Analysis of cumulative oxygen uptake in cocoa beans (Theobroma cacao L.) fermentation using a packed bed reactor

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Abstract. The research aims to determine cumulative oxygen uptake in cocoa beans fermentation using packed bed reactor. Observed variables were including: length times of cocoa fruit ripening before fermentation (X₁) i.e., 1; 5; 9 days, aeration rate (X₂) i.e. 0.2; 0.3; 0.4 liters/minute, and aeration mode (X₃) is intermittent 1; intermittent 2; continuous. The response surface methodology was used to figure out and to describe the relationship between cumulative oxygen uptake to the variables. The research results showed that the highest oxygen consumption occurred on day 2 and day 3 of the fermentation process, where the average peak oxygen demand occurred at the hour 25-72 fermentation, then continue to decline until end of the fermentation process (at fermentation time to 120 hours). The value of cumulative oxygen uptake during the fermentation process using a response surface methodology was obtained at 7.291–25.350 g O₂/kg vs on various treatments. The results of response surface methodology also showed that the highest oxygen consumption during the cocoa beans fermentation was obtained on cocoa fruit ripening of 1-3 days, aeration rate of 0.3-0.4 litres/minute and continuous aeration mode. While the lowest oxygen consumption was obtained on cocoa beans ripening of 1 day, aeration rate of 0.2 to 0.3 litres/minute and aeration mode of intermittent 1.

1. Introduction
Cocoa beans fermentation is one of the important processes must be done to produce high quality chocolate products [1]; [2]. Unfermented or under fermented cocoa beans will have a distinctive flavor of low chocolate when roasted and even covered with excessively astringent and bitter tastes [3]; [4]. Cocoa beans fermentation aims to develop a distinctive flavor of chocolate, hollow seed chip and brown color, reduce excessively astringent and bitter tastes, and produce good quality and aroma cocoa beans [5]; [6]. Even the fermentation process is considerably very important as it can improve biochemical changes and concentration of flavor precursors in cocoa beans [7]; [8]; [9].

The success of cocoa bean fermentation is generally influenced by various factors, including the cocoa fruit ripening, fermentation equipment, the amount of cocoa beans, changes in air temperature,
and stirring cocoa beans factors. The increase in the fermentation air temperature will occur optimally when the fermentation air demand is properly fulfilled. Changes in air temperature fermentation are generally: 25-52 °C [10]; [11]; [12]; The aeration factor is the main thing to consider in a good fermentor design to optimize physical aspects of the fermentation process [4]. Aeration and air temperature changes during fermentation affect the success of the process, so that controlling aeration and air temperature during the process should be done. The purpose of this research is to identify effects of cocoa fruit ripening before fermentation, as well as effect of controlling aeration and aeration mode to amount of oxygen uptake during the cocoa beans fermentation process.

2. Materials and Methods

2.1. Research tools and materials

The research tools used in the research include: packed bed fermentor, airflow meter wiebrock (type AIR 20 °C 1 atm, 0.1-0.8 NL/min), air pump, oxygen/carbon dioxide analyzer Quantek Instruments Model 902D, O₂ meter DO5509, and other supporting tools. Packed bed fermentor used in the research consists of reactor tubes placed in the chamber, a set of air temperature control systems using data logger, fan, lamp, and a set of aeration systems [14]. The material used in the research were cocoa fruits obtained from a farmer plantation in Hargobinangun Village, Pakem, Sleman, Yogyakarta, Indonesia. The uniform type and maturity level of cocoa fruits were chosen as samples.

2.2. Cocoa beans fermentation process

Harvested cocoa fruits were stored at room temperature for 1, 5, and 9 days. Furthermore, 1 kg of cocoa beans that have been separated from the skin and placenta, inserted into a tightly sealed reactor tube, without the addition of air temperature and aeration for 24 hours. On the second day of the fermentation, air was constantly flowed into the reactor with a 0.2 aeration rate; 0.3; and 0.4 L/min until the end of the process according to aeration treatments, which is regulated using airflow meters throughout the fermentation process.

2.3. Aeration during cocoa beans fermentation

Aeration during the fermentation process was done by flowing the air constantly into reactor tube, with three variations: intermittent 1 (1 hour on, 2 hours off), intermittent 2 (2 hours on, 1 hour off), and continuous. The aeration process was aimed to determine amount of oxygen needed during the fermentation process. It is calculated based on the difference in oxygen gas concentration (O₂) between the inlet and outlet when the system has been in steady state [15]. At first, the air was streamed at a determined pace through an inlet, then passing through a cocoa pile and out through an outlet. The average inlet air contains 20.9% oxygen concentration in accordance with the general oxygen concentration in the air. While the concentration of oxygen at its large outlets varies widely and is generally smaller than that of in the inlet (Oxygen concentration measurements were performed hourly). This indicates that the cocoa beans fermentation process requires air (oxygen) as a source of nutrient for microbial respiration living in the reactor tubes during the process.

2.4. Calculation of oxygen uptake rate (OUR) during fermentation process

Calculation of amount of OUR was done using different systems in each condition of aeration mode, i.e. flowing or flushed system and closed or static system [15]. The calculation using a flowing system was done when aeration was given/ flowed, while a closed system was used when aeration was discontinued (“off mode”). Calculation of oxygen uptake rate (OUR) during the fermentation process with continuous aeration mode and when "on mode" is calculated using the equation 1 and 2 [14].

\[
CO_2 = \frac{MO_2 \cdot PCO_2 \cdot vol}{100R(273.15+T)}
\] .............................................................. (1)

\[
OUR = \frac{Q (CO_2a - CO_2)}{VS_0}
\] .............................................................. (2)
Or it can be expressed as follows:

\[
OUR^{"aerasi on/\text{kontinu"}} = \frac{Q (CO_{2a} - CO_{2})}{VS_0}
\]

Where: CO\textsubscript{2} is oxygen concentration (g/m\textsuperscript{3}); MO\textsubscript{2} is oxygen molecular mass (32 g/mol); P is atmosphere pressure (101325 Pa); CO\textsubscript{2vol} is oxygen concentration with a percent unit of volume (%); R is gas constant (8.314472 J/mol/K); T is air temperature, inlet/outlet (ºC); OUR is oxygen uptake rate (consumption O\textsubscript{2}) (g/h/kg); Q is air flow rate (m\textsuperscript{3}/h); CO\textsubscript{2a} is average oxygen concentration in environment (279 m/g\textsuperscript{3}); VS\textsubscript{o} is initial volatile solid in Kg\textsubscript{vs} (VS\textsubscript{o} cocoa beans is 0.96 Kg\textsubscript{vs}).

Furthermore, when the calculation of OUR was at the "off mode", the system was considered as a closed system, because there was no air flowing into the tube through an inlet. Thus, the calculation of OUR amount was done using Eq. 4 [15].

\[
RO_2 = \frac{(YO_{2t_i} - YO_{2t_f}) x V}{100 x M x (t_f - t_i)}
\]

In this research, symbol of eq. 4 was modified following the symbol of eq. 2 to obtain a more uniform unit, as follows:

\[
OUR_{aerasi \text{ off}} = \frac{(CO_{2a} - CO_{2}) x V}{VS_0 x (t_f - t_i)}
\]

Where: RO\textsubscript{2} is oxygen consumption rate (m\textsuperscript{3}/kg/dt); YO\textsubscript{2t_i} is volumetric oxygen concentrations at initial t time (% v/v); YO\textsubscript{2t_f} Volumetric oxygen concentrations at the final t time (% v/v); M is cocoa weight (the cocoa sample used was 1 kg); t\textsubscript{i} is initial time (second); t\textsubscript{f} is final time (second); V is free volume (m\textsuperscript{3}); free volume of reactor tube is the volume of upper and bottom tubes of the unallocated cocoa beans strainer. The free volume on the reactor tube part can be calculated using the following equation of cylinder volume:

\[
V = \pi r^2 t = \frac{1}{4} \pi d^2 t
\]

Where: \(\pi = 3.14\); r is a radius of reactor tube (5.5 cm); d is diameter of reactor tube (11 cm); and t is height of reactor tube (25 cm).

2.5. Data analysis
Data analysis was carried out using the RSM to determine effects of length time of the cocoa beans ripening before fermentation (X\textsubscript{1}), aeration (X\textsubscript{2}), and aeration mode (X\textsubscript{3}) variables to cumulative oxygen uptake during the fermentation process: consisting of 20 treatments. The data analysis using the RSM will deliver a mathematical equation [16]; [17]; [18] as follows:

\[
Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3
\]

Where: Y is response treatment value to cumulative oxygen uptake during fermentation process. \(\beta_0\) is intercept/constant, \(\beta_1, \beta_2, \beta_3\) are linear coefficients, \(\beta_{11}, \beta_{22}, \beta_{33}\) are quadratic coefficients, \(\beta_{12}, \beta_{13}, \beta_{23}\) are a interaction treatment coefficients.
3. Results and Discussion

3.1. Oxygen uptake rate (OUR) in cocoa beans fermentation

Calculation of the amount of oxygen required during the cocoa beans fermentation was done at the beginning of 2nd day of fermentation, i.e. in hours 25 – 120 in each treatment. The result shows that the hourly oxygen uptake rate (OUR) during the fermentation process are as shown in figure 1. The highest oxygen consumption occurs on day 2 and day 3, where the average peak of oxygen demand occurs at the hour 25-72, then continue to decline until the end of the fermentation process (at time of 120 hours). The amount of oxygen required during the fermentation process in the "on mode" is greater than that of in "mode off ". Furthermore, the aeration variation in "continuous mode" is higher than that of in the "intermittent mode".

![Figure 1. Oxygen uptake rate during cocoa beans fermentation using packed bed reactor](image1)

![Figure 2. Changes of cumulative oxygen uptake values during cocoa beans fermentation using packed bed reactor](image2)

OUR value documented every hour can be used to recognize the magnitude of cumulative oxygen uptake value or the total amount of oxygen required during fermentation per kg of cocoa beans, as shown in Fig. 2. Fig. 2 shows that the highest cumulative oxygen uptake value is obtained at treatment 7, which is 27.978 gr O₂/kg vs cocoa beans, with the treatment of cocoa fruit samples ripened for 1 day, aeration rate of 0.4 liters/minute, and aeration mode continuous. While the smallest value of cumulative oxygen uptake is obtained at 9.145 gr O₂/kg vs cocoa beans, which is obtained at treatment 1, with 1 day of ripening time, aeration rate of 0.2 liters/minute, and aeration mode of intermittent 1.
The continuous aeration has a greater effect on the amount of oxygen consumed during the fermentation process. It is presumed due to the continuous aeration has a longer contact time with cocoa beans and microbes in the reactor tube, so that the required air fulfilled.

3.2. Cumulative oxygen uptake analysis using response surface methodology (RSM)

The experimental values and results of the model prediction for cumulative oxygen uptake during the fermentation are as shown in Table 1. Table 1 shows that the cumulative oxygen uptake value obtained based on the experiment or the prediction model has a good fit, where the both difference is 2.040%.

| Treatment | X₁ (days) | X₂ (l/min) | X₃ (on/off; hour/hour) | Cumulative oxygen uptake, g O₂ kg⁻¹ (Y) |
|-----------|-----------|-------------|------------------------|----------------------------------------|
|           | 1         | 1 (-1)      | Intermittent 1 (-1)    | 9.145                                  |
|           | 2         | 9 (+1)      | Intermittent 1 (-1)    | 9.684                                  |
|           | 3         | 1 (-1)      | Intermittent 1 (-1)    | 15.006                                 |
|           | 4         | 9 (+1)      | Intermittent 1 (-1)    | 10.052                                 |
|           | 5         | 1 (-1)      | Kontinu (+1)           | 17.486                                 |
|           | 6         | 9 (+1)      | Kontinu (+1)           | 15.349                                 |
|           | 7         | 1 (-1)      | Kontinu (+1)           | 27.978                                 |
|           | 8         | 9 (+1)      | Kontinu (+1)           | 27.933                                 |
|           | 9         | 1 (-1)      | Intermittent 2 (0)     | 25.223                                 |
|           | 10        | 9 (+1)      | Intermittent 2 (0)     | 23.929                                 |
|           | 11        | 5 (0)       | Intermittent 2 (0)     | 16.760                                 |
|           | 12        | 5 (0)       | Intermittent 2 (0)     | 23.336                                 |
|           | 13        | 5 (0)       | Intermittent 1 (-1)    | 11.591                                 |
|           | 14        | 5 (0)       | Kontinu (+1)           | 22.241                                 |
|           | 15        | 5 (0)       | Intermittent 2 (0)     | 15.553                                 |
|           | 16        | 5 (0)       | Intermittent 2 (0)     | 16.325                                 |
|           | 17        | 5 (0)       | Intermittent 2 (0)     | 16.022                                 |
|           | 18        | 5 (0)       | Intermittent 2 (0)     | 16.060                                 |
|           | 19        | 5 (0)       | Intermittent 2 (0)     | 17.980                                 |
|           | 20        | 5 (0)       | Intermittent 2 (0)     | 15.982                                 |

Based on variance analysis for the cumulative oxygen uptake, it shows that R² value is 84.7% as shows in Table 2. It indicates that constants variable (X₁, X₂, dan X₃) has about 84.7% effect to the model. The mathematical model obtained to predict the cumulative oxygen uptake is as follows:

\[
Y_1 = -18.2251 -1.4091X_1 + 2.9681X_2 + 4.9309X_3 + 3.4938X_1X_3 -1.0342X_2X_3
-4.1662X_1X_3 -1.2001X_1X_2 -0.4959X_1X_3 + 1.3309X_2X_3
\]

\[
\text{………(8)}
\]
Table 2. Variance analysis of cumulative oxygen uptake in cocoa beans fermentation

| Source         | DF | Seq SS  | Adj MS | P   |
|----------------|----|---------|--------|-----|
| Regression     | 9  | 448.754 | 49.862 | 0.004|
| Linier         | 3  | 351.090 | 117.030| 0.001|
| Square         | 3  | 70.005  | 23.335 | 0.089|
| Interaction    | 3  | 27.659  | 9.220  | 0.379|
| Residual Error | 10 | 80.780  | 8.078  |      |
| Lack-of-Fit    | 5  | 77.165  | 15.433 | 0.002|
| Pure Error     | 5  | 3.615   | 0.723  |      |
| Total          | 19 | 529.534 |        |      |

R-Sq = 84.7%

Figure 3. Plot contour and surface of cumulative oxygen uptake ($Y_1$) relationships with length times of ripening ($X_1$) and aeration rate ($X_2$), at aeration mode ($X_3$) = Intermittent 2

Figure 3 indicates that 1-3 days cocoa fruit ripening for with an aeration rate 0.245-0.375 liters/minute, resulting in a higher cumulative oxygen uptake value compared to 4-9 days cocoa fruit ripening. It may because the cocoa fruits stored for 1-3 days (before the fermentation) generally has more cocoa pulp and moist with a fresh and sour aroma. This condition stimulates microorganisms playing active roles during the fermentation process to grow and develop faster, resulting in higher oxygen consumption. While the cocoa samples stored longer usually cause the cocoa beans and pulp is somewhat dry, and sometimes it has been overgrown mushrooms. It is estimated that the growth of microorganisms was less during fermentation.

Figure 4. Plot contour and surface of cumulative oxygen uptake ($Y_1$) relationships with length times of ripening ($X_1$) and aeration ($X_3$), at aeration ($X_2$) = 0.3 liter/minute

Figure 4 shows that the highest cumulative oxygen uptake of 24.415 g O₂/kg vs obtained at 1-day cocoa fruits ripening time, aeration rate of 0.3 liters/minute, and continuous aeration mode. The lowest Cumulative oxygen uptake is at < 12.5 g O₂/kg vs, with an aeration rate of 0.3 liters/minute, aeration mode of intermittent 1, and length times of cocoa fruits ripening of > 2 days. Furthermore, figure 5
shows that the highest cumulative oxygen uptake value for cocoa fruit stored 5 days before fermentation is $21 - 22.444 \text{ g O}_2/\text{kg}$ vs obtained in continuous aeration mode and aeration rate of 0.35-0.4 liters/minute. While the lowest obtained value is $7.291 \text{ g O}_2/\text{kg}$ vs in aeration mode of "Intermittent 1" (1 hour on, 2 hours off), and aeration rate of < 0.3 liters/minute.

**Figure 5.** Plot contour and surface of cumulative oxygen uptake ($Y_1$) relationships with aeration rate ($X_2$), and aeration mode ($X_3$), at length times of ripening ($X_1$) = 5 days

The analysis results of the RSM indicates that the cocoa fruit immersion is < 3 days, the aeration rate > 0.3 liters/minute, and the continuous aeration mode indicates that the oxygen required during the fermentation process is greater than those in other treatments. Previously, it was discovered that every 100 kg of cocoa beans required 700 liters of air during 5-7 days of fermentation, where most of the air was used in the last 4 days of the fermentation. However, it is not known clearly what methods were used and how to determine the amount of aeration [19]. If the air is translated in to the oxygen concentration ($\rho_{\text{oxygen}} = 0.00143 \text{ kg/l}$) used during the last 4 days of fermentation, then the cocoa beans fermentation process requires about 10.01 g O$_2$/kg vs cocoa beans.

4. **Conclusions**

1. Cumulative oxygen uptake values obtained during the fermentation process using the response surface methodology were respectively: $7.291 - 25.350 \text{ g O}_2/\text{kg}$ vs at various treatments.
2. The results analysis of the response surface methodology indicate that the highest oxygen consumption during the fermentation of cocoa beans obtained in cocoa fruit is squeezed < 3 days, the aeration rate 0.3-0.4 liters/minute, and continuous aeration mode. While the lowest oxygen consumption obtained in 1 day cocoa beans ripening, 0.2 liters/minute aeration rate and intermittent 1 aeration mode.

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