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Tetty Marta Linda, Nabila Noor Amalina and Lazuardi Umar

Measurement of Oxygen Consumption of *Saccharomyces cerevisiae* Using Biochip-C Under Influenced of Sodium Chloride and Glucose

Tetty Marta Linda[1,a)], Nabila Noor Amalina[2,b)], and Lazuardi Umar[2,c)]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, University of Riau, Jl. Prof. Dr. Muchtar Lutfi, Panam, Pekanbaru 28293, Indonesia*
[2]*Department of Physics, Faculty of Mathematics and Natural Sciences, University of Riau, Jl. Prof. Dr. Muchtar Lutfi, Panam, Pekanbaru 28293, Indonesia*

[a)]Corresponding author: tetty.martalinda@lecturer.unri.ac.id
[b)]nabilanooramalina17@gmail.com
[c)]lazuardi@unri.ac.id

Abstract. This paper aims to investigate the effects of sodium chloride (NaCl) and glucose on a single strain of yeast *Saccharomyces cerevisiae* during the respiration period. A Biochip-C sensor was used to measure the oxygen consumption rate and calibrated with PbS solution of pH 7.3 and 2.3 M before the experiment, which has a reference voltage of 1840 mV. A 150 µL of *S.cerevisiae* solution with a density of $5{\cdot}10^7$ cells/ml was immobilized into the Biochip-C chamber and left for 600 s at the initial experiment. An amount of 100 µL sodium chloride and glucose with concentrations of 0.1 M, 0.2 M, and 0.3 M were added into the yeast solution and measured until the stationary phase. For every single oxygen consumption, the models were then simulated to predict their stationary phase. The results show the addition of glucose causes an increase in the sensor potential level by a maximum of 3.9 % from the reference represented a higher oxygen consumption, while salt decreases 2.39 % lower, which is shows an inhibit fermentation process and reaches the stationary phase at 1200 s. This work comprises a model study of respiration for the fermentation phase of yeast, especially food screening applications, and to detect the amount of sugar in food.

INTRODUCTION

Yeast *S. cerevisiae* known as bread yeast is the simplest eukaryote microorganisms, which play an important role in the process of fermentation [1,2]. The metabolic activity of yeast cells is influenced by glucose, which is a carbon source for microorganisms to increase the ability of microbes to produce carbon dioxide (CO_2) and more ethanol. The metabolism can change the acidification factor (pH) and also the oxygen levels in the cell environment [3]. Salt on metabolism affects the development of yeast cells because of its nature as a water binder and reasons for the formation of CO_2 [4]. Metabolism of glucose and salt by yeast cells results in environmental changes that can be expressed as cell vitality factors observed through metabolic symptoms.

Yeast cell metabolic activity can be monitored using one of the metabolic parameters, namely dissolved oxygen (DO) levels. Research on DO measurement using an oxygen-based biosensor has been carried out in [5] by observing the respiration activity of the yeast cells due to the addition of sucrose. These device has an advantage on the stable potential reading and can respond to analytes with a wide concentration. However, the results of potential readings can be incorrectly in the presence of external resistance outside the internal resistance of the electrolyte solution [6]. Therefore, it is necessary to do a detection method using biosensors that can take measurements precisely using the principle of amperometric biosensors.

Yeast as a living transducer was used in this study as a biological component that can recognize the analytes samples to produce transducer signals. The use of living cells in these measurements is one approach to acquire a

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reading of the measurement continuously and in real time [7]. Previous studies have used the Biochip-G with a lab-on-chip principle for monitoring of river pollution using green algae cells as bioreceptors [8] and also online cancer cell monitoring for pharmacological research [9]. In this research, a Biochip-C type sensor with a lab-on-chip concept device has been used, which has more sensitive data analysis capabilities, faster analysis time, and lower costs. The Biochip-C sensor is purchased from the cellasys GmbH Company in Munich, Germany, and can have several measuring parameters, namely dissolved oxygen (DO), the acidity value (pH), impedance (Z), and temperature (T) [7]. Cell metabolism represented by Oxygen Consumption was measured under the influence of additional variations in salt and glucose concentration through DO levels using the Biochip-C sensor.

Detection of yeast cell metabolism is an important and interesting study method due to cell responses in certain environmental conditions and can be used as an approach to higher eukaryote organisms [10]. Therefore, the process of detecting DO in yeast cells is later expected to be applied as a glucose biosensor in clinical applications for the diagnosis of Diabetes Mellitus (DM), which requires precise control of blood glucose levels. Another application is in the field of toxicology for the detection of poisons through their effects on living cells, chemosensitivity for measuring the effectiveness of cancer drugs and pharmacology for the investigation of the effects of new substances and environmental monitoring.

METHOD

Test Organism as Bioreceptors

Bioreceptors used in biosensors can be in the form of enzymes, tissues, microorganisms, antibodies, cells, DNA, and other biological microorganisms, that enable to recognize a specific analyte and reject inappropriate biomolecules [11]. In this study, the bioreceptors used were *S. cerevisiae* yeast cells which is immobilized into biosensors, added with the analytics samples in the form of glucose and NaCl solution. The morphology of *S. cerevisiae* cells normally ranges between 1– 5µm in diameter, as shown in Fig. 1.

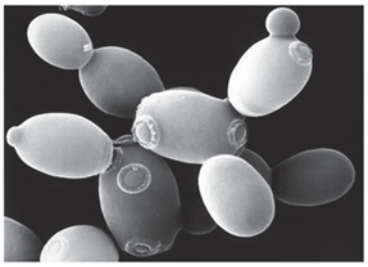


FIGURE 1. *S.cerevisiae* under the microscope [12]

Interaction between bioreceptors and analytes is called biorecognition such as chemical reactions and metabolism which are then converted into electrical signals by transducers in the form of changes in output potential. The resulting electronic signal is proportional to the concentration of specific analytes [13,14].

Preparation and Growth of Yeast Cell Medium

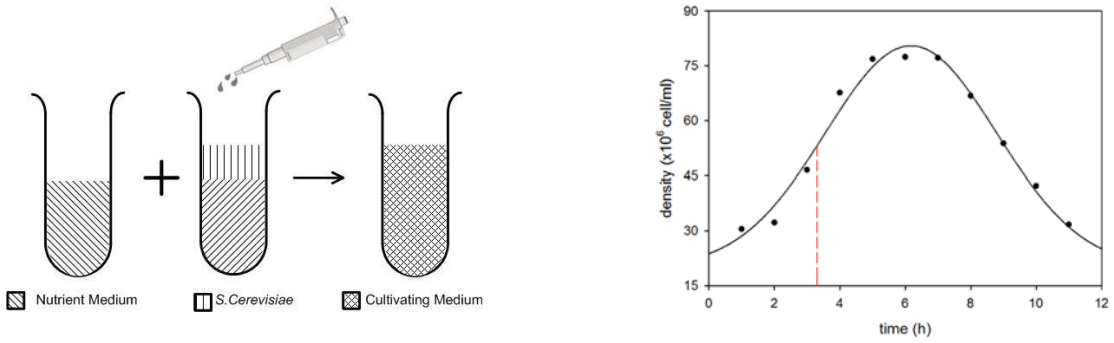
Yeast is a facultative anaerobic microorganism that can carry out aerobic respiration using oxygen and ferment under anaerobic conditions [15]. Aerobic metabolism is the process of converting carbohydrates to CO_2 and H_2O with the help of oxygen. However, anaerobic metabolism is metabolism without oxygen, which converts carbohydrates to CO_2 and ethanol [16]. The biochemical reaction processes that take place in aerobic and anaerobic metabolism are respectively shown in the following reactions:

$$C_{12}H_{22}O_{11} + 12O_2 \xrightarrow{yeast} 12CO_2 + 11H_2O \quad (1)$$

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{yeast} 4C_2H_6O + 4CO_2 \quad (2)$$

S. cerevisiae yeast strains in this study were cultivated on growth media based on Ciabatta bread recipes, while yeast seed culture is stored in agar culture medium on the sterile conditions at 4 °C. Selection of the Ciabatta bread recipe as a growth medium because each ingredient contains compounds that can support the sustainability of yeast metabolism, such as the element carbon (C) obtained from carbohydrates [17]. Ciabatta bread is typical Italian bread made from wheat flour and yeast used as ingredients and flavor providers through anaerobic metabolic processes.

For this experiment, the nutrient medium needed by the yeast for metabolism has been modified from the original recipes of Ciabatta bread. The modified ingredients of the nutrient are made by mixing 1.1 grams of glucose (G5767 Glucose Sigma Aldrich), 1.1 grams of NaCl (salt), 1 gram of yeast and 5 grams of starch (Merck) that have been weighed using an analytical balance into a glass beaker containing 50 mL of distilled water. Figure 2a shows the preparation of the medium.



(a) (b)

FIGURE 2. The process of growing yeast cells (a) and growth curve (b)

The growth of *S. cerevisiae* yeast cells in growth media was carried out aseptically on a test tube by mixing 300 µL of yeast cell strain and 3000 µL of an aqueous solution [18]. Solution mixture of yeast cell seeds and medium tightly closed using plastic wrap and aluminum foil to avoid contamination and stored at 30°C. Yeast culture stock is prepared by cultivating the seeds in the medium for 12 hours as shown in Fig. 2b. For yeast testing, after 3 hours of cultivation time the yeast sample is ready to be immobilized into the biochip. About 10^7 yeast cells are needed for the measurement with the biochip with a maximum chamber volume of 350 µL.

Making of Glucose and NaCl Solution

Glucose and NaCl solutions are prepared as test samples which are purchased from Sigma Aldrich. Both solutions were made with various concentration of 0.1 M, 0.2 M, and 0.3 M, which is calculated after [19].

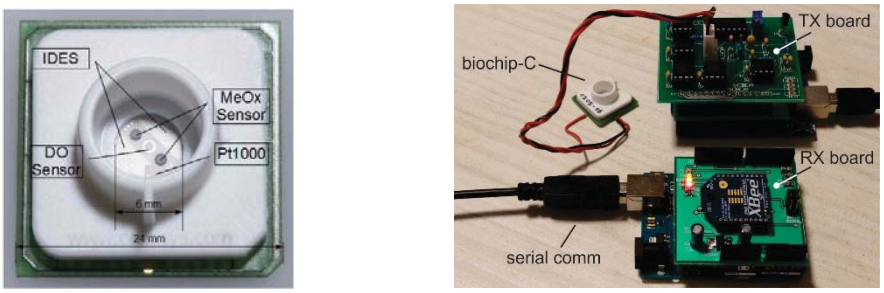
$$M = \frac{m}{Mr} x \frac{1000}{V(ml)} \tag{3}$$

The preparing process begins by weighing the mass of glucose (Molecular Weight 180.16) and NaCl (Molecular Weight 58.5) using an electronic scale and mixed with distilled water determined according to equation (3).

Biochip-C Electrodes and Electronic Systems

The main component of this research is Biochip-C produced by the cellasys GmbH Munich Germany, which is made of ceramic material with thin filaments designed for simultaneous multiparameter measurements. Figure 3a shows the physical shape and structure of the electrodes on Biochip-C, which has four different sensor electrode components namely, RTD PT 1000 temperature sensors to measure temperature changes, two potentiometric sensors to measure the degree of pH change, two MeOx sensors for impedance measurements and amperometric sensors for DO measurement [20]. The oxygen sensor uses an amperometric sensor consisting of three electrodes, the Reference Electrode (RE) provides constant electrical potential into the electrolyte cell, the Auxiliary Electrode (AE) works to keep the electrical potential of the reference electrode at a constant value and the Working Electrode (WE) measures

the change in current in the electrolyte solution. For the measurement, the biochip is connected with an electronic module as a signal processing circuit using wireless electronic modules (433 MHz), as depicted in Fig. 3b. The transimpedance based electronic module has a transmitter section, which is connected to the Biochip-C sensor, while the receiver module is connected directly to the computer that collects the measurement signal and displays the data in graphical form.



(a) (b)

FIGURE 3. (a) Biochip-C electrodes [21] and (b) wireless biosensor module transmitter (TX) and receiver (RX)

Through the building of CO_2 by yeast metabolism, it comes to changes by the pH-value and at the oxygen respiration, which will be evaluated by the BioChip-C sensors. The electrical voltage output increases by CO_2 production. That means the pH-value will decrease when CO_2 builds and also the oxygen respiration in the medium decreases.

DO Detection of Yeast Cell Metabolism

The initial stage of measurement is to calibrate the instrument which aims to ensure the condition of the Biochip as the electrode can function properly. Calibration is done by measuring the module output voltage under blank conditions (without solution inside) and using a standard Phosphate buffered saline (PBS) solution which will produce a sensor reference voltage ready to be used.

Detection of yeast cells is carried out to find oxygen consumption by immobilized cells into the Biochip-C chamber. The yeast cell mixture that had been grown in the Ciabatta medium was immobilized as much as 150 µL into Biochip-C gently using an Eppendorf dropper pipette which has a density of $5 \cdot 10^7$ cells/ml and left for 600 s at room temperature of 28 °C. After the process of detecting yeast cells experience a stationary phase, glucose and NaCl solution of 100 µL with concentrations of 0.1 M, 0.2 M and 0.3 M are separately added to Biochip-C, the detection process ends when the curve has reached stationary conditions. Measurements were made repeatedly for glucose and NaCl solutions based on the concentration of the solution. For analysis, the change in DO rate is represented by the value of the electric current that has been converted into the form of the electrical potential detected by Biochip-C. The setup of measurement with a wireless biosensor module is shown in Fig. 4.

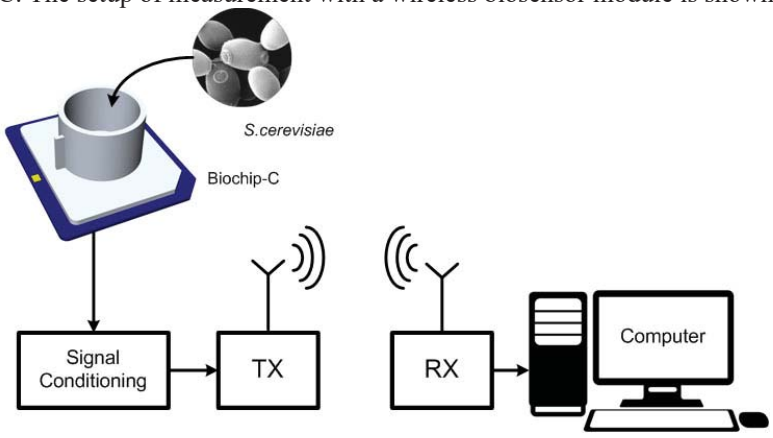
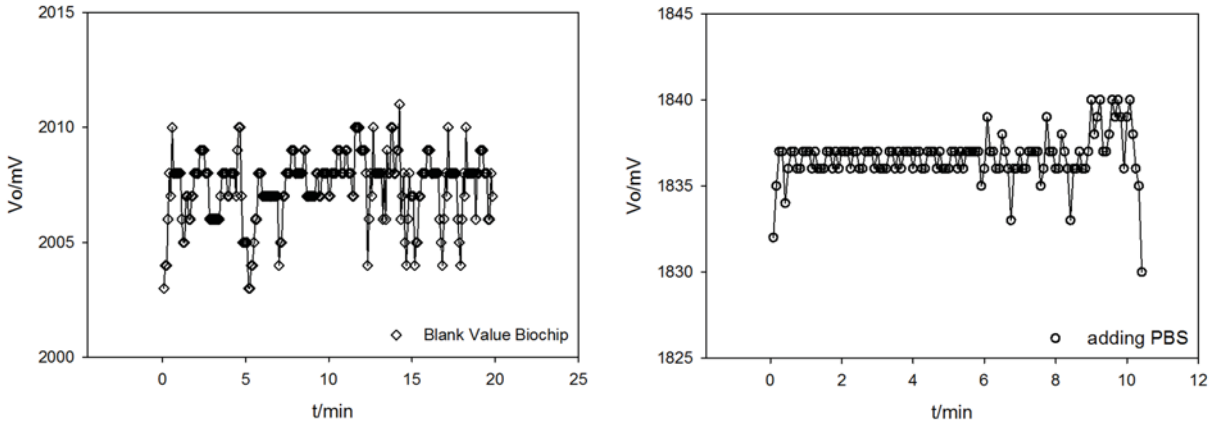


FIGURE 4. Set up of DO measurement

RESULTS AND DISCUSSION

The measurement process using Biochip-C under empty conditions and PBS solution pH 7.3 and 2.3M is shown in Fig. 5. Figure 5a shows a test on a Biochip-C blank that produces a potential value at the 2007 ± 4 mV intervals, while the adding of PBS solution caused a decrease in potential value up to 1837 mV (Fig. 5b). A decrease in potential value due to the content of ions undergoing electrolysis in the PBS solution is detected by the amperometric electrode on the Biochip. The change in value indicates that Biochip-C responds to the dissolved oxygen content in the PBS solution quickly and immediately adjusts it to obtain an average potential value of 1837 mV. This potential value is compared with references from Cellasys GmbH for calibration conditions of 1845 mV ± 100 mV.



(a) (b)

FIGURE 5. Biochip-C test (a) blank value and (b) using PBS solution

Figure 6 shows the DO level obtained from the metabolic process of *S.cerevisiae* yeast cells represented the highest CO_2 level. However, when added to glucose, there is an increase in DO consumption by yeast cells, so that reduced DO level and increased CO_2 production characterized by increased potential. Glucose in yeast cell metabolism plays a role in triggering the formation of more CO_2 gas metabolite products, which causes fewer oxygen ions dissolved oxygen, so the potential generated is higher with increasing glucose concentration.

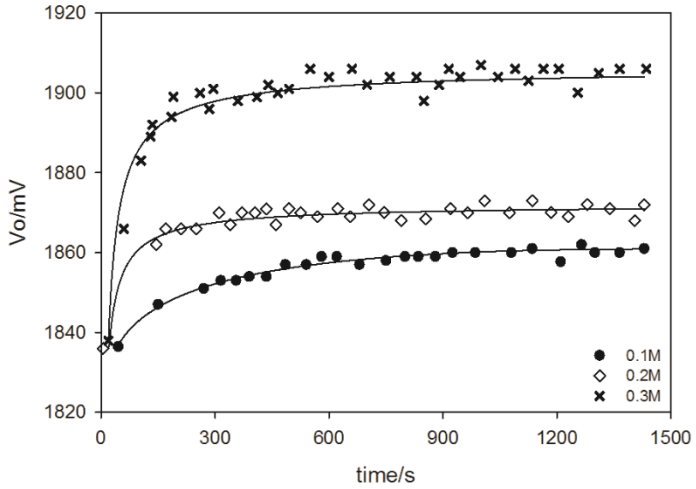


FIGURE 6. DO measurements of yeast cells with the addition of variations in glucose concentration t

The tendency of higher potential values over time in the metabolic process indicates the Biochip's response to the dissolved oxygen ions produced by cells and indicates a decrease in the level of dissolved oxygen in the environment of yeast cells due to metabolite products that produce more CO_2 gas [22]. Besides, due to the influence of the addition of nutrients to the anaerobic metabolism of bread yeast cells. Anaerobic metabolism (fermentation) as it is known is a biological process where sugar is converted into cellular energy and metabolic products such as ethanol [3]. Glucose solution has an influence on the environment of yeast cells, both the process of respiration and anaerobic metabolism. Yeast cells use nutrients that are immobilized as energy to metabolize, even though they provide only a slight potential change. Glucose solution 0.3 M when added to yeast cells, produces a potential that tends to rise higher by 3.76 % compared to the addition of 0.1 M and 0.2 M only 1.31 % and 1.91 % based on reference potential, as shown in Table 1. Research conducted by [23] explains that yeast cells respond very well to glucose for metabolism and are very active at higher sugar concentrations. However, the higher concentrations of ethanol that accumulate in the media will inhibit cell growth, thereby reducing cell viability and causing a slowdown in the rate of fermentation [24]. This is what causes the potential to be detected to be in a stationary condition after 1200 s.

TABLE 1. The potential increase of yeast cell by adding glucose

Glucose Concentration (M)	Reference (mV)	Potential (mV)	Potential Increase (%)
0.1		1861	1.31
0.2	1837	1872	1.91
0.3		1906	3.76

Measurement of DO yeast cells with variations in the concentration of NaCl shown in Fig. 7 produces a potential that is inversely proportional to the addition of glucose. The change in potential value is caused by the addition of NaCl tends to bind free oxygen in the water. This reason the supply of oxygen cannot be utilized by yeast cells. The presence of sodium chloride in the yeast cell growth medium decreases the rate of fermentation and inhibits the survival of yeast cells, so the number of cells will decrease and experience a death phase, which results in not being able to produce metabolic products such as ethanol and CO_2.

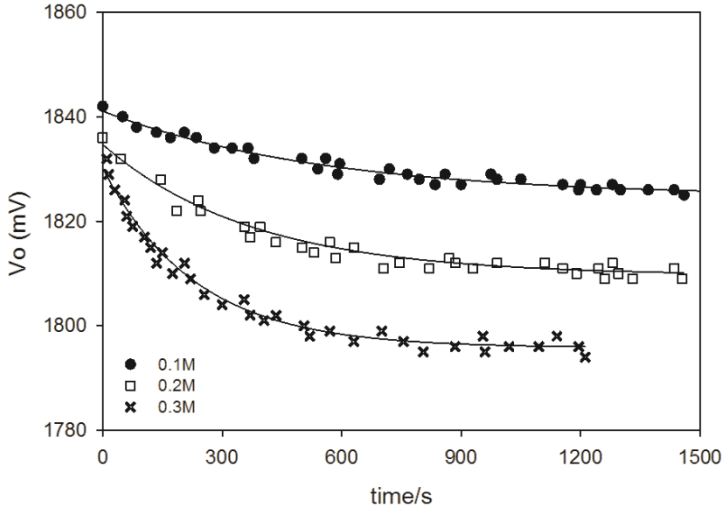


FIGURE 7. DO measurement by adding variations in the concentration of NaCl

Based on TABLE 2, the more concentrated the concentration of NaCl solution, the potential value decreases, which means increasing levels of dissolved oxygen in the cell environment due to the addition of salt as an inhibitor of the anaerobic metabolism process of yeast cells.

TABLE 2. The potential decrease of the biochip by adding NaCl

NaCl Concentration (M)	Reference (mV)	Potential (mV)	Potential Decreased (%)
0.1		1826	0.60
0.2	1837	1809	1.52
0.3		1796	2.23

In [25], *S. cerevisiae* yeast cells will experience a decreased ability in metabolism due to the addition of NaCl reaching a maximum of 2.23 % of yeast cell potential at the highest concentration of 0.3 M. This study agrees with [26] which shows that the ability of bread yeast is reduced for a metabolic process, but due to the addition of NaCl, CO_2 production decreases marked by increasing oxygen levels around the yeast cells so that the potential value reads on the amperometric oxygen sensor decreases.

CONCLUSIONS

The addition of glucose causes an increase in the basal sensor potential level by a maximum of 3.76 % of the reference representing higher oxygen consumption. Meanwhile, the addition of salt gave a 2.23 % lower decrease indicating the inhibiting fermentation process. The second curve of treatment reached the stationary phase at 1200 s. This result is an initial stage of the study of respiration models for the fermentation phase of yeast, especially food filtration applications, to detect the amount of sugar in food.

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