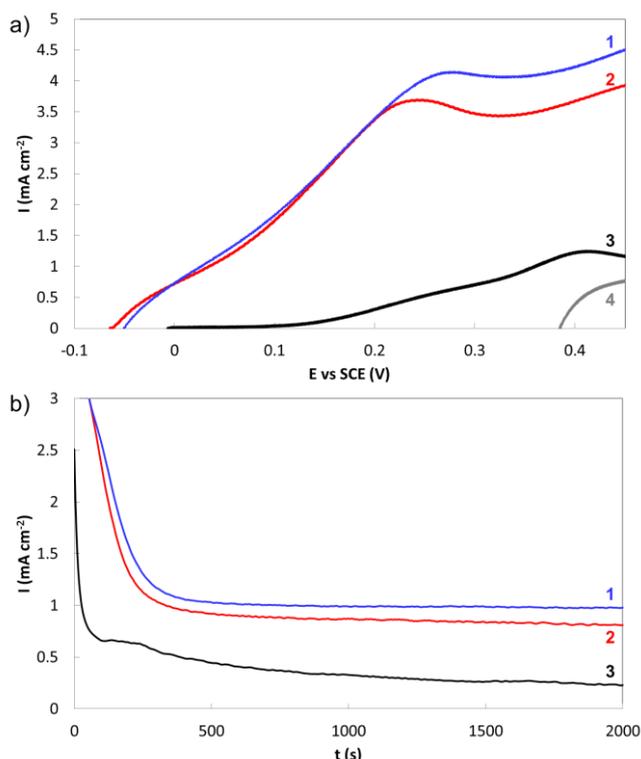


Figure 1. a) Polarization curves of bioanodes: centrifuged at 13500 rpm (1), centrifuged at 5000 rpm (2) and casted on GC electrode (3) in 0.05 mol L⁻¹ glucose solution. Polarization curve in absence of glucose (4) for bioanode centrifuged at 13500 rpm. Scan rate 2 mV s⁻¹. b) Chronoamperometry response at 0.155 V vs SCE for bioanodes: in 0.05 mol L⁻¹ glucose solution. Electrolyte solution composed of 0.1 mol L⁻¹ PBS (pH 6.5) in nitrogen-saturated condition.

Information). This means that there were no significant direct electron transfers (DET) between the redox active sites of GOx and the Graphene-CS matrix when no the redox mediator was present.

Glucose oxidation was detected when the synthesized bioanodes (ctf-13500, ctf-5000 and casted sample) were tested in glucose solution. The ctf-13500 bioanode had an open circuit potential (OCP) of 0.38 V in the absence of glucose (curve 4, Figure 1a). When glucose was introduced in the testing environment, all synthesized bioanodes exhibited more negative OCP and increased current density. The negative shifting of OCP (~0.4 V) and increase in current density showed that the bioanodes indeed undergo glucose oxidation when glucose was present.^[6a, 9a, 11]

Table 1. Catalytic performances for the different bioanodes from Figure 1A

Electrode	OCP (V vs. SCE)	I (mA cm ⁻²) @ 0.4 V vs. SCE
ctf-13500 (1)	-0.05	4.21
ctf-5000 (2)	-0.06	3.67
Casted (3)	0	1.23
Graphite/Fc/GOx ^[9a]	0	~ 1.85

The centrifuged samples exhibited superior catalytic activity towards glucose oxidation compared to the casted sample. The

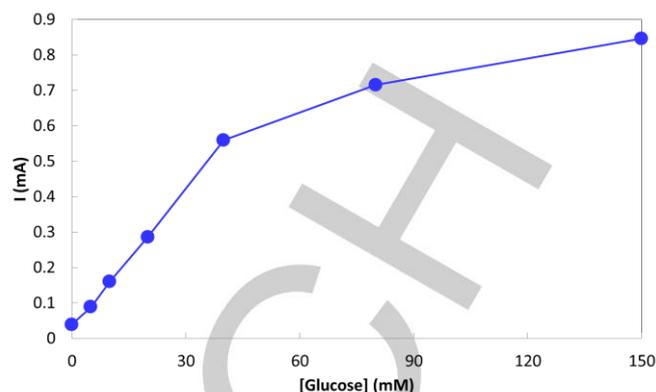


Figure 2. Plot of current towards glucose concentrations for bioanodes of 13500 rpm centrifuged sample. Electrolyte solution composed of 0.1 mol L⁻¹ PBS (pH 6.5) in air-saturated condition.

OCP and current density for ctf-13500, ctf-5000 and casted sample were shown in Table 1.

The centrifuged samples exhibited superior catalytic activity towards glucose oxidation compared to the casted sample. The OCP and current density for ctf-13500, ctf-5000 and casted sample were shown in Table 1. The catalytic current observed in the centrifuged samples (ctf-13500 and ctf-5000) were ~3 times higher compared to that of casted samples, thus showing the superior electrocatalytic activity of centrifuged samples. The bioanode with redox mediators exhibited ~7 higher catalytic activities than bioanode without redox mediators (see Figure S1 in Supporting Information), this means the MET process has greater efficiency in electron transfer compared to DET process. In addition, the centrifuged bioanodes (ctf-13500 and ctf-5000) exhibited higher catalytic activity towards glucose oxidation when compared to ferrocene-mediated bioanode fabricated through mechanical compression.^[9a] This could be likely due to the improved immobilisation of enzyme and redox mediators within the matrix through the centrifuge compression.

The chronoamperometric responses (CA) of the bioanodes were tested at 0.155 V vs SCE^[9b, 11] with presence of glucose (Figure 1b). The bioanodes exhibited a steady-state catalytic current of 0.98, 0.81 and 0.23 mA cm⁻² for ctf-13500, ctf-5000 and casted electrode respectively. Similar to the maximum catalytic current in the polarization curve, the steady-state currents of centrifuged samples were also ~3 times higher compared to that of casted samples. The CA result confirms the greater catalytic activity in centrifuged sample compared to casted sample for glucose oxidation. The result from LSV and CA proved that by centrifuging the mixture solution at high rotational speed, the respective components were compressed within the solution, achieving sufficient electrical interconnectivity to exhibit higher catalytic current.

The apparent Michaelis-Menten (K_M^{app}) was investigated to understand the catalytic efficiency of the immobilized enzymes in the bioanode (Figure 2). The K_M^{app} of ctf-13500 bioanodes were computed based of Lineweaver-Burke (double reciprocal) method^[12] from the CA experiments and the values obtained were within the range of 60 – 80 mM. The high K_M^{app} values were similar to literature^[11] (\approx 60 mM) and this is possibly due to the slow diffusion of glucose molecules into the 3D bioanode.^[9b, 13] This implies that the GOx immobilized within the Graphene-CS

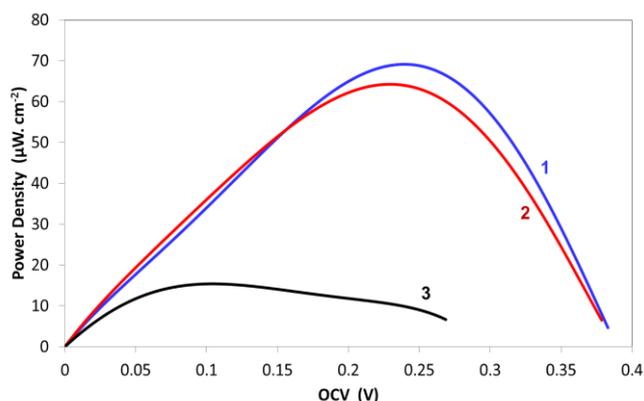


Figure 3. Dependence of power density on operating voltage for bioanodes: centrifuged at 13500 rpm (1), centrifuged at 5000 rpm (2) and casted on GC electrode (3) against Pt wire as cathode in 0.01 mol L⁻¹ glucose solution. Electrolyte solution composed of 0.1 mol L⁻¹ PBS (pH 6.5) in air-saturated condition.

matrix (not only the surface of bioanode) also contributed to glucose oxidation reaction and the slow diffusion of glucose into the matrix (reaching GOx deep inside) was hindering the catalytic efficiency of the bioanodes.

The glucose/O₂ biofuel cells comprising the synthesized bioanodes (pairing with Pt-wire as cathode for oxygen reduction reaction) were investigated without any separating membrane. The recorded open-circuit voltages (OCV) were 0.38±0.02 V for centrifuged bioanode (ctf-13500 and ctf-5000) and 0.27±0.01 V for casted bioanode (Figure 3). The OCP for Pt wire in oxygen reduction reaction (ORR) in oxygen-saturated PBS, pH 6.5, is ~0.30 V (see Figure S2 of Supporting information), thus the obtained OCV for the GBFC was in agreement with the OCP obtain from anodic and cathodic half-cell reactions. The maximum power densities of the bioanodes are 69.1, 64.2, and 15.4 µW cm⁻² for ctf-13500, ctf-5000 and casted respectively. The results showed that the maximum power density of centrifuged bioanode was ~4 times higher compared casted bioanodes and the OCV of the biofuel for centrifuged bioanode was also superior. The power density at 10 mM glucose observed in centrifuged bioanodes is considerably higher compared to literatures (that uses Pt as cathode).^[11] This is in agreement with previous result of centrifuging improves the electrical connectivity with the bioanode and exhibit higher catalytic activity in glucose oxidation.

The GBFC exhibited high power density and OCV even days after the preparation (Figure 4). The OCV of the GBFC remained relatively constant for the first 3 days which then decreased the following days after. The mean OCV recorded on Day 7 was 0.32 V, which is a decrease of ~0.07 V from Day 1. In regards to the maximum power density, it remained quasi-constant for the first 4 days^[11, 14] and gradually decreases for the next 3 days. At Day 7, the maximum power density dropped to around 62 % of the first day. The decrease in power output was possibly due to a few reasons; firstly, the loss in enzyme activity, redox mediator or both from the bioanodes itself during testing.^[11] Secondly, the electrical disconnection or rearrangement between the enzyme^[9b] and the graphene-chitosan matrix caused by the swelling effect of chitosan in aqueous solution,^[15] thus affecting the electron transfers process.

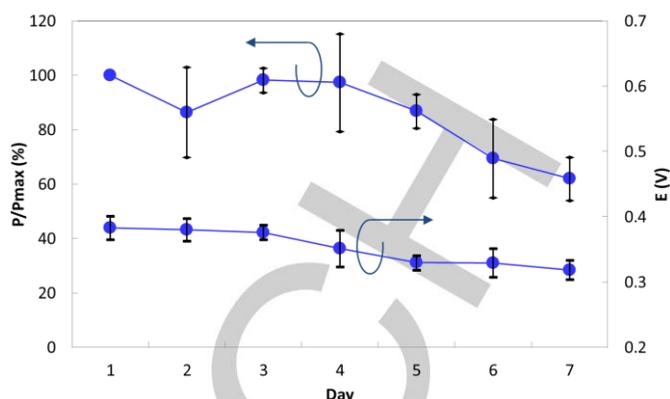


Figure 4. Durability of GBFC against time of bioanodes centrifuged at 13500 rpm with Pt wire as cathode in 0.01 mol L⁻¹ glucose PBS. The error bars represent the standard deviation of different trials.

Furthermore, the swelling effect may also disrupt the connection between the 3D bioanodes and the Pt wire connection, thus causing the loss of electrons transfers.

Nevertheless, the observed maximum power density in Day 7 was ≥34.1 µW cm⁻²; this value was higher than the reported value of Day 1 in literature.^[11] This means that the ctf-13500 bioanode was able to retain substantial catalytic activity even after 7 days.

In summary, we have demonstrated that centrifugation process is effective in improving the interaction of enzyme, redox mediator and the graphene-chitosan matrix in the bioanode from the increased in catalytic activity for glucose oxidation. The power generation of assembled GBFC with Pt wire as cathode also showed the similar results. The fabricated bioanode produced a power output comparable with literature and is also able to retain ~60 % of its maximum activity after 7 days. The proposed method indicates a possible approach to fabricate electrodes for diverse enzyme-mediator conjugate in EBCs.

Experimental Section

Enzyme and chemicals

Glucose Oxidase (GOx) from *Aspergillus niger* (108 units mg⁻¹) was purchased from AMRESCO. Acetic acid (100 %) was purchased from Merck. Graphene Nanopowder (11 – 15 nm) was purchased from SkySpring Nanomaterials, Inc. Hydroxymethylferrocene (99 %) was purchased from Strem Chemicals, Inc. Chitosan (medium molecular weight), D-(+)-Glucose, sodium phosphate monobasic (≥99 %) and sodium phosphate dibasic heptahydrate (98 – 102 %) were purchased from Sigma-Aldrich. Supporting electrolyte of 0.1 mol L⁻¹ phosphate buffer solution (PBS) with pH 6.5 was obtained from sodium phosphate monobasic and sodium phosphate dibasic heptahydrate based on the information from Sigma-Aldrich. All chemicals are analytical grade and used as received without further purification.

Material synthesis

Preparation of chitosan solution: Viscous chitosan (CS) solution is prepared by dissolving 0.5 wt% of chitosan in 0.2 M acetic acid at 50 °C under constant stirring for 30 minutes. Then, the dissolved CS solution was left under constant stirring at ambient temperature for 2 hours^[6b].

Graphene nanopowders were added to the CS solution at 10 g L^{-1} concentration under stirring for 15 minutes. The Graphene-CS matrix solution was placed in sonication bath for 30 minutes and subsequently, probe sonication (power $\sim 60 \text{ W}$) for another 30 minutes before use.

Synthesis of centrifuges bioanodes: A mixture solution containing 3 mL of Graphene-CS matrix solution, 2 mL of GOx from *Aspergillus niger* solution (10 g L^{-1} prepared in pH 6.5 PBS) and 0.5 mL of Hydroxymethylferrocene (Fc-MeOH, 0.05 mol L^{-1} prepared in ethanol/deionized water in the ratio 1: 1) was placed in fridge at $4 \text{ }^\circ\text{C}$ for 1 hour. The mixture solution was then subjected to centrifugation at $15 \text{ }^\circ\text{C}$ in the sequence of (i) 1500 RPM for 20 minutes, (ii) 5000 RPM for 20 minutes, (iii) investigated speed (5000 or 13500 RPM) for 1 hour and (iv) investigated speed (same as step iii) for 1 hour after removing the remaining solvent. The bioanode was constructed by collecting the centrifuged sample and mold it to a spherical structure. A Pt wire was pierced through the center to act as the electrical connection for the bioanode before leaving it to dry at $4 \text{ }^\circ\text{C}$ in air for 2 days before testing. The spherical bioanodes were measured to have diameter between 0.4 – 0.45 cm. All centrifuge of prepared mixture were conducted by Sorvall Legend X1R from Thermo Fisher Scientific.

Synthesis of casted bioanodes: For the casted samples, glassy carbon electrodes (GCEs, $d = 3 \text{ mm}$, from Bioanalytical Systems, Inc) were polished with Alumina slurry ($0.05 \text{ }\mu\text{m}$, Bioanalytical Systems, Inc.), rinsed and sonicated in ethanol, subsequently in deionized (DI) water (Millie-Q Gradient A10) for 5 minutes respectively. The clean GCEs were casted with $40 \text{ }\mu\text{L}$ of mixture solution (Graphene-CS, GOx and Fc-MeOH) on the glassy carbon surface and allowed to dry at ambient temperature before storing in $4 \text{ }^\circ\text{C}$.

Electrochemical characterization

All electrochemical measurements were obtained using Princeton Applied Research VersaSTAT3-200 potentiostat with Ag/AgCl (KCl-saturated) as reference electrode and Pt wire as counter electrode. Potentials were converted to saturated calomel electrode (SCE) for comparison with other literatures. All experiments were conducted at ambient temperature in 0.1 mol L^{-1} phosphate buffer solution (PBS) with pH 6.5 as the support electrolyte. Linear scan voltammetry (LSV; for polarization curve) and chronoamperometry (CA) measurements were conducted in nitrogen-saturated PBS (pH 6.5) in absence and presence of glucose. The glucose concentration for LSV and CA conducted in presence of glucose is 0.05 mol L^{-1} . The scan rate for LSV measurements was 2 mV s^{-1} . CA measurements for different glucose concentration were conducted in air-saturated PBS (pH 6.5) until either the current stabilized or for 45 minutes (whichever condition was reached). Power generation tests were conducted in air-saturated PBS (pH 6.5) in the presence of glucose at 0.01 mol L^{-1} . The glucose biofuel cell was assembled by connecting the respective bioanodes with Pt wire as cathode without separating membrane.

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Keywords: biofuel cell • anode • glucose oxidase • ferrocenemethanol • graphene • centrifuge compression

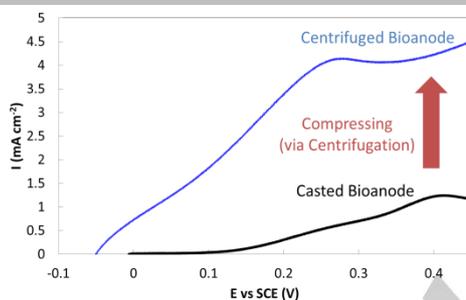
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Table of Contents

COMMUNICATION

Graphene-based bioanode thru centrifugation was synthesized as catalyst for glucose oxidation. The centrifugal force increased the interaction of the enzymes, redox mediator and supporting substrate, enhancing catalytic activity to a greater extent than mechanical compression.



Desmond C. L. Tan and Hirotaka Sato**

Page No. – Page No.

Enhancing catalytic activity of bioanode for glucose biofuel cell by compressing enzyme, mediator and carbon support thru centrifugation