BIOSENSOR FOR THIAMINE DETECTION USING YEAST SACCHAROMYCES CEREVISIAE

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ABSTRACT

Nowadays, people's lifestyles have undergone significant changes, such as decreased physical activity and increased sedentary behavior, as well as an increase in nutritionally unbalanced diets, especially in vitamin B1 (thiamine) intake. Deficiency and excess of thiamine can cause various diseases. Based on this, it is important to know the level of thiamine intake in dietary supplements and medicines. This study used a biosensor with amperometric principles to determine the effect of thiamine on the yeast Saccharomyces cerevisiae as a cell model. Measurements were made by adding controlled vitamins to cell metabolic activity using an amperometric biosensor based on Pt/Ag electrodes. An amperometric biosensor will measure the current from a reduction and oxidation reaction with a constant potential. The effect of adding vitamins to yeast cells was observed in the form of cellular respiration, which was expressed as a parameter of dissolved oxygen level (DO). Vitamins used as samples were given various concentrations of 30 mM, 45 mM, and 60 mM. The addition of thiamine causes an increase in the potential value for each increase in concentration, indicating that DO levels in the cell environment have decreased due to yeast cells consuming O_2 during the respiration process. The results of this study indicate that yeast cell-based biosensors can detect variations in the concentration of thiamine for further health applications.

Keywords: *Amperometric biosensor; Saccharomyces cerevisiae; yeast; vitamin B1; dissolved oxygen*

INTRODUCTION

Vitamins are necessary organic compounds that support enzymatic reactions. In general, mammalian cells are unable to produce vitamins, thus these nutrients must be obtained through diet. Yeast, cereal grains, nuts, and meat are just a few of the foods that contain vitamin B1, also known as thiamine. In addition to being consumed through food, thiamine can also be taken as a supplement along with other B vitamins and folic acid. Thiamine is frequently used to treat thiamine deficiency diseases, such as beriberi disease and nerve inflammation (neuritis) brought on by pellagra or pregnancy. Based on this, it is very important to measure the detection of thiamine levels consumed to reduce the risk of thiamine deficiency or excess particularly in dietary supplements and drugs. This research uses the principle of biosensor by utilizing the yeast as a bioreceptor.

A biosensor is an analytical tool conjured up of biological components called bioreceptors for detecting the analyte and transducers that interact each other (Kaur et al., 2015). In this case, yeast functions as a bioreceptor that can identify an analyte through the level of dissolved oxygen. The microorganism's biological signal is transformed into a transducer-measurable signal. Saccharomyces cerevisiae, or baker's yeast, is used as a cell model because the basic principle of its cells resembles higher eukaryotic organisms such as humans (Mohammadi et al., 2015).

Thiamine is an essential factor for the growth of yeast cells, especially in helping the initiation of alcoholic fermentation and limiting the risk of slow fermentation. The addition of thiamine nutrients causes an increase in the rate of reproducibility and the growth rate of yeast cells.

A previous study on thiamine detection was carried out by Ahmad (2015) using a spectrophotometric method to detect pure solutions and vitamin supplements based on thiochrome formation, but the results of the study were not accurate on the molar absorbivity of thiochrome. Halma (2017) conducted research using an enzymatic biosensor, namely the transketolase enzyme, but its stability and long-term use was low due to the enzyme reacting with thiamine every time it was used.

This study uses the electrochemical method for the detection of DO levels by using an oxygen sensor integrated in an amperometric sensor. The advantages of this method are that it has high sensitivity and is able to produce precise in situ

measurements compared to other electrochemical methods (Orellana et al., 2011). The principle of the Clark oxygen sensor is used on the DO sensor electrode by using three electrodes, namely a platinum-based working electrode (WE), a reference electrode (RE) and an auxiliary electrode (AE) based on an Ag/AgCl electrode. The data obtained from the measurements illustrate the relationship between thiamine concentration and DO levels produced by yeast cell metabolism.

METHODS

Saccharomyces cerevisiae Cultivation

The yeast strain used in this study was *Saccharomyces cerevisiae* FNCC – 3049 from Gadjah Mada University, then grown using Potato Dextrose Broth (PDB) medium. Yeast cell cultivation medium uses PDB medium, which is a popular medium for yeast cell growth and is rich in glucose and starch. Traditionally, PDB medium is made from potato starch and glucose, which makes PDB medium a high quality carbohydrate. Carbohydrate content in the medium plays a critical role in yeast cell conidiogenesis.

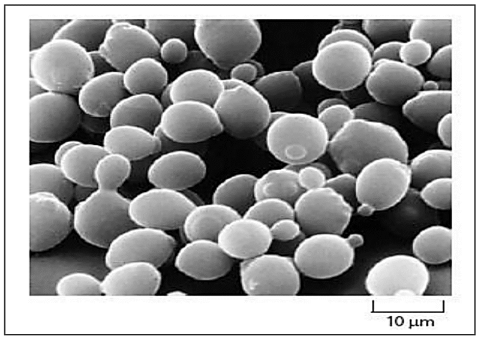

Figure 1. Yeast Saccharomyces cerevisiae under microscope.

The growth process was carried out by aseptic inoculation of 1 ose of pure culture into 20 mL of PDB medium at pH 6.5 and temperature 30° C for 4 hours. The grown yeast was then calculated for cell density using a hemocytometer and observed using a microscope. A total of 24.8 x 10^6 cells/mL with a volume of 150 µL was then immobilized into the sensor electrode using an Eppendorf pipette to measure DO levels from the metabolic process of yeast cells.

Analyte Sample Preparation

The analyte sample used in this study was thiamine purchased from Sigma-Aldrich. Thiamine was prepared with various concentrations of 30 mM, 45 mM, and 60 mM.

Thiamine solution was prepared by measuring the concentration of thiamine (Mr = 337,2699) using a digital scale. The mass of thiamine that will be used to make a solution concentration of 30 mM, 45 mM, and 60 mM is 0.10 g, 0.15 g, and 0.20 g, respectively.

Figure 2. Foods that contain thiamine

Thiamine can be obtained from various food sources as shown in Figure 2. Foods that contain high quantities of thiamine include: eggs, fish, nuts, cereals, and whole grains. On the other hand, foods that cointain low quantities of thiamine include: polished rice, fruits, and vegetables.

Yeast Cell Immobilization

Immobilization of yeast cells into the sensor electrode chamber was carried out by taking 150 µL of yeast cell solution during 4 hours of yeast cell cultivation in PDB medium.

System Design of Amperometric Biosensor

Amperometric sensors work on the principle of a potentiostat where the sensor is connected to an analog signal converter circuit. The potentiostat has three electrodes, namely the auxiliary electrode (AE), the reference electrode (RE) and the working electrode (WE). The reference potential is used at the non-polarized Ag/AgCl electrode.

Sensor Electrodes

This study uses an oxygen sensor with the Clark oxygen principle consisting of a platinum (Pt) electrode as a redox indicator electrode and an Ag/AgCl electrode as a reference electrode and a reference electrode to observe yeast cell metabolism as bioreceptors as has been developed in previous studies (Rosandi et al., 2021).

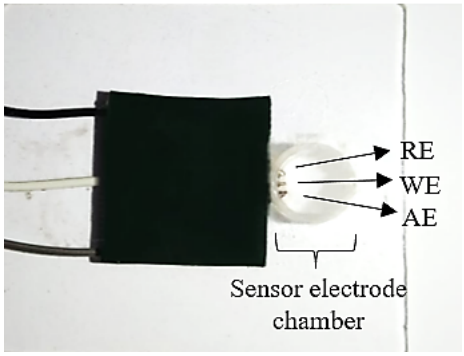

Figure 3. DO electrode sensor

The Ag/AgCl electrode is used as a reference and comparison electrode, where the Ag electrode is very stable and does not change with time or with temperature. The main advantage of using Ag/AgCl electrodes is the low noise level during measurement readings of biological signals resulting from cell metabolism**.** Yeast cell measurement scheme with the addition of thiamine as shown in Figure 4.

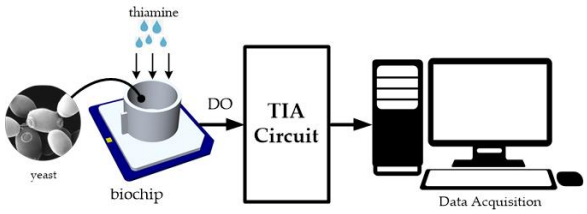

Figure 4. Set up measurement

The metabolic response of yeast cells due to the addition of foreign analytes causes yeast cells to re-adapt to their environment and causes changes in yeast cell metabolism which are indicated by changes in potential values due to changes in the oxygen gas produced. The yeast cell mixture was immobilized into the biochip using an eppendorf pipette. The sample solution was then added after the potential in the yeast cells experienced a stationary point with a concentration variation of 30 mM, 45 mM, and 60 mM. Data collection was carried out for each analyte concentration with the same treatment.

The result was analysed using analysis of variance (ANOVA) which is based on a comparison of two estimates of the population variance for statistically significant differences. The result of the ANOVA test is the calculated F value which is compared with the f table value, then it is also proven by the p-value < 0.001.

ANOVA was processed using Minitab 16 software using 25 data in a stationary state from the measurement of each concentration, where there were 75 data to be tested. ANOVA test results will be proven if the p-value <0.001.

RESULTS AND DISCUSSION

Sensor Calibration

Initial tests were carried out by calibrating PDB, DO calibration Cal0 and Cal100 as in previous research by Rosandi (2021), to ensure that the data measured on the sensor was the result of yeast metabolism when thiamine was added.

Cal0 and Cal100 calibrations were performed to determine the minimum (0%) and maximum (100%) DO values as shown in Figure 5. In measuring the minimum value of 0% DO, the sensor is calibrated with Sodium Sulfite (Na_2SO_3) Merck (126.043 g/mol), while for the measurement of 100% DO the sensor is given a solution of aerated distilled water.

The potential output values for 0% and 100% oxygen are 1990 mV and 1737 mV, respectively.

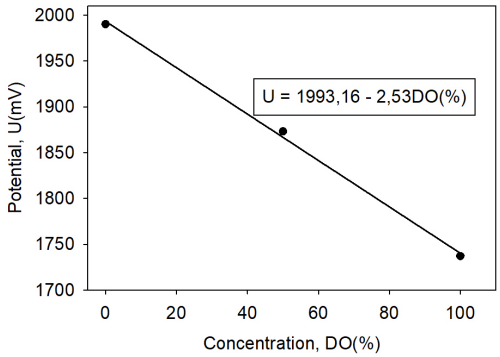

Figure 5. Calibration of Cal0 and Cal100

The addition of PDB medium solution into the biosensor chamber causes a decrease in the measurement of the potential value, which is caused by the effect of the dissolved oxygen concentration in the PDB medium. The measurement results are shown in Figure 6, where the reference potential value obtained is 1797 mV.

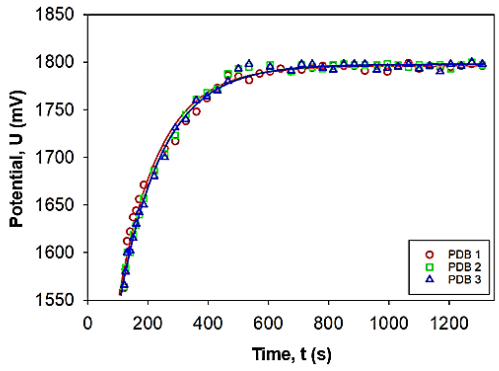

Figure 6. PDB medium calibration

Thiamine Detection

In the determination of thiamine, pure thiamine calibration was carried out for concentrations of 0 mM (control), 30 mM, 45 mM, and 60 mM. The resulting potential for control is 1884 mV. The resulting potential for thiamine with a concentration of 30 mM is 1971 mV. The resulting potential for thiamine with a concentration of 45 mM is 1978 mV. Meanwhile, the potential for thiamine with a concentration of 60 mM is 1984 mV. Based on this, it can be seen that the addition of thiamine concentration will increase yeast cell metabolism in consuming dissolved oxygen. Increase in potential value up to 4.74% at 60 mM concentration from control. The measurement results are shown in Figure 7.

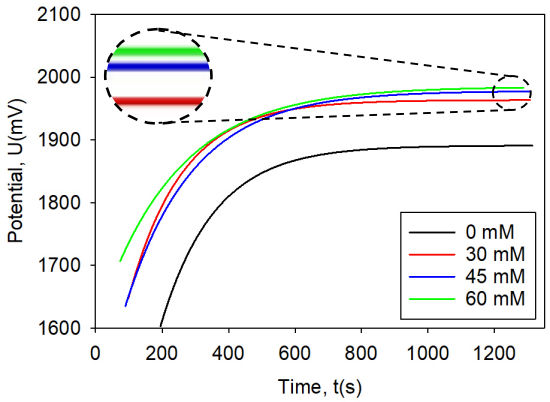

Figure 7. Measurement of DO level with the addition of thiamine was carried out for 20 minutes

The potential value increases as the concentration of thiamine increases, which indicates that dissolved oxygen levels in the cell environment are decreasing. The addition of thiamine solution caused an increase in the rate of reproducibility and growth rate of yeast cells. When the thiamine solution was injected into the whole cell biosensor system, there was an increase in yeast cell respiration activity from metabolic activity. Thiamine acts as a cofactor for the enzyme transketolase, which plays an important role in metabolic processes. Yeast cells use oxygen and the enzyme transketolase to convert glucose to glycerylaldehyde. The more thiamine increases, the yeast cell metabolism will increase. This causes more oxygen to be absorbed by the yeast cells which can be seen from the increase in the yeast cell potential value which indicates a decrease in the observed DO concentration.

Tabel 1. DO level result for thiamine addition

Concentration (mM)	Potential (mV)	DO (%)
0	1890	40.77
30	1971	8.76
45	1978	5.99
60	1984	3.62

The results of the ANOVA test using the Minitab software are shown in Table 2.

Table 2. ANOVA test result for thiamine

Source	DF	SS	MS	F	P
Conc	2	2143.44	1071.71	785.46	< 0.001
Error	72	98.24	1.36	-	
Total	74	2241.68	-	-	

The results obtained show p-value <0.001, which indicates that the data for each concentration is significantly different.

CONCLUSION

The addition of thiamine samples makes it easier for yeast cells to carry out cell metabolic activities that can be used as a source of nutrition (food) by yeast cells and can be detected by biosensor electrodes as changes in DO levels. The increase in potential value indicates DO levels in the cell environment have decreased due to cell metabolic activity in breaking down thiamine. The results of this research data are expected to be applied and used as a reference for controlling levels of vitamin consumption that affect health aspects.

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