THE DEVELOPMENT OF BIOCHEMICAL OXYGEN DEMAND SENSOR USING LOCAL YEAST: *Candida fukuyamaensis,* UICC Y-247

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Abstract

In order to shorten the measurement time of biochemical oxygen demand (BOD), a BOD sensor based on yeast metabolism was developed. Local yeast, Indonesian Origin, *Candida fukuyamaensis* UICC Y-247, was used as a transducer. The yeast was immobilized as a thin film in agarose matrix with the auxiliary of Nafion® acting as the membrane for ion exchange process. The film was then attached to gold-modified glassy carbons and used as transducer on the working electrodes. The measurements were conducted by observing the depletion of glucose concentration using multipulse amperometric method and then converted to BOD values. Optimum condition was observed in a waiting measurement time of 30 min at an applied potential of 450 mV (*vs.* Ag/AgCl). Linearity was shown in glucose concentration range of 0.1–0.5 mM, which was equivalent to BOD concentration range of 10–50 mg/L. A detection limit of 1.13 mg/L BOD could be achieved. Good repeatability was shown by a relative standard deviation (RSD) of 2.7% (n = 15). However, decreasing current response of ~50% was found after 3 days. Comparing to the conventional BOD measurement, this BOD sensor can be used as an alternative method for BOD measurements.

Keywords: amperometr, BOD sensor, Candida fukuyamaensis UICC Y-247*, gold-modified carbon, Indonesian yeast*

1. Introduction

Biochemical oxygen demand (BOD) is an important parameter for monitoring water pollution. BOD was measured as the amount of oxygen consumed by microorganisms to oxidize organic materials in water. Conventional BOD method observes the depletion of oxygen concentration in water after a period of time [1]. This method generally requires 5 days to achieve optimum growth of the microorganisms in water. This five day measurements caused many disadvantages, particularly when immediate results are required. Therefore, an alternative method, which is more practicable, faster, easier, and can be developed for in situ measurements, is proposed. Those criteria are fulfilled by electrochemical biosensor [2-6]. This kind of sensors applied a transducer consists of microorganisms immobilized at a surface of an oxygen-measurable electrode. The signal responses are generally fast with high sensitivity, and hence, the BOD determination can be conducted in less than 30 min. Furthermore, some groups have even reported faster measurements with measurement time of 5 to 20 min [5]. However, to the best of our knowledge, the use of original Indonesian microorganisms for BOD sensors is not yet available. Generally, research of BOD biosensors were conducted by foreign researchers [2-6], therefore involving foreign microorganisms. Consequently, different experiment conditions and possibly higher cost are required to utilize such kind of microorganisms in Indonesia. Moreover, importing certain foreign microorganisms may pose a risk in Indonesia.

In this work, BOD sensor system was fabricated by applying local yeast, *Candida fukuyamaensis* UICC Y-247. It was selected based on its properties, such as easy to obtain, aerobic, and relatively stable and grow easily [7-8]. This yeast was immobilized in agarose film with the aids of Nafion® membrane. The film was then applied as a transducer. The measurements of BOD value were done using glucose solutions as the model of organic samples. The BOD value of glucose solution has been studied, in which 1 mM glucose is equivalent to 100 mg/L BOD [9]. Validation was also conducted by comparing the measurement results of the developed BOD sensor to those of the conventional method. The results showed that the developed BOD is a promising candidate as an alternative method for BOD measurements.

2. Experiment

Determination of growth curve of *C. fukuyamaensis* UICC Y-247. *C. fukuyamaensis* UICC Y-247 was obtained from University of Indonesia Culture

Collection (UICC), Microbiology Laboratory, Dept. of Biology, Faculty of Mathematics and Science, University of Indonesia. The growth curve was determined by incubating the yeast in several erlenmeyer flasks with different incubation time. Each flask contains of 25 mL liquid YMB fermentation media (YMA without agar) and 1.25 mL cell suspension. Fermentation process was conducted in an incubator shaker at 30 ^{o}C for 2 days. The growth of the cells was monitored by observing its turbidity using spectrophotometer at 600 nm.

Yeast Immobilization. Cell suspension, a creamy white suspension, was separated from its liquid medium by centrifugation. After washing with 0.1 M phosphate buffer solution (PBS) pH 7, suspension was immobilized in biomembrane consisting of 2% agarose in PBS. Agarose solution was warmed at 36 ^{o}C. After cooling, 2 mL of yeast suspension was added into the solution. The mixture was then poured on Nafion® membrane. Thickness of the film was varied using different volume of suspension into a membrane area of 3.5 cm x 2.5 cm. Immobilized film was then stored in PBS at room temperature

Fabrication of the working electrode. The working electrode was prepared as previously described [10-11]. Glassy carbon was cleaned and immersed in concentrated allylamine solution and then irradiated under UV light (λ= 254 nm) for 6 h followed by rinsing and drying. The glassy carbon was then modified by gold nanoparticle. The gold nanoparticle was prepared by adding 0.5 mL of 0.01 M $HAuCl_4$ into 18.5 mL deionized water and followed by vigorous stirring. After 5 min, a volume of 0.5 mL sodium citrate solution was added and stirred for 5 min. Then, 0.5 mL of 0.1 M $NaBH_4$ solution was added and stirred until red color appeared. The gold nanoparticle was characterized by UV-Vis spectrometer and Transmission Electron Microscopy (TEM). Modification of glassy carbon by nanoparticle was conducted by immersing the N-modified glassy carbon into a gold nanoparticle solution for 20 min, followed by washing and drying at 60 ^{o}C. Characterization was conducted electrochemically for glucose oxidation. For BOD sensor application, a piece of immobilized yeast film was cut and attached at the gold-modified glassy carbon before measurements (Figure 1).

Application of the BOD Sensor. Activity of the sensor was analyzed by observing the cyclic voltammetric and amperometric current produced upon the oxidation of glucose in PBS pH 7. Platinum wire and Ag/AgCl system was used as counter and reference electrodes, respectively. Prior to the measurement, the solution was purged by nitrogen gas for 2 min before saturated by oxygen gas. Multipulse amperometric technique was used with a potential of 450 mV (*vs.* Ag/AgCl).

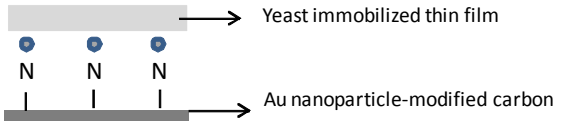

Figure 1. Illustration of a Tranducer-modified BOD Sensor

3. Results and Discussion

The growth curve of *C. fukuyamaensis* UICC Y-247. The growth curve of *C. fukuyamaensis* UICC Y-247 growth was studied using optical density method through observation of the change of turbidity. The curve indicated that the lag phase was not observed, suggesting that the *Candida sp* does not need adaptation (Figure 2). The yeast entered logarithm phase during 0-24 h, shown by elevated number of the cell. After that time, the yeast started to enter the stationer phase.

Influence of the amount of yeast cell and the required waiting time of measurement were also studied.

Preparation of gold-modified carbon electrode. The sensor system was studied by observing of electrochemical oxidation of glucose in PBS pH 7. Glucose solution measurements were used as a model of the organic sample solution assuming that most organic material can be metabolized like glucose by this microorganism [7]. The correlation between glucose concentrations and BOD values is already known [8]. Therefore, glucose concentration in the sample is equivalent to oxygen concentration required by the microorganisms to oxidize glucose. A peak observed at the potential of ~450 mV (vs. Ag/AgCl) indicated that gold modification at carbon was succesfully conducted (data not shown). The data was in agreement to the report of glucose oxidation at gold electrode [12]. It is well already known that glassy carbon electrodes do not give any response at carbon electrodes [13-16]. Linear calibration in the glucose concentration range of 0.1–0.5 mM suggested that the electrode can be applied in this BOD sensor.

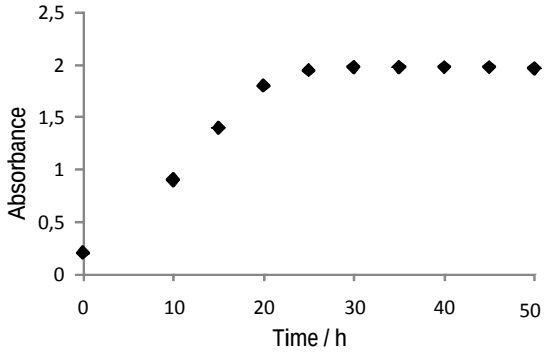

Figure 2. The Growth Curve of *Candida fukuyamaensis* UICC Y-247

BOD measurements with free cell of the yeast. Before immobilization of the yeast, activity of the free cells in various glucose concentrations (0.1-0.5 mM in PBS pH 7) was investigated. These concentrations are equivalent to 10-50 mg L^{-1} BOD based on Miller and Miller standard [9]. Free cell is a condition where the yeast was not immobilized. Yeast cells from the stationer phase were harvested and added to the glucose solutions. Voltammograms of the solutions were shown in Figure 3b. Linearity of the current plots to the glucose concentrations showed strong correlation between glucose concentrations and BOD values (Figure 3b). Current responses were proportionally increased with glucose concentrations. High activity of the yeast cells was shown by high sensitivity of the responses (20 mA/mg L^{-1} BOD), indicating that the oxygen can freely diffuse into the matrix used.

BOD measurements with *C. fukuyamaensis* immobilized in agarose-PBS matrix film. Yeast immobilization was carried out in agarose-PBS biomembrane. As transducer in the BOD sensor, the yeast should obtain maximum oxygen supply from the system. At the same time, the yeast should be protected from contaminations of other organisms in sample solution. Consequently, the yeast matrix should be kept from direct contact with the solution. An addition layer was added to overcome the problem. This layer should be able to separate the matrix with the solution and also to maintain oxygen transfer from solution into the matrix [4]. In this work, Nafion® membrane was utilized due to its good permeability and suitability for gas-liquid separation [17]. Comparison of the current responses with and without Nafion® membrane showed that linearity of the current responses is higher at the BOD sensors with membrane (Figure 4).

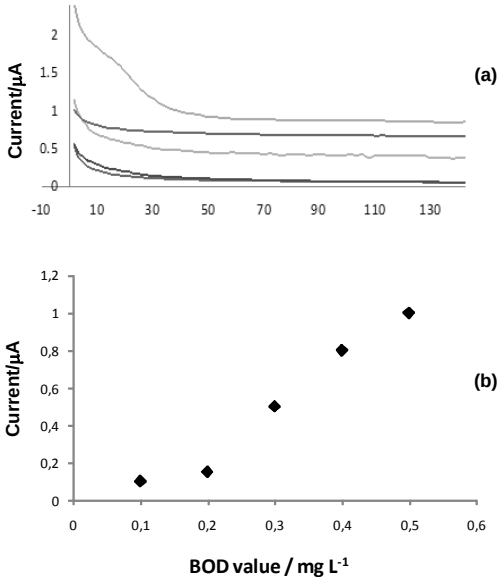

Figure 3. (a) Amperometric Voltammograms of Various Concentrations of Glucose (0.1-0.5 mM) in 0.1 M PBS at Gold-modified Carbon Electrode. A Free Cell Solution of *C. fukuyamaensis* UICC-247 was used as a Transducer. The Applied Potential was 450 mV (vs. Ag/AgCl). (b) Plots of Currents vs. BOD Values Extracted from Figure 3a

Optimum number of the yeast cells was investigated by observing current responses of the BOD sensor against various number of yeast cells. The number of cells was varied by different volume of yeast suspension added in the matrix mixture. The more volume of yeast suspension used, the higher the current produced (Figure 4). The suspensions of 1 mL yeast was chosen for the next experiments due to the limitation of Nafion® membrane thickness (50 µm).

The BOD values in the sensor system cannot be immediately measured since the yeast needs time to consume dissolved oxygen in the solution. Figure 5

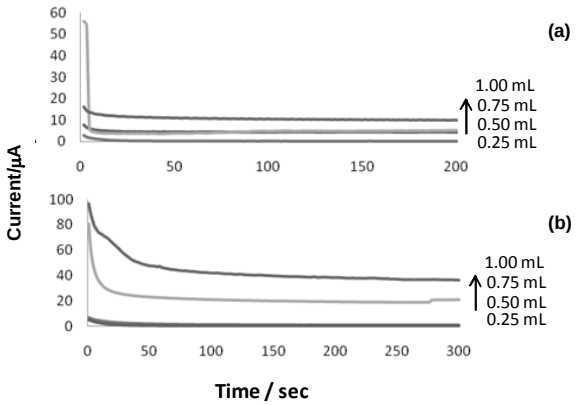

Figure 4. Amperometric Voltamograms of 0.5 mM Glucose in 0.1 M PBS pH 7 with Various Volume of *C. fukuyamaensis* UICC-247 Suspension Immobilized in Thin Films of Agarose (a) with and (b) without Nafion® Membrane at a Gold-modified Glassy Carbon Electrode. The Applied Potential was 450 mV (vs. Ag/AgCl)

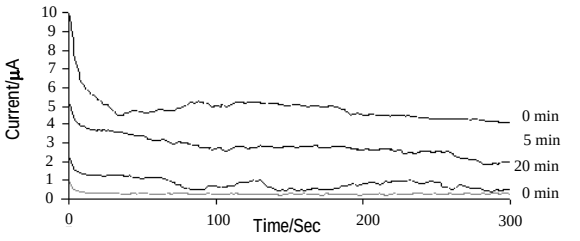

Figure 5. Amperometric Voltammograms of 0.5 mM Glucose in 0.1 M PBS pH 7 in Various Waiting Times. The Working Electrode is Gold-modified Glassy Carbon with *C. fukuyamaensis* UICC-247 Immobilized in a Thin Film of Nafion® Membrane at the Surface. The Applied Potential was 450 mV (vs. Ag/AgCl)

shows comparison of amperometric voltammograms of 0.5 mM glucose solution after four different waiting times. The Figure shows that glucose concentration gradually decreased and this is proportional to the declining of oxygen concentration [7]. Stable current response was shown after waiting time of ~30 min. Therefore, a waiting time of 30 min was fixed for the next measurements.

Linear calibration curve of the immobilized system (Figure 6) were investigated with the same condition as the free cell system in Figure 4. Good linearity (R^2 = 0,996) in the BOD concentration range of 10-50 mg L^{-1} (3 repetitions) can be achieved. However, lower sensitivity of the yeast cells (0.32 mA/mg L^{-1} BOD) was shown compared to that of the free cells, indicating there the matrix may contribute to the oxygen transfer in the system. Detection limit of 11.36 µM glucose, which is analogue to 1.13 mg L^{-1} BOD value, was calculated by S/N=3.

Stability of the sensor was examined by consecutive-BOD measurements using the same film (Figure 7a). Good stability was shown by an RSD value of 2.7% (n=15). However, decreasing of the current responses was observed after 3 days (Figure 7b).

The method was examined by comparing BOD measurements of four different glucose concentrations using conventional and sensor methods. Conventional method used is a titrimetric method which measure BOD-5 value based on the oxygen concentration change [1]. The comparison in Table 1 shows good similarity

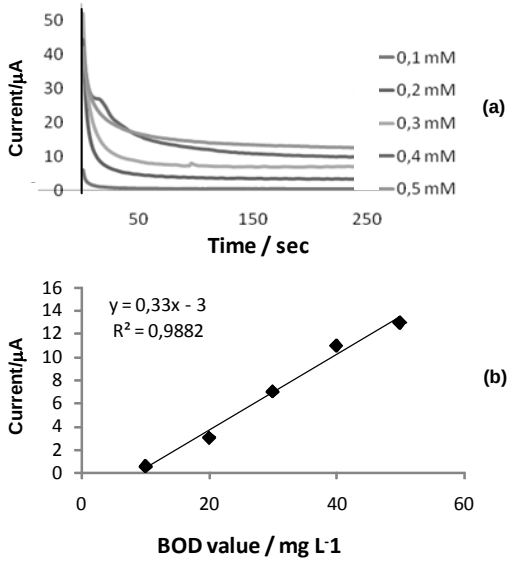

Figure 6. (a) Amperometric Voltammograms of Glucose in Various Concentrations of 0.1-0.5 mM Glucose in 0.1 M PBS pH 7 with the same Condition as in Figure 5. (b) Plots of Currents vs. BOD Values

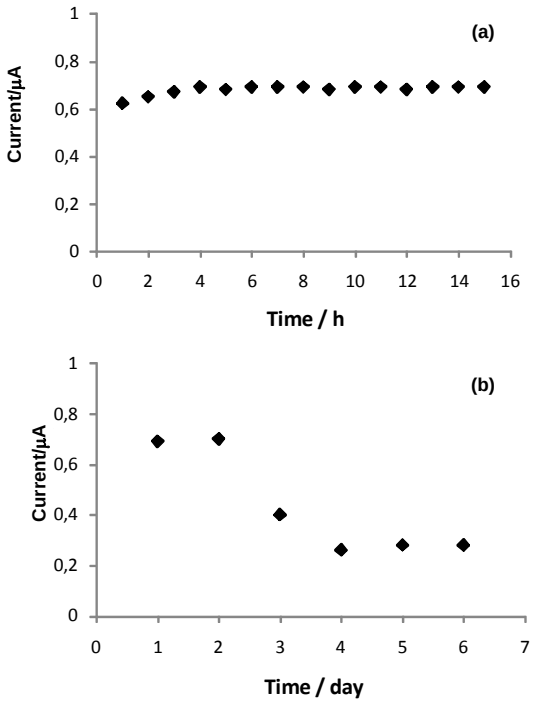

Figure 7. Plots of Current Responses of 0.5 mM Glucose in 0.1 M PBS pH 7 in (a) 15-consecutive Measurements, and (b) 3-Consecutive Day Measurements with the same Condition as in Figure 5. When it was not used, the Electrode was Stored in Deionized Water and the Film was in PBS pH 7

Table 1. Comparison of BOD Values of Different Samples of Glucose Concentrations Measured by Conventional Method and Developed BOD Sensor

BOD values of conventional method	BOD values of developed BOD sensor
17	17
17	18
20	19
21	20

between BOD values of conventional and sensor method, indicating that the developed BOD sensor is promising as an alternative method for real applications of BOD measurements.

4. Conclusion

BOD sensor using Indonesian yeast, *C. fukuyamaensis* UICC Y-247, immobilized as a thin film in agarose-PBS media, was successfully developed. The film was applied as a transducer attached at a gold-modified carbon electrode. At the measurement potential of 450 mV and waiting time of 30 min, BOD value can be obtained via glucose oxidation measurements. Linear

calibration curve can be achieved in the concentration range of 0.1-0.5 mM glucose or equivalent to 10-50 mg L^{-1} BOD with a limit of detection of 1.13 mg L^{-1} BOD. Good equivalency with conventional method suggested that the BOD sensor is promising for real applications.

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