Simple Amperometric Biosensor for Sucrose Concentration Measurement Based on Principal Component Analysis

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Abstract. Sucrose is a type of sugar that is widely used in various types of foods and beverages. In Indonesia, sucrose consumption reaches 2.8 million tons on average per year. Effects of consuming too much sucrose can increase the risk of various diseases such as diabetes, dental caries and obesity. The level of maximum amount of sucrose that is safe for the body equal to 10% of the total energy or the equivalent of 50 g/person/day, so that the required detection system and the identification of the sucrose concentration. In this work, the identification process was carried out using an amperometric biosensor based on the yeast Saccharomyces cerevisiae as a bioreceptor. Measurements were made by immobilizing yeast cells and analyte samples into the biosensor electrodes and observed based on cellular respiration activity which was expressed as a parameter of dissolved oxygen (DO). The biosensor response is generated in the form of an output potential value, then processed using principal component analysis (PCA) to produce a sucrose concentration classification point with a percentage of variance of the two main components of 98.77% which states that the sensor is able to identify sucrose concentrations.

1. Introduction
Sucrose is one of natural sugar that often used in various types of food and beverage products. The maximum sugar consumption per person per day is 10% of the total energy or equivalent to 50 gr/person/day. Excessive consumption of sucrose can lead to diabetes, dental caries, and obesity because a gram of sucrose contains 4 calories of energy [1]. Based on this, it is necessary to monitor sucrose levels to reduce sugar consumption and prevent various diseases caused by excess sucrose. This work uses the principal of a biosensor by utilizing the yeast Saccharomyces cerevisiae as a bioreceptor to identify the amount of sucrose concentration.

The biosensor device is a detection component that requires bioreceptors as the subject of sending signals to the transducer, which is integrated in the biosensor component. The quantity received by the transducer will be converted into an electrical quantity [2]. S. cerevisiae as living cell has eukaryotic cells similar to human cells that needs carbohydrates for the yeast metabolism process. Therefore, it is suited for detecting sucrose sugar levels (C₁₂H₂₂O₁₁) in a solution [3].

Yeast cells will consume oxygen in the respiration process to release energy from sugar, lowering the oxygen level in the cell environment. In this work, sucrose levels were detected using changes in dissolved oxygen (DO) levels, which were transformed from a range of potential values arising from
the addition of sucrose solution to bioreceptors. DO levels have previously been measured using a variety of techniques, including the Winkler method, electrochemistry, and optics. The Winkler method, however, has limitations in the complex titration process, such as a long measurement response time and the inability to perform continuous online detection [4].

Although there are many studies on yeast-based biosensors to detect sugar levels, no research has been reported on yeast biosensors combined with a Principal Component Analysis (PCA) of data processing for identification and classification. Based on this, a simple amperometric biosensor for sucrose detection was developed using a self-made oxygen sensor represented by DO level detection in this research. The data obtained from the measurement depicts the correlation between sucrose concentration and DO levels produced by yeast cell metabolism. While the sucrose concentration classification is processed using PCA algorithm based on a score plot field to show the distribution of concentration levels.

2. Methodology
2.1 Yeast Cultivation
A yeast strain of *S. cerevisiae* FNCC – 3049 in the form of pure agar culture purchased from the Gadjah Mada University Yogyakarta, was used as a bioreceptor for the biosensor. Yeast cell was grown in a potato dextrose broth (PDB) medium for providing nutrients to the metabolic activities. Incubation of the yeast cells in a medium based on the substrate is a well-known method to improve the selectivity of the selected cells. Under anaerobic conditions at room temperature of 28 °C, 1 ose of pure yeast cell culture was aseptically inoculated into 20 mL of PDB medium. The *S. cerevisiae* cells were then cultivated for 4 hours at 30°C in 20 mL Iwaki Pyrex test tube prior the experiment. An image of the *S. cerevisiae* cells is given in Figure 1.

![Figure 1. *S. cerevisiae* for bioreceptor [5]](image)

The cultured cell density of *S. cerevisiae* was counted using a hemacytometer and observed using a microscope [6]. Yeast cell density calculation was carried out to determine the number of yeast cell colonies before being used as bioreceptors in the experiment. A yeast cell density of 12.8 x 10⁶ cells/mL and volume of 150 µL was immobilized into the sensor electrode using an Eppendorf pipette and left for approximately 10-15 minutes on the sensor electrodes to achieve a stationary condition.

2.2 Analyte Sample Preparation
The analyte used in this work was the natural sugar sucrose (C₁₂H₂₂O₁₁) purchased from Sigma Aldrich, which is an organic compound known as granulated sugar. Sucrose is incorporated in a disaccharide sugar and consists of a combination from glucose and fructose sugar groups, which is often used as a standard for measuring sweetness levels [7]. Figure 2 shows the commonly sugar used in food and the chemical structure of sucrose.

Standard solutions of sucrose were prepared by varying Molarity concentrations of 0.1, 0.2, 0.3 and 0.4 M, by measuring the mass of each sucrose concentration (molar mass 342 g/mol) using a digital scale and diluted in 5 mL distilled water. The required sample mass is stated in equation (1) [8].
The obtained samples mass for each concentration from 0.1 to 0.2, 0.3 and 0.4 M sucrose were 0.171, 0.342, 0.513 and 0.684 grams, respectively.

Figure 2. (a) Sugar in food and (b) chemical structure of sucrose [9]

A 150 µL sucrose solutions was gently injected into the sensor chamber using an Eppendorf pipette, while the yeast response was measured online and represented by sensor potential.

2.3 Amperometric Biosensor
Dissolved oxygen (DO) levels is measured using an oxygen sensor based on amperometric principle using three electrodes as Working Electrode (WE), Reference Electrode (RE) and Auxiliary Electrode (AE), which determines the change in current at a constant potential, as shown in Figure 3.

Figure 3. Amperometric sensor with three electrodes (RE, AE and WE) [10, 11]

The oxygen sensor with *S. cerevisiae* is connected to a transimpedance amplifier-based analog signal conversion circuit (TIA), which will convert the current output of a sensor into a voltage. The designed TIA circuit applies three op-amp components and works as a voltage difference controller on RE and WE, as well as a current to voltage converter or commonly known as an I/V converter [12].

2.4 Design of Amperometric Biosensor
The sensor electrodes WE, RE and AE were designed and fabricated after [13] and have length of 2.3 mm. A platinum (Pt) needle of 0.2 mm diameter was used as RE electrode in this experiment, while RE and AE were finished from non-polarized Ag electrodes of 0.45 mm diameter. All electrodes were mounted at the bottom of the electrode chamber, which was constructed from an acrylic cylinder with a diameter of 11.6 mm and a maximum volume of 400 µL, as illustrated in Figure 4.
2.5 Measurement of Dissolved Oxygen (DO) Levels

Figure 5 shows the experimental setup for detecting DO levels of yeast metabolism, which comprises of an amperometric electronic circuit with a remote device linked to sensor electrodes and a receiver that had previously been established [14]. For data acquiring, a BioSensor software was created to collect real-time data from the sensor readings. Since the sucrose concentrations varied from 0.1 to 0.4 M, the metabolic activities changed in response to the concentration of the analytes.

The signal captured depicts the metabolic rate of immobilized yeast cells at various sucrose concentrations. When the sucrose solution is introduced to yeast, the cell metabolism produces carbon dioxide and consumes oxygen, lowering the oxygen concentration in the vicinity of the cell environment, as well as the measured potential during metabolism. However, a calibration is required to convert the measured potential value be a percentage of oxygen (% DO). Calibration was carried out using a solution of Cal0 and Cal100 method, which shows the minimum (0 %) and maximum (100 %) of DO levels. The Cal0 solution was prepared by dissolving 0.05 grams of Sodium Sulfite (Na$_2$SO$_3$) (M = 126.043 g/mol) into 10 mL of distilled water, while the Cal100 solution was made using aerated distilled water [15].

2.6 Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a statistical data processing method that uses mathematical procedures to simplify data variables by transforming a large number of variables into simple variables without eliminating important information in the data. The PCA procedure is carried out by reducing the dimensions so that the main independent variable that is correlated becomes a new independent variable that has no correlation at all (principal component) [16]. The first step to simplify the dimensions by minimizing the residual projections generated by the variables from each variant. Based on equation (2), the mean squared error (MSE) can be determined as follows.

\[
MSE(\hat{w}) = \frac{1}{n} \left( \sum_{i=1}^{n} \| \tilde{x}_i \|^2 - \sum_{i=1}^{n} (\hat{w} \cdot \tilde{x}_i)^2 \right)
\]
The residual mean square value is obtained from equation (3) below.

\[
\frac{1}{n} \sum_{i=1}^{n} (\hat{w} \cdot \hat{x}_i)^2 = \left( \frac{1}{n} \sum_{i=1}^{n} \hat{x}_i \cdot \hat{w} \right)^2 + \text{Var} \left[ \hat{w} \cdot \hat{x}_i \right]
\]

(3)

The variable \( w \) is a vector unit on a plane line, while \( x_i \) is a projection of the data unit in vector form, then the product of the vector unit \( x_i \cdot w \) is the length of the projection line in scalar form.

Data transformation using the PCA method produces the first principal component based on the first largest eigenvalue while the second principal component is obtained from the second largest eigenvalue and so on, the data obtained will be analyzed in new coordinates [17]. In this work, the PCA processing method is displayed in a graph score plot function.

The score plot graph shows the relationship between the value of the first principal component versus the second principal component, if the two data show differences in data variance, the graph of the score plot will produce groups of points forming clusters, outliers and trends from the data. The process of forming a score plot is carried out by standardizing the main data based on the average value of the data and the standard deviation. The mean value and standard deviation are obtained from equation (4) and (5) [18].

\[
\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}
\]

(4)

\[
SD = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}}
\]

(5)

Based on equation (4) and (5), the standardized value of the data variance can be determined using equation (6).

\[
Z = \frac{x - \bar{x}}{SD}
\]

(6)

Variable \( Z \) is the standardization, \( \bar{x} \) is the mean value and SD is the standard deviation. The standardized value obtained will be the first principal component and the second principal component in the coordinates of the score plot graph [19].

3. Results and Discussion
A calibration curve of DO level is presented in Figure 6. At low level of 0 % oxygen concentration, the oxygen ions contained in a solution of distilled water interact with Na\(_2\)SO\(_3\) which implies an oxygen free water, while at 100% DO level the aerated water will perform oxygen saturation resulting a lower potential value.

![Figure 6. Calibration of minimum (0%) and maximum (100%) DO](image-url)
Based on the resulted calibration curve, a linear equation was used converts the measured biosensor potential (in mV) into a DO percentage.

\[ DO(\%) = \frac{(1993.16 - U)}{2.53} \]  

Figure 7 shows the amounts of dissolved oxygen at various sucrose concentrations. The sensor potential is initially relatively low, and rises slowly towards a stationary state after 1200 seconds.

![Figure 7](image)

**Figure 7.** (a) DO level measurement curve in the form of output potential with the addition of sucrose concentration (C₁₂H₂₂O₁₁), and (b) saturated sucrose concentration indicates DO levels (%)

**Table 1.** DO level measurement data with the addition of sucrose concentration

| Sucrose Concentration (M) | Output Potential (mV) | DO level (%) |
|---------------------------|-----------------------|--------------|
| 0.1                       | 1875 ± 0.7            | 46.70        |
| 0.2                       | 1892 ± 1              | 39.95        |
| 0.3                       | 1915.55 ± 1.1         | 30.67        |
| 0.4                       | 1934.18 ± 1.13        | 23.31        |

Figure 7(a) and Table 1 depict the sensor potentials at various sucrose concentrations. The concentration of 0.4 M shows a lower DO level than 0.1 M, 0.2 M and 0.3 M, since the yeast cells consumes more oxygen from the vicinity to break down sugar into energy [20]. During the metabolic processes, the sucrose is converted to a simple sugars; glucose and fructose resulting in CO₂ production which raises the pH value and lowers DO levels. The higher the sugar content, the lower the detected oxygen level, which is inversely proportional to sensor voltage, as depicted in Figure 7(b).

This research results obtained can be used to detect sucrose concentration, which is similar with the results obtained by Rotariu [20], that had previously detected sucrose at different concentrations concentration from 10⁻⁵ M to 10⁻² M using a potentiometric biosensor. Fawole [21] used TeraHertz waves with a range from 0.75 to 1.1 THz for monitoring yeast cell activity. However, exposure to high frequency waves for a long time can defect yeast cells [22, 23]. Therefore, the amperometric method in this study more safety to use for maintain the vitality of yeast cells. Portaccio [24] carried out measurements based on an amperometric biosensor using graphite as a working electrode, where the device used was able to detect glucose and lactose concentrations in a small range of 1 µM. Biosensor with multienzyme bioreceptors have a more complex structure, where enzymes are used mostly in biosensor observation process due to their high sensitivity to detect analytes. However, most of the enzymes used in biosensor applications are unstable and relatively expensive for routine analysis of the target analyte. In this work, yeast cells used as stable bioreceptors and relatively low cost is more suitable for the detection of sugar analyte.
Principal Component Analysis (PCA) method was used to determine the data variance classification of each sucrose concentration, as presented in Figure 8.

![Figure 8. Graph of score plot of sucrose concentration classification](image)

A separate classification of sucrose concentrations based on the quadrant of the score plot graph shows a higher concentration cluster which leads to the positive x-axis. The difference in the position of the cluster for each concentration indicates that the data processed using the PCA method can distinguish variations in the concentration of the natural sugar sucrose. The results of the PCA analysis resulted in the percentage of data variance from the first component and second component of 98.77%. It means that the sensors and yeast bioreceptors used can respond to differences in each concentration.

4. Conclusion
This study has succeeded in identifying sucrose sugar using a yeast *S. cerevisiae* based biosensor and classifying differences in sugar concentrations using the PCA algorithm. The percentage values of DO levels produced from sucrose concentrations of 0.1, 0.2, 0.3 and 0.4 M were 46.70%, 39.98%, 30.67% and 23.31%, respectively. Classification of sucrose concentration using PCA based on the potential values resulted in the variance percentage of the two main components of 98.77%, which was calculated based on the eigenvalues of the correlation matrix as an indicator of the sensitivity of sensors and bioreceptors in identifying changes in sucrose concentration.

Acknowledgments
The authors acknowledge the Ministry of Education and Culture, Republic of Indonesia for their funding through the 2021 DRPM Basic Research Program, contract no. 144/E4.1/AK.04.PT/2021.

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