Enzymatic activity of Glucose Oxidase from *Aspergillus niger* IPBCC.08.610 On Modified Carbon Paste Electrode as Glucose Biosensor

T Rohmayanti¹, L Ambarsari¹* and A Maddu²

¹Department of Biochemistry, Faculty of Science, Bogor Agricultural University, Indonesia
²Department of Physics, Faculty of Science, Bogor Agricultural University, Indonesia

Email: ami_icha@yahoo.com

Abstract. Glucose oxidase (GOx) has been developed as glucose sensor for measuring blood glucose level because of its specificity to glucose oxidation. This research aimed to determine kinetic parameters of GOx activity voltametrically and further test its potential as a glucose biosensor. GOx, in this research, was produced by local fungi *Aspergillus niger* IPBCC.08.610 which was isolated from local vine in Tarakan, East Borneo, Indonesia. GOx was immobilized with glutaraldehyde, which cross-linked onto modified carbon paste electrode (MCPE) nanofiber polyaniline. Intracellular GOx activity was higher than extracellular ones. Immobilized GOx used glutaraldehyde 2.5% and dripped on the surface of MCPE nanofiber polyaniline. MCPE have a high conductance in copper with the diameter of 3 mm. The concentration of glucose in the lowest concentration of 0.2 mM generated a current value of 0.413 mA while 2 mM of glucose induced a current of 3.869 mA value. Km and Imax of GOx in MCPE activities polyaniline nanofiber were 2.88 mM and 3.869 mA, respectively, with turnover (Kcat) of 13 s⁻¹. Sensitivity was 1.09 mA/mM and response time to produce a maximum peak current was 25 seconds. Km value was then converted into units of mg/dL and obtained 56.4 mg/dL. GOx immobilized MCPE electrode is potential to be able to detect blood glucose level in a normal condition and hypoglycemia conditions

Keywords: Glucose oxidase, Glucose Biosensor, Electrode Paste Carbon

1. Introduction

Indonesia is a tropical country, which consists of diverse microorganism. Microorganisms may be valuable and potential sources for various purposes, which can be revealed through comprehensive research. Production of various enzymes using microorganisms is one thing that can be developed. Ironically, to date Indonesia has not been able to produce enzymes industrially yet still fully dependent on imported products [1]. One of widely used enzymes is glucose oxidase (GOx). In this research, GOx was produced directly from local *Aspergillus niger* which was isolated from local vine *Dryobalanops* in Tarakan, East Borneo, Indonesia. Previous study showed that this enzyme has activity and yield of 27.77 U/mg and 72.86%, respectively.
GOx is an enzyme that catalyzes the oxidation of β-D-glucose into D-gluconolactone and hydrogen peroxide using molecular oxygen as an electron acceptor [2]. Nowadays, GOx is generally applied to biosensor and other commercial applications. In addition, the enzyme is also used in biomedicine for determining blood glucose levels. Determination of blood glucose levels using GOx through either colorimetric or amperometric assay is more effective than that non-enzymatic methods because GOx has a high substrate specificity towards β-D-glucose [3]. Thus, biosensor based on GOx could be used in analyzing glucose content of a blood. GOx can oxidize glucose in blood to gluconic acid and hydrogen peroxide by using oxygen molecule as the electron acceptor which will be taken as the signal, and it is then decoded in computer software [4].

Application of GOx onto electrode in biosensor system needs immobilization process [5]. GOx immobilization into polyaniline-nanofiber at carbon pasta electrode surface is presumably to rise electrode conductance in biosensor. Nanometer-sized polyaniline widens electrode surface so that its application may be more economically efficient. GOx immobilized into carbon graphite can be repeatedly used so that it is cheaper and more efficient.

This research has a novelty as a biosensor because of GOx produced from local fungi A. niger IPBCC.08.610. This research aimed to determine kinetic parameters of GOx activity voltametrically, to further test its potential as glucose biosensor in blood. It can be developed potential diversity of microorganisms to make high value product.

2. Materials and Methods

2.1. Materials

Local fungi A. niger IPBCC.08.610 purchased from the fungal collection of Biology Department of Bogor Agricultural University (BAU), was isolated from Tarakan East Borneo, polyaniline-nanofiber, glutaraldehyde, carbon graphite, ortho-dianisidine and hydrogen peroxida (H₂O₂). The other materials used such as Potato Dextrose Agar media, sucrose, peptone, (NH₄)₂HPO₄, MgSO₄·7H₂O, KH₂PO₄, CaCO₃, ammonium sulfate, glucose, Bradford reagent, Bovine Serum Albumin (BSA) standard, paraffin wax, acetic buffer 0.1 M, KCl, (K₃Fe(CN)₆).

2.1.1. Glucose oxidase production, isolation, and purification. This assay was done based on Rohmayanti 2016 with modifications. Production media of A. niger contained 0.4 g/L (NH₄)₂HPO₄; 0.2 g/L KH₂PO₄; 0.2 g/L MgSO₄·7H₂O; 40 g/L CaCO₃; 3.3 % sucrose; and 0.35 % glucose. Culture was then incubated at 37°C on 120 rpm for 40 hours. After being incubated, mycelium was filtered by using gauze. The supernatant contained the crude extract of extracellular glucose oxidase. Filtered mycelium was homogenized using quartz sand and sodium phosphate buffer 0.1 M pH 6.0, then centrifuged at 12,000 x g temperature of 4°C for 20 minutes. The supernatant obtained was crude extract of intracellular. The purification of both extracellular glucose oxidase and intracellular crude extract was done through precipitation with saturated 80% (NH₄)₂SO₄. Protein content was measured according to Bradford method (1976) and its activity based on Bergmeyer method (1988).

2.1.2. Measurement of Carbon Paste Electrode (CPE) and Modified Carbon Pasta Electrode (MCPE) Cyclic Voltammogram. This experiment was conducted based on Colak et al. [6] with modifications. CPE was prepared by mixing 100 µL paraffin and 0.15 gram graphite powder into mortar until pasta shaped. Furthermore, the carbon paste electrodes was inserted into the prepared tube of glass capillary size variation copper diameter of 1 mm and 3 mm. The best diameter copper size of EPK was then modified by the addition of polyaniline nanofiber by 2 mg polyaniline. Whereas, MCPE was made of 2 mg polyaniline which mixed into mortar with 100 µL paraffin and 0.15 g graphite powder. Furthermore, each graphite pasta was added into electrode tube which prepared from capillary glass with its diameter and height were respectively 3 mm and 10 mm. CPE and MCPE (as working electrode alternately), Ag/AgCl electrode (reference electrode) and platina electrode (counter...
electrode) were placed into solution test contained 3 mL potassium chlorid (KCl) solution. These electrodes then linked to three distinct wires that had been connected to potentiostat.

2.1.3. Immobilization of Glucose Oxidase into MCPE. This assay was done based on Ambarsari 2016 with modifications. About 50 µL from each GOX extract was mixed with 1 mg BSA, 50 µL of 0.1 M acetic buffer (pH 4.5), and 30 µL of 2.5% glutaraldehyde. The mixture was placed into micro tubes and then shaked slowly. It was then dropped down (4 µL) to the upper surface of MCPE. Electrode utilized immobilized-GOx from local isolate was called GOx_{immobilized} | MCPE. Furthermore, the GOx_{immobilized} | MCPE was dried at 4°C. Then, it was washed several times with acetic buffer pH 4.5 to get rid of unimmobilized enzyme. Electrode was then kept in refrigerator at 4°C in 0.1 M acetic buffer (pH 4.5). The prototype electrode is showed at figure 1.

![Figure 1. MCPE Performance and GOD reaction](image_url)

2.1.4. Immobilized Glucose Oxidase Kinetics in MCPE. Cyclic Voltammogram of GOx_{immobilized} | MCPE was measured in 1 mL 0.1 M acetic buffer (pH 4.5) supplemented with 1 mL 0.1 M potassium ferisianida and 180 µL glucose standard solution with concentration of 0.2-8 mM. Cyclic Voltammogram analyses was then subjected for designing the Michaelis-Menten curve. Electrode kinetics parameter determination of GOx_{immobilized} | MCPE required linier line of Michaelis-Menten chart, then plotted into Lineweaver-Burk equation to obtain K_m and I_{max} value.

3. Result and Discussion

3.1. GOx Fraction of Intracellular and Extracellular Crude Extract and Saturated (NH_4)_2SO_4 80% Precipitate

GOx was produced by A. niger IPBCC.08.610 in intracellular and extracellular manner. Intracellular GOx activity was higher than extracellular, about 1.3084 U/mL. Intracellular and extracellular GOD activity of crude extract was 1.31 U/mL and 0.17 U/mL, respectively. Biomass produced by A.niger
be large enough round cells and white. Then GOx intracellular crude extract was purified by saturated (NH₄)₂SO₄ 80%.

Precipitation of intracellular GOx crude extract was the initial step to purify enzyme that significantly increased protein concentration, reduced the volume, and separate the protein target from partial undesired contaminant. Indeed, the purity level of precipitated GOx was 20 times higher than GOx crude extract, with specific activity and yield of 27.77 U/mg and 72.86%, respectively.

Based on the chart Lineweaver-Burk, the activity of the GOX is glucose concentration dependent. Enzymatic activity at low glucose concentration showed linear pattern in corresponds to the increased glucose concentrations. However, enzymatic activity at more than 0.06 M glucose was reduced V_maks and Km value of precipitated GOx was 20.747 U/mg and 56 mM, respectively. Moreover, the GOx k_cat value of ammonium sulfate 80% fraction was predicted about 53 s⁻¹.

3.2. Cyclic Voltammogram of CPE and MCPE

Cyclic Voltammogram may visualize the produced current by electrochemical cell as current value. Conductance change of this electrode was measured by using cyclic voltammetry method because it could evaluate electrochemical reaction on the surface of electrolyte solution and electrode [7]. Figure 2 shows that cyclic voltammogram of CPE and MCPE has higher current evolving than cyclic voltammogram of MCPE. The increasing current is conceived by the change of the width area of cyclic voltammogram. This is due to an improvement of polyaniline into nanofiber that had role to raise its conduction capacity. Moreover, the variation of the size would affect the mass and thickness of carbon graphite which added to electrode capillary, so that it causes distinct electron transfer that could be analyzed from its current and potential.

![Cyclic voltammogram diameter of CPE](image)

**Figure 2.** Cyclic voltammogram diameter of CPE ——— 1mm, ———— 3mm.

3.3. Immobilized Enzyme Kinetics Parameter in MCPE

Immobilized GOx was dripped onto the surface of modified carbon paste electrodes (MCPE) nanofiber polyaniline. MCPE have a high conductance in copper with the diameter of 3 mm. GOx which was immobilized on MCPE was called as GOx_immo-MCPE. GOx kinetic parameters (K_m and I_max value) was determined using cyclic voltammetry. within range of glucose concentration of 0.2 - 8.0 mM
According to Michaelis-Menten curve, below 2 mM of glucose concentration the GOx_{immob-AU]|MCPE electrode performance to its substrate would evolve by the increasing concentration of glucose, on the other hand, enzymatic activity increased following elevated concentration of glucose. However, when glucose concentration was about 2 mM, GOx activity has reached its maximum activity, thus additional glucose concentration had no effects to the oxidation current value of GOx_{immob-AU}|MCPE electrode (figure 3).

![Cyclic Voltamogram performance of GOx_{immob-AU}|MCPE.](image)

Linier chart shows glucose substrate concentration to electrode current value of GOx_{immob-AU}|MCPE determined with Michaelis-Menten equation (figure 4). The range of electrode performance was in the concentration of 0.2 – 2 mM, with regression value (R^2) of 0.986. Km and I_{maks} value of GOD electrode in this research is better than previous study by Colak et al., 2012, which was 0.61 mM and 1.22 µA, respectively. On the other hand, Ambarsari (2016) result indicated lower Km value of 0.70 mM and higher I_{maks} value of 4.24 mA which was probably due to the utilization of GOx commercial with high thickness and high activity (5204.3 U/mL). In addition, GOD_{immob-AU}|MCPE sensitivity was 1.099 mA mM^{-1}.
Km value of GOx immo-IPB|MCPE was 56.4 mg/dL, thus it is potential for this electrode to be used as glucose biosensor. According to the Km values, GOx immo-IPB|MCPE electrode is able to detect blood glucose level in a normal condition, hyperglycemia, and hypoglycemia conditions. Human normal blood glucose level is about 70-110 mg/dL, and hypoglycemia blood glucose condition to be in a state if glucose level under 60 mg/dL and in a state of hyperglycemia when glucose level above 270 mg/dL.

### Table 1. Kinetics parameter of GOx immo-IPB|MCPE electrode.

| Electrode               | Kcat (s⁻¹) | Range [glukosa] (mM) | Sensitivity (mA mM⁻¹) | Km (mM) | Imax (mA⁻¹) |
|-------------------------|------------|----------------------|------------------------|---------|-------------|
| GOx immo-IPB|MCPE        | 13                    | 0.2-1.6                | 1.09    | 2.88        | 3.869       |
| GOD (NH₄)₂SO₄     | 53         | 2-100                | -                      | 30      | -           |

### 4. Conclusion

Biochemical characteristics of GOx of A. niger (IPBCC.08.610) consists of enzymatic activity, kinetics parameter and its immobilization in carbon pasta electrode. The enzymatic activity of intracellular GOx was higher than extracellular one. MCPE have a high conductance value in copper with the diameter of 3 mm. Linear region of the electrode performance GOx immo-IPB|MCPE, which is half of the maximum speed, is at a concentration of 1.6 mM or 56.4 mg/dL. GOx immo-IPB|MCPE electrode is highly potential to be used as glucose biosensor to to detect blood glucose level in a normal condition and hyperglycemia.

### References

[1] Saryono 2008 *J Natur Indonesia* 11 19-23
[2] Sabir S, Bhatti H N, Zia M A and Sheikh MA 2007 *Indo J Chem* 45(4) 443-446
[3] Ahmad A, Syaiful A, Firman A, and Patong AR 2007 *Indo J Chem* 7 97-104
[4] Simpson C, Jordaan J, Gardiner N S and Whiteley C 2007 *Protein Express Purif* 51 260-266
[5] Ambarsari L, Setyawati I, Kurniasih R, Kurniati P A and Maddu A 2016 *Indo J Chem* 1 1-16
[6] Colak O, Arslan H, Zengin H and Zengin G 2012 *Int J Electrochem Sci* 7 6988-6997
[7] Zhu J, Chen M, Qu H, Zhang X, Wei H, Luo Z, Colorado HA, Wei S and Guo Z 2012 *Polymer* 53 5953-5964