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Biofuel Cells Composed by Using Glucose Oxidase on Chitosan Coated Carbon Fiber Cloth

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This study presents a high-performance biofuel cell construction based on the covalent immobilizing of glucose oxidase (GOx) on chitosan-coated carbon cloth which is used as an anodic catalyst. The chitosan was coated by the coagulation of an aqueous chitosan solution on the carbon cloth surface. The GOx was coupled to chitosan using N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) and N-hydroxysuccinimide (NHS) as the condensing agents. Response surface methodology (RSM) and Box-Behnken design were employed to search for the optimal conditions, and to understand the significance of the factors affecting the activity of immobilized GOx. From ridge max analysis, the optimal immobilization condition was found at a reaction time of 50 min, a pH of 5.9, and an enzyme/support ratio of 3 (w/w). Under optimal conditions, the predicted and the experimental immobilized GOx activities were 34.42±1.07 and 33.50±0.92 U g^{-1} support, respectively. Based on the regression model, the carbon cloth electrodes with various GOx activities were prepared, and the effect of GOx activity on the power density generated from the biofuel cell was investigated. The power density was increased with GOx activity, and the maximum power of 1.87 mW cm^{-2} was obtained at a cell voltage of 0.44 V.

Keywords: Enzymatic biofuel cell, glucose oxidase, immobilization, response surface methodology (RSM), chitosan-coated carbon cloth

1. INTRODUCTION

Biofuel cells provide an environmentally-friendly alternative energy source for producing electricity from renewable fuel sources. Biofuel cells are bio-electrochemical devices capable of transforming chemical energy into electrical energy via electrochemical reactions involving biochemical pathways [1, 2]. The unique characteristic of enzymatic biofuel cells is that enzymes are employed, rather than conventional noble metal catalysts; redox enzymes such as glucose dehydrogenase [3, 4], glucose oxidase (GOx) [5, 6], lacase [7], fructose dehydrogenase [8] and alcohol dehydrogenase [9] have been used in the design of biofuel cells. However, there are several bottlenecks yet to be overcome; these include high enzyme costs, poor power density and short lifetime, which are related to enzyme loading, activity and stability, respectively.

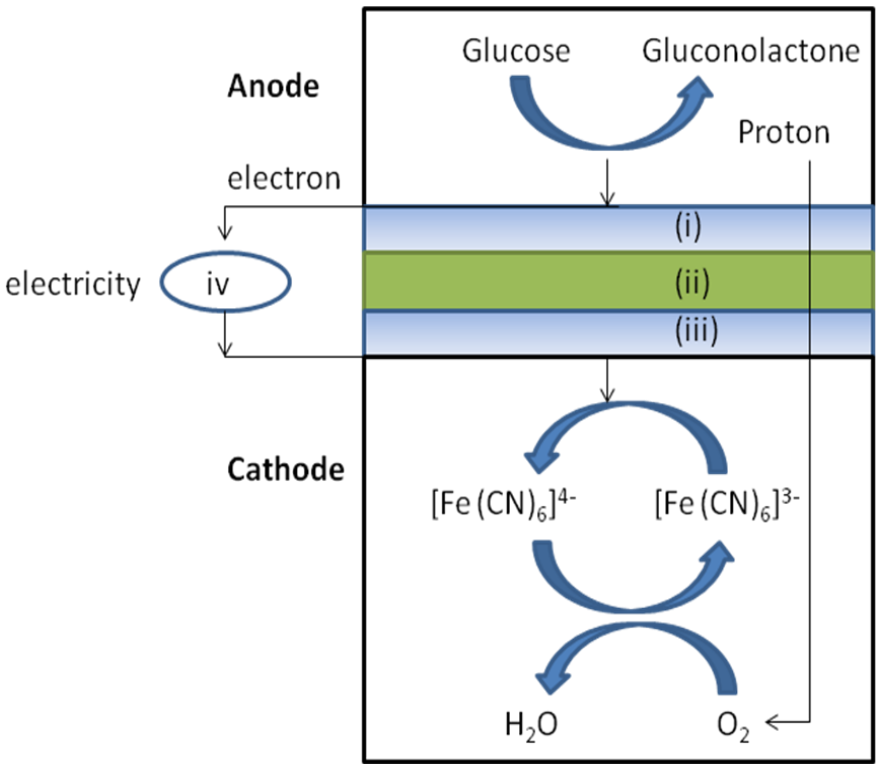


Figure 1. Reaction scheme of the biofuel cell. (i) GOx immobilized carbon-cloth electrode, (ii) Nafion membrane, (iii) carbon-cloth electrode.

Compared with other redox enzymes, the GOx is inexpensive and provides a low-cost advantage when applied as a biocatalyst in biofuel cells. GOx is a redox enzyme capable of oxidizing glucose using oxygen; it has been widely used for the blood glucose assay [10]. The GOx oxidizes glucose and can generate electricity via electron transfer; such device used in this study is depicted in Fig. 1. At the anode, the GOx reacts with glucose to produce protons and electrons. At the cathode, incoming electrons (via the external circuit) reduce ferricyanide to ferrocyanide. Simultaneously, the protons migrate through the proton exchange membrane into the cathode compartment. Protons are consumed in the cathode compartment, reducing oxygen to water and reoxidizing ferrocyanide to

ferricyanide. Previous investigators have immobilized GOx on various electrode materials for biosensor or biofuel cell applications. These materials include: carbon nanotubes [11], glassy carbon [12] and carbon paste electrode [13]. Compared with native enzymes, immobilization increases the enzyme stability to prolong the lifetime of biofuel cells, or to resist enzyme denaturing. However, the substrate access to enzymes is limited after immobilization because the enzyme on the electrode is immovable [14, 15]. With a lower GOx activity, the generation of electrons from glucose oxidation will slow down, resulting in poor power density. Enzyme immobilization can be achieved via adsorption, entrapment, or covalent binding. Most of the immobilized enzymes used in the biofuel cells reported so far have been constructed using adsorption or entrapment, because the electrode materials require functional groups for covalent binding; therefore, the modification of carbon electrode materials is inevitable.

Chitosan (2-amino-2-deoxy-(1→4)-β-d-glucan) is a polysaccharide carrying amino group useful for conjugation with proteins via cross-linking agents such as N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), or glutaldehyde. GOx immobilization on chitosan-coated electrode materials has so far been used as a biosensor; it shows a fast heterogeneous electron transfer rate between the redox center of the GOx and the electrode [16, 17]. However, few attempts have been made to apply the chitosan coated electrodes in the biofuel cell [18]. The immobilized GOx activity on the chitosan coated electrodes will likely require improvement before it can be effectively used in a biofuel cell.

To solve this problem, response surface methodology was employed to enhance the immobilized GOx activity for realizing the purposes of this study. Response surface methodology (RSM) is a collection of mathematical and statistical techniques for designing experiments, building models, evaluating the relative significance of several independent variables and determining the optimum conditions for desirable responses. It is used for evaluating the effects of various parameters and their interactions in a process, while requiring a small number of experiments. Compared with a one-factor-at-a-time design, which is adopted most frequently in the literature, the experimental design and RSM are more efficient in reducing both the number of experimental runs and the amount of time required to investigate the optimal conditions [19]. In this study, the covalent immobilization of GOx onto chitosan-coated carbon cloth was investigated, and the immobilization parameters affecting the immobilization were evaluated using RSM and a Box-Behnken design. Optimum immobilization conditions were deduced by ridge analysis and then verified. Furthermore, the electricity produced as a result of various GOx activities of anode electrodes was evaluated in the enzymatic biofuel cell.

2. EXPERIMENTAL

2.1. Materials

GOx from *Aspergillus niger* (Gluzyme Mono® 10000 BG) was purchased from Novo Nordisk Bioindustrials Inc. (Copenhagen, Denmark). Horseradish peroxidase (250 Pyrogallol U mg^{-1}) was purchased from TOYOBO Co., LTD. (Osaka, Japan). Carbon cloth was purchased from CeTech Co. (Taichung, Taiwan). N-hydroxysuccinimide (NHS), N-(3-dimethylaminopropyl)-N'-

ethylcarbodiimide hydrochloride (EDC), sodium tripolyphosphate (TPP) and *o*-dianisidine dihydrochloride were purchased from Sigma–Aldrich (St. Louis, MO). Chitosan powder (MW 140000 dal/mol) with 90% deacetylation that had been sieved by a 100-mesh filter was obtained from Shin ERA Tech. Co. Ltd. (Taipei, Taiwan). Nafion® N117 membranes (DuPont) were pre-treated at 80°C with 5 wt% H_2O_2 and 2 M H_2SO_4 solutions for 1 h, then rinsed and stored in D.I. water. Unless otherwise noted, all reagents and chemicals were of analytic grade.

2.2. Preparation of Chitosan-Coated Carbon Cloth

The carbon cloth was cut into small pieces (1 X 1cm^2), with each piece weighing 13.0 mg. The chitosan solution was prepared by dissolving 0.5 g of chitosan in 50 ml of 1% (v/v) acetic acid, followed by the addition of 12.5 ml of 1 mg ml^{-1} TPP solution as a crosslinker to enhance colloidal stability [20]. It was then stirred at room temperature for 10 min. The small piece of carbon cloth was immersed into a 1% chitosan solution in a sonication bath for 5 min, and then transferred into 1 N NaOH solution to precipitate the coated chitosan. Following this, the chitosan-coated carbon cloth was put into D.I. water for 3 h. The wash procedure was repeated 3 times to remove the residue from the NaOH solution.

2.3. GOx Immobilization

Two mg of EDC (2.6 mM) were added to 4 ml of 50 mM phosphate buffer solution containing 13-39 mg of GOx (pH and amount of GOx were determined from experimental design), and the solution was incubated at 25°C for 1 h with shaking (150 rpm). Next, 2.4 mg of NHS (5.2 mM) were added to the solution and the incubation continued for a further 1 h. After that, the chitosan-coated carbon cloth was put into activated GOx solution and shaken at 150 rpm with different immobilization times.

2.4. Experimental Design and Data Analysis

In order to evaluate the effects of the immobilization time (x_1), immobilization pH (x_2) and the enzyme/support ratio (x_3) on GOx immobilization, a 3-factor and 3-level Box-Behnken design and RSM were applied to investigate the optimum levels of these variables and their relationships. The variables and the levels of each selected for the study were as follows: an immobilization time of 0.5–1.5 h, an immobilization pH of 6–8 and an enzyme/support ratio of 1–3, w/w. Table 1 shows the levels of the independent factors and experimental designs as coded (0, 1, and −1) and uncoded (actual value). A total of 15 experimental runs, including combinations of different levels of the three factors, were carried out in duplicate. The experimental data (Table 1) were analyzed by response surface regression (RSREG) with SAS software (SAS Institute, Cary, NC, USA) to fit the following second-order polynomial equation:

$$Y = \beta_{ko} + \sum_{i=1}^{3} \beta_{ki} X_i + \sum_{i=1}^{3} \beta_{kii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{kij} X_i X_j \quad (1)$$

where Y is the response (activity of GOx); β_{k0}, β_{ki}, β_{kii} and βk_{ij} are constant coefficients; and Xi and Xj are uncoded independent variables. The option of ridge max was employed to compute the estimated ridge of maximum response, in order to increase the radius from the centre of the original design.

Table 1. Box-Behnken design and observed experimental data for 3-level–3-factor response surface analysis

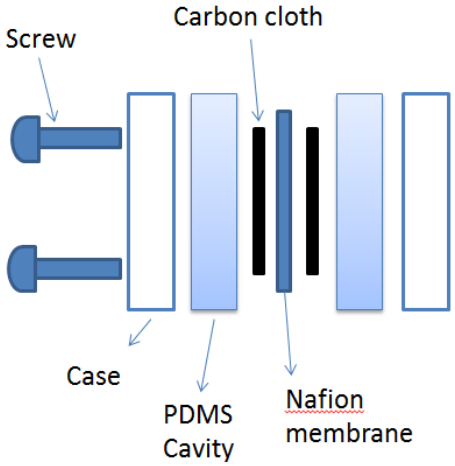
Treatment No. [a]	Factor			Experimental Values Activity (U g^{-1} support)
	Time (min), x_1	pH, x_2	Enzyme/support ratio (w/w), x_3	
1	[b]0(60)	-1(6)	-1(1)	20.44±0.63
2	-1(30)	0(7)	-1(1)	11.76±0.51
3	1(90)	0(7)	-1(1)	16.52±1.06
4	0(60)	1(8)	-1(1)	3.36±0.33
5	-1(30)	-1(6)	0(2)	27.18±0.49
6	1(90)	-1(6)	0(2)	28.80±0.63
7	0(60)	0(7)	0(2)	20.82±1.40
8	0(60)	0(7)	0(2)	19.60±1.18
9	0(60)	0(7)	0(2)	19.60±0.63
10	-1(30)	1(8)	0(2)	5.48±0.11
11	1(90)	1(8)	0(2)	8.45±1.15
12	0(60)	-1(6)	1(3)	32.81±0.18
13	-1(30)	0(7)	1(3)	26.55±0.92
14	1(90)	0(7)	1(3)	23.58±0.54
15	0(60)	1(8)	1(3)	6.95±0.40

[a]The run order of treatments were sorted from right to left.
[b]The values -1, 0, and 1 are coded levels.

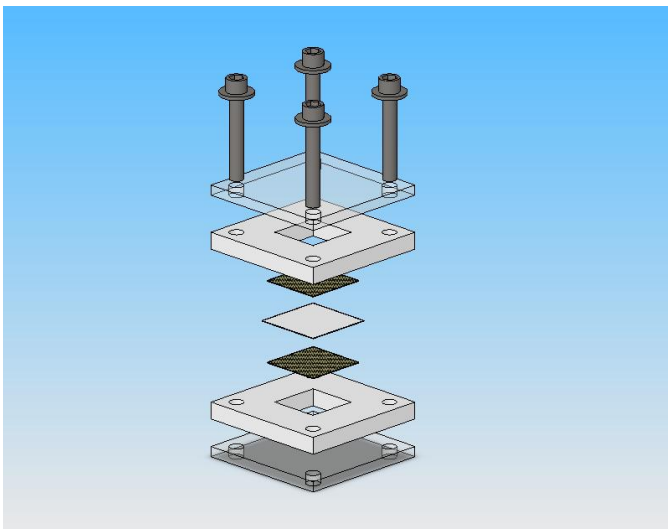
2.5. GOx Activity Assay

The procedure for the analysis of GOx activity is based on the method of Bergmeyer [21]. GOx catalyzes the oxidation of glucose to gluconolactone and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide reacts with a reduced dye (*o*-dianisidine) to produce an oxidized dye which has absorption maxima at 436 nm. The 3 ml of enzyme assay reagent containing 2.4 ml of 0.208 mM *o*-dianisidine solution, the 0.5 ml of 10% glucose solution and the 0.1 ml of peroxidase solution (60 Pyrogallol U ml^{-1}), were all prepared using a 0.1 M, pH 7 phosphate buffer. The immobilized GOx prepared from Table 1 was reacted with 3 ml of enzyme assay reagent at room temperature for 1 min, and the change in absorbance at 436 nm was measured with a UV/VIS spectrophotometer (Metertek SP-830, Metertech Inc., Taiwan). The molar extinction coefficient (ε) for *o*-dianisidine is 8.3 $\mu mol^{-1}cm^2$. One unit (U) of enzyme activity is defined as the amount of GOx causing the oxidation of one micromole of glucose per minute under the assay conditions.

2.6. Enzymatic Biofuel Cell Assembly



(A)



(B)

Figure 2. Schematic diagrams of the enzymatic biofuel cell; (A) cross-sectional view, (B) assembly diagram.

The design of an enzymatic biofuel cell is illustrated in Fig. 2 A. A sandwich structure is composed of a Nafion membrane, two carbon cloth electrodes, two polydimethylsiloxane (PDMS) cavities and two acrylic cases. Four screws were used to fix the enzymatic biofuel cell. The PDMS cavity was designed with the desired pattern using CAD software, followed by machining to create mold inserts by the CNC machine. The cavity was replicated from the mold insert and formed in PDMS material. This design pattern features a 1.5 x 1.5 cm^2 cavity with a depth of 0.5 cm for both anode and cathode. The total volume of the cavity is 1.125 cm^3. Since the PDMS is flexible enough so that it can be used as a cushion, it makes for a better fit in the assembly. The cathode electrode material is the carbon cloth, and the anode electrode is comprised of the chitosan-coated carbon cloth with the GOx immobilized. The proton exchange membrane (Nafion membrane) and two electrodes

as well as the PDMS cavities are compressed by the outer acrylic cases. Then, the cell is fixed using four screws, as shown in Fig. 2 B. The anode solution consists of 0.1 M glucose, 0.1M phosphate buffer solution (pH 7) and 0.1 M NaCl. The cathode solution consists of 0.1M potassium hexacyanoferrate ($K_3Fe[CN_6]$) and 0.1M phosphate buffer solution (pH 7). Both solutions are injected into the PDMS cavities.

2.7. GOx Anode Electrode Preparation

In order to study the electrochemical behavior of the enzymatic electrode in the biofuel cell, the carbon cloth was magnified to a piece 3.5 x 3.5 cm^2 in size, weighing approximately 146.0 mg. The chitosan coating procedure was described as Section 2.2. Based on the weight of the carbon cloth, the EDC, NHS and GOx amount and reaction volume were increased 11 fold; the GOx immobilization procedure was described in Section 2.3.

2.8. Enzymatic Fuel Cell Measurement

The electrical characteristic measurements involved in the discharge performance of the biofuel cell included an open-circuit voltage stability and polarization curve. The open-circuit voltage stability measurement was done to determine the relationship between the output voltage and the elapsed time. Cell activation and output voltage stability required 3 min. The load test was measured by a micro-fuel cell test station (EL505R3, Beam Associate Co. Ltd., Taiwan) to obtain the cell polarization curve, by using the Tafel method [22]. The loading voltage was set from high to low and low to high, repeatedly. Then, the cell voltage was gradually decreased as the applied load was increased. By calculating the current and voltage, the polarization curve was obtained; the highest point of the curve indicated the high-power density of the fuel cell.

3. RESULTS AND DISCUSSION

3.1. Chemistry of GOx Immobilization on Chitosan-Coated Carbon Cloth

Since chitosan is insoluble in an alkaline solution, the chitosan-coated carbon cloth was generated by adjusting the pH for precipitating chitosan onto the surface of the carbon cloth. EDC is an activation reagent for carboxyl groups, and is frequently used in peptide synthesis, enzyme immobilization or cross-linking proteins to nucleic acids [23, 24]. In the presence of NHS, EDC can be used to couple carboxyl groups to primary amines. The hypothesis of GOx immobilized to chitosan-coated carbon cloth assumes that the carboxyl groups of GOx were activated using EDC and NHS to form an activated GOx-NHS ester, which is susceptible to attack by amines. The amine groups from chitosan on the surface of carbon cloth could react with this ester and form an amide bond.

3.2. Statistical Analysis of GOx Immobilization

To improve the electric properties of the enzymatic biofuel cell, an increase in the biocatalyst activity on the electrode is necessary. Three parameters: immobilization time, pH and the enzyme/support ratio were selected to investigate their influence on the immobilization, and then subsequent analysis was performed using RSM. The Box-Behnken design was used to study the effect of the three variables on the immobilized GOx activity. A statistical approach was used to understand the relationship between the immobilization parameters. The efficiency of immobilized GOx prepared by the 15 experiments of immobilization protocols is shown in Table 1. Among the various treatments, the greatest immobilized GOx activity (32.81 U g^{-1} support) was treatment #12 (1 h, pH 6 and enzyme/support ratio 3) and the smallest immobilized GOx activity (only 3.36 U g^{-1} support) was #4 (1 h, pH 8 and enzyme/support ratio 1). From the SAS software (SAS Institute, Cary, NC, USA) output of RSREG, the second-order polynomial Eq. (2) is given below:

$$Y = -105.026646 - 0.001950x_1 + 36.797382x_2 + 27.958758x_3 + 0.000656x_1x_1 + 0.011237x_2x_1 - 3.121525x_2x_2 - 0.064411x_3x_1 - 2.196478x_3x_2 - 0.998031x_3x_3 \quad (2)$$

where Y is the immobilized GOx activity (U g^{-1} support), x_1 is the immobilization time, x_2 is the immobilization pH and x_3 is the enzyme/support ratio. By ANOVA analysis, this quadratic polynomial model was found to be highly significant, and to sufficiently represent the actual relationship between the response and the three parameters with a very low p-value (<0.001) and a satisfactory coefficient of determination (R^2 = 0.99). Therefore, this model was able to adequately predict the immobilization results within the range of the variables employed.

Table 2. ANOVA for joint test of all independent variables

Factor	Degrees of freedom	Sum of Squares	Prob. > F [a]
Time (x_1)	4	21.76	0.0728
pH (x_2)	4	958.46	<0.0001*
E/S (x_3)	4	216.64	0.0005*

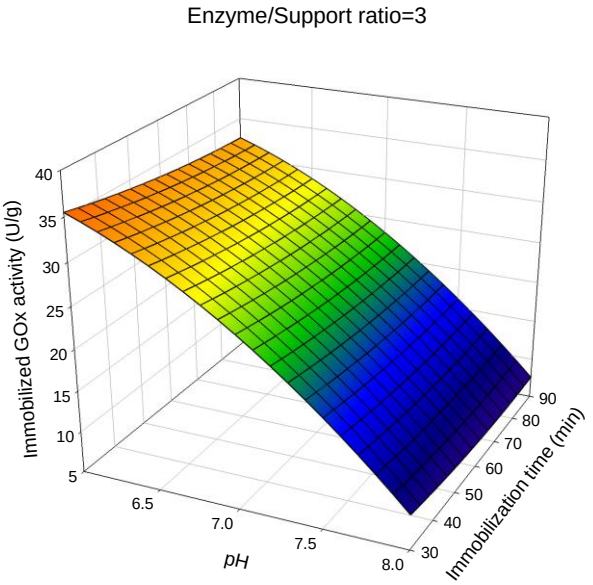
[a] (Prob. > F) = level of significance.
* Significant at *p*-Value less than 0.05.

The overall effect of the immobilization variables on the immobilized GOx activity was further analyzed by a joint test. As shown in Table 2, pH (x_2) and Enzyme/Support ratio (x_3) were the most important factors concerning the response ($P < 0.05$), whereas the immobilization time (x_1) did not have an apparent influence ($P > 0.05$).

3.3. Relationships between Immobilization Factors and Response

Using surface response plots of the quadric polynomial model, the relationships between the immobilization factors and the response (immobilized GOx activity) can be better understood by holding one variable constant and studying the function between the other two variables. Fig. 3(A)

shows the effects of pH, immobilization time and their mutual interaction on the immobilization at an enzyme/support ratio of 3; Fig. 3(B) shows the effects of the enzyme/support ratio, immobilization time and their mutual interaction on the immobilization at pH 6.



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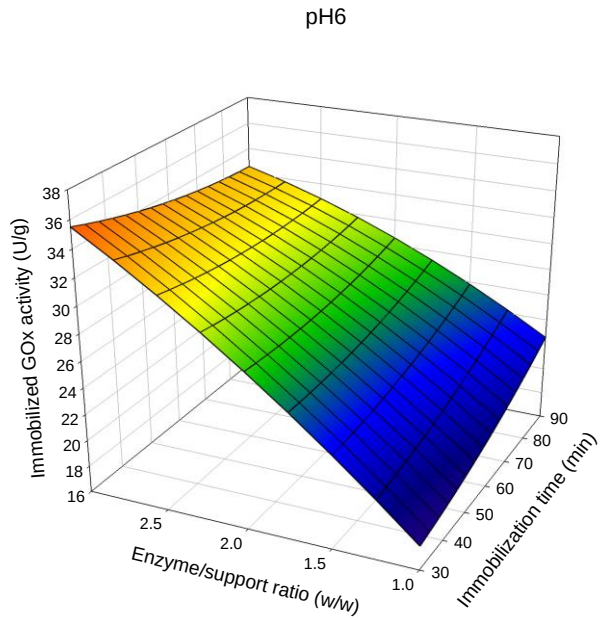

(B)

Figure 3. Response surface plots show (A) the effect of pH, immobilization time, and their mutual interaction on immobilized GOx; (B) the effect of E/S ratio and immobilization time and their mutual interaction on the immobilized GOx.

With a fixed immobilization time of 0.5 h, a decrease in pH (from 8 to 6) and an increase in the enzyme/support ratio (from 1 to 3) led to an increase in GOx activity (from 10.0 to 35.0 U g^{-1} support

and from 18 to 35.5 U g^{-1} support, respectively). The immobilization pH and enzyme/support ratio appeared to have been important factors in the immobilization; however, the immobilization time appeared to have had little effect on the immobilization. The immobilized GOx activity's linear increase with enzyme/support ratio indicated that the enzyme loading had not yet reached saturation at enzyme/support ratio of 3. The pH plays two important roles in the immobilization procedure. First, the EDC and NHS are activated in a weak acid solution. Most references describe the optimal reaction medium for EDC and NHS being at a pH between 4.7 and 6 [25]. Second, NHS-ester is very unstable and hydrolyzes within hours or minutes, depending on the pH of the reaction solution: a half-time of NHS-ester hydrolysis at pH 7.5 is 14 min and pH 9 is 1 min [26]. Therefore, a decrease in pH which led to an increase in immobilization efficiency was found. The shorter half-time of NHS-ester result in the immobilization time showed less influence on the GOx immobilization.

3.4. Attaining Optimum Immobilization Conditions

The optimum immobilization conditions were determined by ridge max analysis, which computed the estimated ridge of maximum response for an increasing radius from the center of the original design. The immobilized GOx activity (response; Y) at distances of 0, 1.5, 2.0 and 2.5 is calculated according to the immobilization model [Eq. (2)] shown in Table 3.

Table 3. Estimated ridge of maximum response Y (Immobilized GOx activity; U/g support)

Coded Radius	Time x_1	pH x_2	E/S ratio x_3	Estimated Response Y	Observed Response Y
0.0	60.00	7.00	2.00	20.01±0.65	20.00±0.57
1.5	50.27	5.92	2.99	34.42±1.07	33.50±0.92
2.0	39.43	5.71	3.37	38.82±1.84	35.32±0.53
2.5	26.89	5.55	3.71	43.49±3.70	35.79±0.02

The actual experimental value of the GOx activity increased as the radius increased, and reached its maximum at a radius distance of 2.0. When the radius distance was greater than 2.0, GOx activity (observed) gradually leveled off as the radius distance increased. When the radius distance was over 2.0, the experimental pH was 5.71 and 5.55, while the E/S ratio was 3.37 and 3.71, respectively corresponding to radius distances of 2.0 and 2.5. Because the EDC and NHS were activated in a weak acid solution, decreasing the pH and increasing the E/S ratio would increase the amount of enzyme immobilized on the chitosan-coated carbon cloth. However, the over- loading of GOx to the support might cause steric hindrance and protein aggregation, thereby decreasing the immobilized GOx activity. Based on the ridge max analysis, the optimal immobilization conditions are obtained at an immobilization time of 50 min, an immobilization pH of 5.9, and an enzyme/support ratio of 2.99, with GOx activity of 33.50±0.92 U g^{-1} support.

3.5. Enzymatic Biofuel Cell Performance

According to the experimental results and RSM regression model, three different activities of the GOx anode electrode were prepared under the selected immobilization conditions, as Table 4 shows. The effect of GOx activity in the power production was observed from the polarization and power density curve.

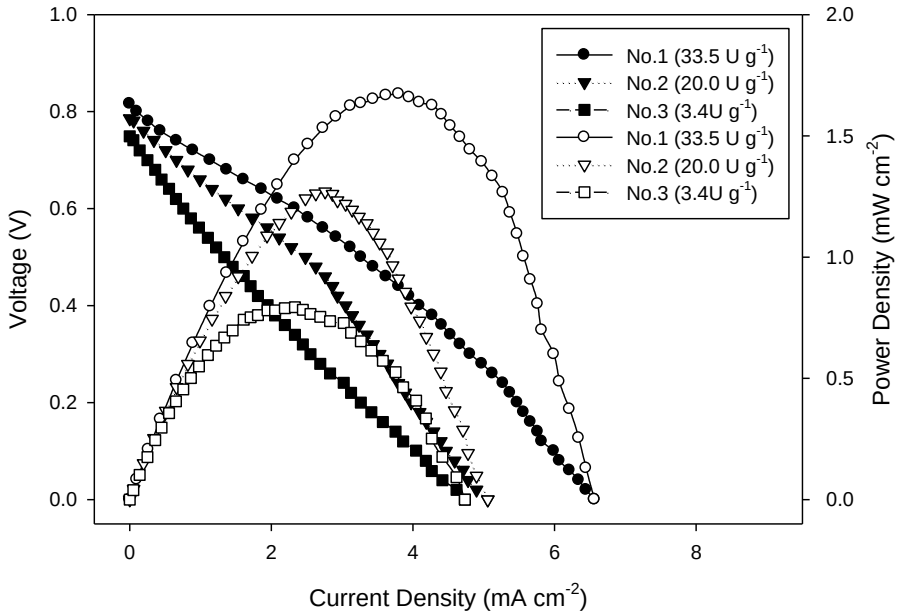

Figure 4. Polarization (fill symbol) and power density (empty symbol) curves of different activity of GOx anode electrodes in the enzymatic biofuel cell at 0.1 M glucose concentration. The GOx immobilization conditions of No.1, NO.2 and NO.3 electrode are listed in table 4.

The results are shown as Fig. 4; the open-cell potentials of the cell are between 0.75 to 0.82 V, which is similar to the glucose biofuel cell reported in the literature [27]. The cell potential of No. 1 electrode (GOx activity 33.5 U/g) decreases more slowly than that of the No. 3 electrode (GOx activity 3.4 U/g) as the applied load increases.

Table 4. Experimental conditions used for prepare GOx anode electrode for the enzyme fuel cell test.

Treatment No.	Time (min)	pH	Enzyme/support	Experimental Activity (U g^{-1})
1	50	5.9	3	33.5
2	60	7.0	2	20.0
3	60	8.0	1	3.4

The results indicate that increasing the GOx activity on the electrode would enhance the cell voltage stability and current density. Fig. 4 also plots the power density curves. The highest GOx

activity of the anode electrode (No.1) prepared under optimal conditions displayed a maximum power density of 1.672 mW cm^{-2}. As immobilized GOx activity decreased to 20.0 U/g (electrode No.2), the maximum power density decreased to 1.27 mW cm^{-2}. However, the lowest GOx activity of the anode electrode (No.3) showed the lowest maximum power density of 0.793 mW cm^{-2}. In the absence of GOx, the maximum power density yielded from chitosan-coated carbon cloth (control) was only 0.02 mW cm^{-2}. The results demonstrate that the performance of the enzymatic biofuel cell is directly proportional to immobilized GOx activity because the glucose oxidation reaction can then proceed effectively and more efficiently.

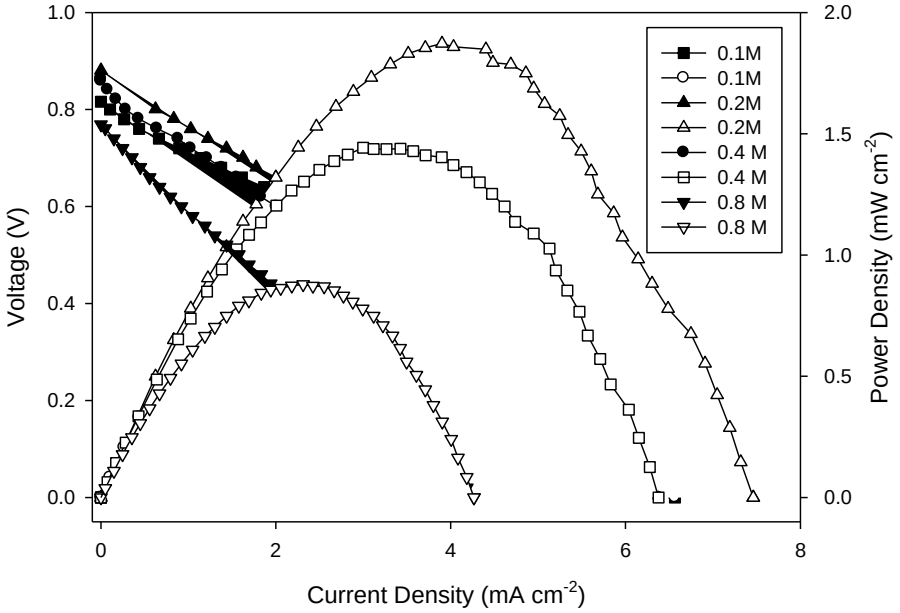

Figure 5. Effect of glucose concentration on the polarization (fill symbol) and power density (empty symbol) performance in the enzymatic biofuel cell. The GOx activity on anode electrodes was 33.5 U/g.

Table 5. Comparison of biofuel cell performance

Fuel/Oxidant	Enzymes Anode/Cathode	Mediators Anode/Cathode	Power density mW cm^{-2}	Ref.
Glucose/O_2	GOx/-	-/-	0.12	[2]
Glucose/K_3 [$Fe(CN)_6$]	GDH/BOD	VK_3/-	1.45	[4]
Glucose/O_2	GOx/-	Benzoquinone /-	0.25	[5]
Glucose/O_2	GOx/Tyrosinase	-/-	0.16	[28]
Glucose/O_2	GOx/-	Benzoquinone/-	0.90	[29]
Glucose H_2O_2/O_2	GOx Catalase/Laccase	-/-	1.25	[30]
Glucose/K_3 [$Fe(CN)_6$]	GOx/-	-/-	1.87	This study

The effect of glucose concentrations on the biofuel cell performance is shown in Fig. 5. As glucose concentration decreases, the maximum power density is shifted from 0.88 mW cm^{-2} at 0.8 M

glucose to 1.672 mW cm^{-2} at 0.1 M glucose. The lower cell performance at 0.8 M glucose concentration may be due to the high glucose concentration decreasing electron conductivity or inhibiting enzyme activity. The maximum power density at 0.2 M glucose was determined to be 1.87 mW cm^{-2}. This power density is comparable to that previously reported, as shown in Table 5, and is better or comparable to the power density of previously designed biofuel cells.

4. CONCLUSIONS

A chitosan-coated carbon cloth electrode was successfully prepared and used for the covalent immobilization of GOx on its surface for enzymatic biofuel cell application. GOx was covalently bound to the chitosan-coated electrode via EDC and NHS activation at room temperature. The effects of immobilization time, pH, and the enzyme/support ratio on the efficacy of immobilization were investigated by a 3-level-3-factor Box-Behnken design and response surface methodology. The immobilized pH and enzyme/support ratio significantly affected the immobilization efficacy, but immobilization time ranging from 0.5 to 1.5 h, did not. A second-order model depicting the relationship between the response (immobilized GOx activity) and the three immobilization parameters was established. Based upon the model, the optimum conditions for immobilized GOx was determined to be 33.5 U g^{-1}. The performance of enzymatic biofuel cells can be improved by increasing GOx activity on the electrode. Using the optimum conditions for preparation of an anode electrode, a maximum power density of 1.87 mW cm^{-2} was obtained at 0.2 M glucose. It was also noticed that the immobilized GOx based on the chitosan-coated electrode is easily prepared. This immobilized GOx electrode also demonstrated its potential as an excellent anodic material for the construction of biofuel cells. Taking the above results into consideration, this enzyme immobilization technique could be key to the production of high-powered, stable and cost-effective commercial enzymatic biofuel cells in the future.

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