Research Article

The Kinetic Study of Dampit Robusta Coffee Caffeine Degradation by *Saccharomyces cerevisiae*

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Abstract
About 2–2.8% of robusta coffee contains caffeine. The maximum caffeine content in coffee should be between 0.45–2% according to the standard. Consequently, the fermentation process is required to lower the caffeine content in robusta coffee. *Saccharomyces cerevisiae* produces enzymes during fermentation process that can break down caffeine into uric acid, 7-methylxanthine, and xanthine. In this study, simulation is used to obtain data on fermentation's kinetics. The goal of this study is to determine the effect of Dampit robusta coffee fermentation using *S. cerevisiae* on the caffeine level in coffee beans and to simulate the fermentation process using the Monod equation. This study employs two techniques: laboratory-scale investigation and simulation of fermentation kinetics. Ultraviolet-visible spectrophotometry is used to analyze the caffeine content. The caffeine-level data is used in fermentation kinetic simulation using microsoft Excel software. Simulation data is validated using the coefficient of determination (R^2) on the regression results and the least significant difference method. Caffeine-level data is tested with 24, 36, 48, 60, and 72 h as the fermentation time variables. The study's findings indicate that fermentation using *S. cerevisiae* can reduce caffeine content to 15.0063 mg/g. Simulation of robusta coffee fermentation using the Monod equation yields the valid values of reaction rate constant (k) and Monod constant (C_M) as −0.01047 and −24.978, respectively. Monod fermentation is suitable for simulating the fermentation of Dampit robusta coffee using *S. cerevisiae* with a fermentation time of 24–60 h.

Keywords: Decaffeine, Fermentation, Monod Kinetics, *Saccharomyces cerevisiae*

1 Introduction

Caffeine (1,3,7-Trimethyl Xanthine) is a derivative compound of alkaloids that are naturally contained in more than 60 varieties of fruits, beans, and leaves, such as coffee, tea, and cacao [1]. The molecular structure of caffeine is shown in Figure 1 [2]. The most popular types of coffee are arabica coffee and robusta coffee. Arabica coffee contains 10–12 mg/g caffeine less than robusta coffee, which contains 19–21 mg/g coffee [3], [4]. Typically, there are 100 mg of caffeine in a 180 mL or 6 oz cup of coffee [5]. So, if someone consumes two to three cups per day, the caffeine consumed is around 200–300 mg. According to SNI-01-7152-2006, the daily limit of caffeine that people consume is 150 mg per day.

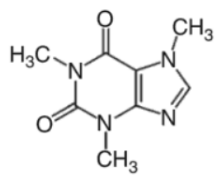

Figure 1: Chemical structure of caffeine (1,3,7-Trimethyl Xanthine) [2].

Overconsumption of caffeine leads to many health problems, such as fever, unstable emotions, insomnia, nervousness, acute anxiety, muscle problems, and digestive problems [6]–[8]. In research done by Claudia, the caffeine content in Dampit robusta coffee is measured at 5.65% w/w [9]. This number exceeds

the maximum amount of 0.45–2% w/w regulated in SNI-01-354-2004. Based on this fact, robusta coffee consumers face serious health hazards because caffeine overconsumption has a greater possibility to happen in robusta coffee consumption. Therefore, a treatment process should be done to robusta coffee beans to reduce the caffeine level. Fermentation is the approach to lessen the caffeine content in coffee beans.

The fermentation process is a popular method to reduce the caffeine content in coffee beans. Although caffeine is toxic for some bacteria, it is not rare to find bacterial strains resistant to caffeine. Some bacterial strains had been used as the fermentation agent such as *Pseudomonas sp*., *Serratia marcescens*, and yeast (*Saccharomyces cerevisiae*). The fermentation method shows an effective way to degrade the caffeine content in coffee and produces products and wastes that are safe to be consumed by humans and animals [10]. The fermentation process plays a role in breaking the protopectin and sugar components in the mucosa layer. This component-breaking mechanism is caused by the catalase enzyme. The catalase enzyme breaks the protopectin in the mucosa layer. After the mucosa layer ruptures because of the catalase enzyme, the process of breaking down sugar compounds occurs. This sugar compound acts as a substrate for the bacteria. The fermentation process converts sugar into lactic acid and acetic acid. For the next process, acetic acid will be converted into other acids by the bacteria, such as butyric acid, propionic acid, and ethanol [11].

One of the external factors that can be affecting the coffee bean fermentation's outcome is fermentation time. The longer fermentation time means lesser caffeine in the coffee beans. During fermentation, caffeine will break down into uric acid, 7-methylxanthine, and xanthine. However, there is a limit to the fermentation time to get the best caffeine level. If the fermentation is uncontrolled, the fermentation process will take too long and coffee will have a bad smell and high acidity because of the aliphatic acid formation that will decrease the quality of coffee's flavor [12]. Tulungrejo and Aepodu used coffee fermentation using *S. cerevisiae* as a method to reduce the caffeine level in robusta coffee. Until now, there is no research yet using *S. cerevisiae* as an agent in Dampit robusta coffee fermentation.

There are some methods to calculate the fermentation kinetics, such as Monod; Tessier; Moser second, third, and fourth-order; and Contois [13]. Monod kinetics has many advantages in explaining microbial growth and substrate consumption. There are some research works that analyze the fermentation process using Monod kinetics as the basic theory. Garnier and Gaillet proposed a combination of the Monod-Luedeking-Piret equation based on Monod kinetics for analyzing cell culture data in batch fermentation that could be used for research studies and industrial needs [14]. Zentou *et al.*, presented a comparison between the Monod kinetics and Andrew kinetics for molasses fermentation which resulted in higher accuracy for the Monod equation when the initial concentration of substrates is low [15]. Miller *et al.*, presented the use of the Monod equation in many wine industries using *S. cerevisiae* [16]. Most of the past studies used the Monod equation as the basic theory for the derived equation to explain the fermentation process. These equations also have been applied to many industries that use fermentation process [14], [16]. An important benefit of using Monod kinetics is the ability to simulate a fermentation process that uses low initial substrate and batch fermentor with high accuracy.

In this research, fermentation kinetics data (fermentation rate) is necessary to forecast the fermentation process in the decaffeination of coffee, so that the fermentation process will run smoothly without overflowing and/or economic loss problems. The important step in fermentation kinetics is to monitor and predict the fermentation process. Fermentation kinetics consists of growth kinetics, substrate utility, and product formation. This study uses Monod kinetics as a method to explain the fermentation kinetics in coffee bean fermentation because this method is compatible with modeling microbial growth and restricting substrate kinetics.

2 Materials and Methods

2.1 *Research materials*

The study was conducted using the quantitative method. This research uses two methods: laboratory-scale research and kinetic simulation. The kinetic simulation is done using Microsoft Excel software.

The materials used in this study were coffee cherries (*Coffea robusta*) collected from Dampit, South Malang. *S. cerevisiae* (sorbitan monostearate E491) is

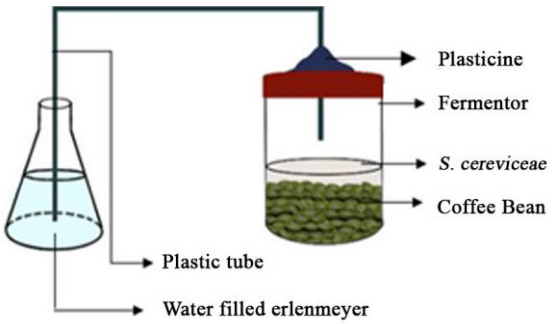

Figure 2: Fermentor construction.

used in this fermentation. Fermentation was done using five polypropylene vessels (as batch fermentors) and is constructed as shown in Figure 2.

Fermentors were filled with a mixed substrate comprising 5 g of sugar, 250 mL distilled water (aquadest), 4 mL buffer citrate pH 4.5, and 250 g of coffee beans with 5 g of yeast. These fermented beans were measured for their caffeine levels using an ultraviolet-visible (UV-Vis) spectrophotometer (*Shimadzu* instrument double-beam). The caffeine-level data were employed to model the fermentation process. The coffee bean fermentation simulation is done using Monod's equation. Data were calculated and evaluated using Microsoft Excel.

2.2 *Experiment process*

2.2.1 Coffee bean fermentation and caffeine-level determination

S. cerevisiae starter was used to fermentate the sorted and clean coffee beans. The starter was created by combining 5 g of sucrose, 250 mL distilled water, and 5 g of *S. cerevisiae*. Four grams of buffer citrate was added into the starter to keep the acidity of the fermentation media. Fermentation was started by combining the starter with 250 g of coffee beans in a fermentor. Fermentation was performed after 24 h for fermentor 1, 36 h for fermentor 2, 48 h for fermentor 3, 60 h for fermentor 4, and 72 h for fermentor 5. The fermented coffee beans would be cleaned and proceeded into coffee powder by roasting and grinding process. For the roasting process, the fermented coffee beans were heated in an oven at 100 °C temperature until their moisture content reached 12%. The coffee beans were

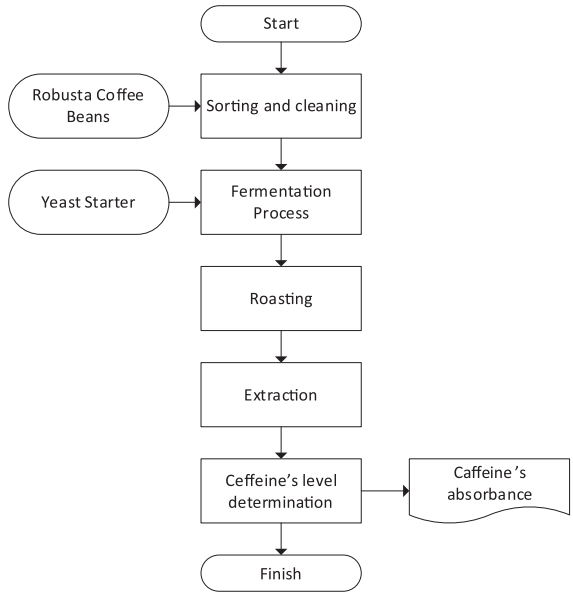

Figure 3: Fermentation and caffeine level determination process.

then roasted in 210 °C roaster for 20 min. The roasted coffee beans were converted into coffee powder using the grinder. The ideal size of the coffee powder is 20 meshes. The experimental stages in the laboratory are shown in Figure 3.

The caffeine level was determined using the maximum absorbance of coffee extract. To produce a coffee extract, a vacuum brewer method was used with a ratio of coffee to solvent as 1:18 [17]. The extraction process has done by using 10 grams of coffee and 180 mL of distilled water to get the coffee extract. The absorbance of the caffeine was determined using a spectrophotometer UV-Vis in a wavelength range of 200–400 nm [18]. The absorbance data were used to calculate the caffeine level using Equation (1) [19]:

$$\text{Caffeine Level} = \frac{M.V.f_p}{m} \quad (1)$$

where caffeine's level is in mg/g, M is for the concentration of coffee extract (ppm or mg/L), V is volume (L), f_p is the dilution factor, and m is the weight of the sample (g).

2.2.2 Fermentation kinetic model

The microbe fermentation reaction is defined as follows [20]:

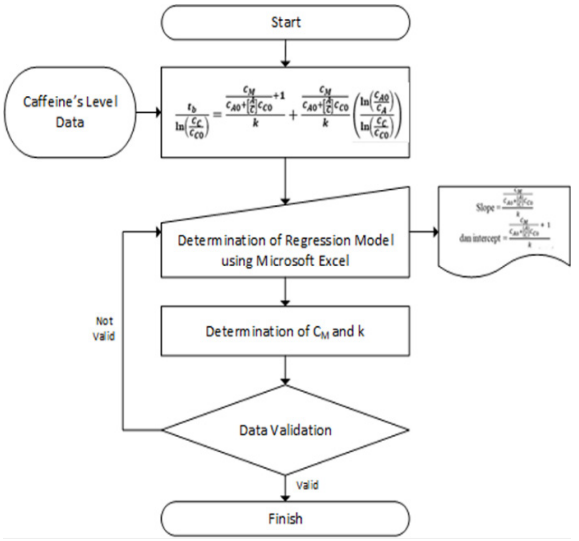

Figure 4: Simulation of coffee bean fermentation process.

$$A \xrightarrow{C} cC + rR$$

Notation *A* defines substrates/food's quantity for microbes *C*. Notation *C* denotes the presence of a particular type of microbe that speeds up the fermentation process (biocatalyst). In a fermentation process, products are notated as *R*. These products occasionally have an obstructing quality since they can kill the microbes and halt the fermentation process. In robusta coffee bean fermentation, coffee has a role as the substrate with *S. cerevisiae* as the microbe *C* and biocatalyst during the fermentation process [21]:

$$\text{Caffeine} \xrightarrow{S.cerevisiae} S.cerevisiae + \text{simple organic acid}$$

The fermentation process depends on two elements: food availability and the production of the products (*R*). These two factors can inhibit the microbe's multiplication process. Monod fermentation kinetics is based on the Monod equation, which has a similar expression to the Michaelis–Menten equation. The general expression of the Monod equation is

$$r_C = (-r_A)\left[\frac{C}{A}\right] = (r_R)\left[\frac{C}{R}\right] = k\frac{C_C C_A}{C_A + C_M} \quad (2)$$

Symbols

$-r_A$ Reaction rate of substrate consumption

r_C Reaction rate of microbe's development

r_R Reaction rate of product's formation

Equation (2) shows that an element in the product formation process is substrate availability. Cell production will decrease along with decreasing total substrate and/or increasing product (*R*). Additionally, Equation (2) is employed for finding a derivative equation to establish the parameters. The simulation of Monod Kinetics is shown in Figure 4.

2.2.3 Validity test

In analyzing data, the coefficient of determination (R^2) was used to validate the regression result. The equation for R^2 uses the sum of square value: the sum of square residual (SS_{Res}) and sum of square total (SS_T) [22]:

$$R^2 = 1 - \frac{SS_{Res}}{SS_T} \quad (3)$$

Data validation also uses Fischer's LSD method or the LSD method. The LSD testing method uses mean square error (MS_E) value in Equation (4) [22]:

$$LSD = t_{\frac{\alpha}{2},N-a}\sqrt{\left(\frac{2MS_E}{n}\right)} \quad (4)$$

3 Results and Discussion

3.1 *Determination of caffeine content based on UV-Vis spectrophotometer testing*

The determination of caffeine content in the extract of Dampit robusta coffee was carried out using a UV-Vis spectrophotometer. The maximum wavelength was measured using 3 ppm standard caffeine solution, which results in λ = 273 nm. Calibration curves of caffeine standard solution were obtained from caffeine solutions with concentrations of 1, 2, 3, 4, and 5 ppm with λ = 273 nm. Figure 5 shows the calibration curve of the caffeine standard solution.

Figure 5 shows a linear relationship between concentration and absorbance with the correlation coefficient (*r*) = 0.9988 and the equation of the regression line shown in Equation (5).

$$y = 0.0981x + 0.0085 \quad (5)$$

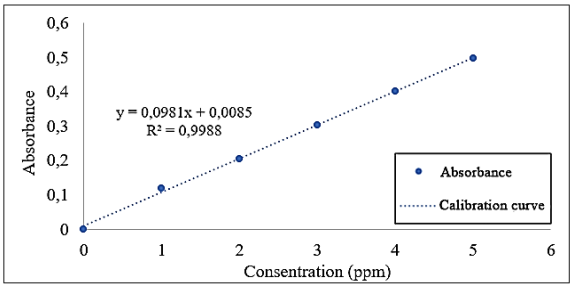

Figure 5: Calibration curve of caffeine standard solution (λ = 273 nm).

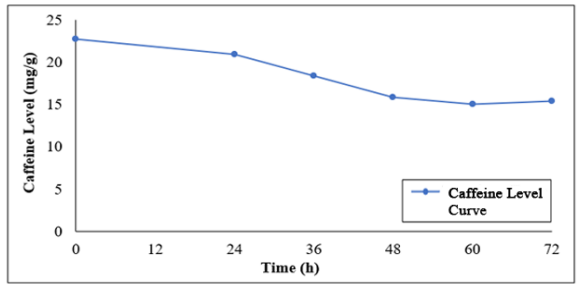

Figure 6: Caffeine level data with fermentation time variation.

Quantitative determination of caffeine content can be measured using a UV-Vis spectrophotometer. This method is the most straightforward of all methods and covers the wavelengths that belong to caffeine [23]. The caffeine content in the sample is determined using the following Equation (1).

3.2 *The effect of fermentation on the decrease in coffee extract caffeine levels*

In this study, robusta coffee was fermented for different times, which were 24, 36, 48, 60, and 72 h fermentation, to determine the best length of time to reduce the caffeine content contained in the coffee extract. The effect of the length of fermentation time on the decline in caffeine levels in the coffee extract is illustrated in Figure 6.

The organic substrate used is robusta Dampit coffee beans, the result of natural processing from a coffee plantation in Dampit District, Malang City, East Java. Figure 6 demonstrates that the amount of caffeine in coffee will diminish over the course of the fermentation process and begin to stabilize after 48 h of fermentation.

The caffeine level decreased significantly until 48 h of fermentation. Previous studies suspected that the proteolytic enzyme activity produced by *S. cerevisiae* has a big role in the decaffeination process. The proteolytic enzyme could break the caffeine structure in the coffee beans cytoplasm because of its similarity with the amide protein structure. The derived compounds could be diffused easier through the cell wall and dissolved in water because they have smaller molecules mass than caffeine [24].

After 48 h of fermentation, there was a minor change in the caffeine level. Moreover, the caffeine level slightly increased after 72 h of fermentation. Based on the study done by Saripah, this phenomenon was caused by the imbalance of microorganism activity. At some points during the fermentation process, the substrate would be limited because of the microorganism consumption. This would start a secondary metabolism that produced alkaloid compounds. The alkaloid compounds were detected as caffeine by the microorganism, which resulted in lower microorganism activity to break the caffeine structure [24].

3.3 *Determination of reaction rate constants and Monod constants in robusta coffee fermentation*

Research on the simulation of fermentation kinetics using *S. cerevisiae* on caffeine levels in robusta coffee (*Coffea robusta*) has the main objective of obtaining k and C_M values in robusta coffee fermentation. Using the data that has been gleaned from the fermentation process and the Monod fermentation kinetic model, it is anticipated that this study can generate valid and reliable k and C_M values. The reaction rate constant (k) and Monod constant (C_M) were determined based on the experiment data in Table 1.

Table 1: Caffeine's level data from dampit robusta coffee extract

Fermentation Time	Caffeine's Level before Fermentation (mg/g) (C_{A0})	Caffeine's Level after Fermentation (mg/g) (C_A)
24	22.7259	20.9372
36	22.7259	18.3954
48	22.7259	15.8535
60	22.7259	15.0063
72	22.7259	15.3828

The values in Table 1 are substituted into Equation (6) as follows [20]:

$$\frac{t_b}{\ln\left(\frac{C_C}{C_{C0}}\right)} = \frac{M+1}{k} + \frac{M}{k}\left(\frac{\ln\left(\frac{C_{A0}}{C_A}\right)}{\ln\left(\frac{C_C}{C_{C0}}\right)}\right) \quad (6)$$

with $M = \frac{C_M}{C_{A0} + \left[\frac{A}{C}\right] C_{C0}}$

Where,

C_{A0} Caffeine level before fermentation
C_A Caffeine level after fermentation
C_C The amount of caffeine level at any time
t_b Fermentation time
C_{C0} The amount of yeast used

The value of product concentration, C_{R0}, is zero because, at the time of zero, no product has been generated. C is the number of microorganisms used, where $C = C_C - C_{C0}$. A is the amount of product that is produced or lost caffeine where $A = C_A - C_{A0}$. The C_C value is obtained from the formula for the derivative of the Monod equation and the exponential phase where C_C is $C_{C0}.e^{\mu t}$ [25].

Fermentation time (t_b) is computed after the microorganisms pass the lag phase. During the lag phase, no growth of microorganisms occurs because microorganisms are still conducting the adaptation phase to the new environment. According to Olivares Marin, the lag phase in the growth of *S. cerevisiae* lasts about 8 h [26]. Thus, the t_b here is calculated by subtracting the observation time (t) from the lag phase (8 h).

The C_A used to calculate [A/C] is the average C_A for all variables since the C_A value may represent C_A for all time variables. In equation $C_C = C_{C0}.e^{\mu t}$, which is used to calculate C_C, μ is the specific growth rate of microorganisms with a value of 0.03 [27], and t is the time when the microorganisms grow (time during the exponential phase). The lag phase occurs at 0 to 8 h, the exponential phase from 8–24 h, and then the stationary phase toward the death phase at 97 h. Therefore, the data used in Equation (6) is t_b = 16 h for all time variables.

Since Equation (6) is a linear equation, in the fitting graph, the y-axis data is the value of $\frac{t_b}{ln\left(\frac{C_C}{C_{C0}}\right)}$, and the x-axis is the value of $\left(\frac{ln\left(\frac{C_{A0}}{C_A}\right)}{ln\left(\frac{C_C}{C_{C0}}\right)}\right)$. The values of the x-axis and y-axis may be used to form a straight-line equation graph through point regression, as shown in Figure 7.

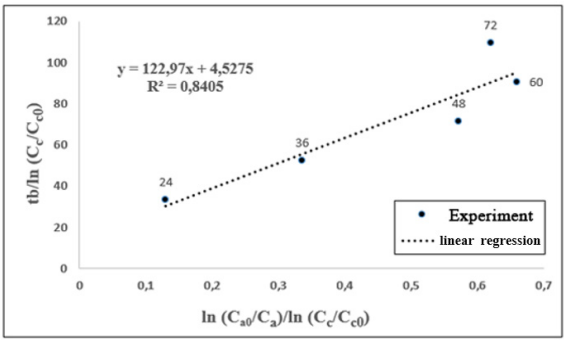

Figure 7: Monod kinetic evaluation graph.

Based on Figure 7, the equation y = 122.97 + 4.5275 results in a value of R^2 = 0.8405. Thus, the slope value obtained is 122.97 and the intercept is 4.5275. The value of 122.97 is the value of the $\frac{M}{k}\left(\frac{ln\left(\frac{C_{A0}}{C_A}\right)}{ln\left(\frac{C_C}{C_{C0}}\right)}\right)$ calculation, and the value of 4.5275 is the value of the $\frac{M+1}{k}$ calculation. The two equations were eliminated and then substituted to obtain a k value of −0.0085 and a C_M value of −25.4989.

3.4 *Analysis and validation of simulation result data with experiment data*

Monod kinetics is used to determine the Monod constant and reaction rate constant. Caffeine-level data from small-scale experiment is used to simulate Monod kinetics. This simulation results in a linear chart containing C_M (Monod constant) and k (reaction rate constant). The analysis and validation of the simulation data were performed after identifying the values of k and C_M. If the validation result is bad, data elimination is applied to the C_A experiment data, so that the C_A data have less deviation and the C_M and k values are valid and reliable. Validation is conducted using the coefficient of determination (R^2) on the regression

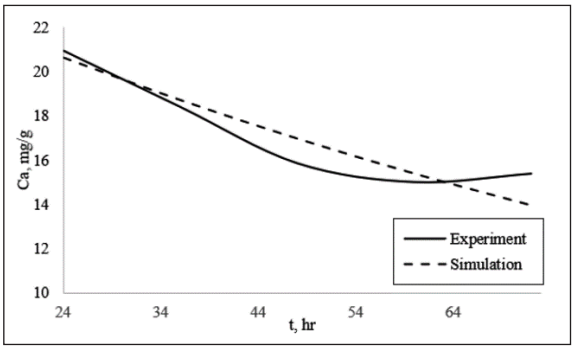

Figure 8: Comparison between simulation and experiment C_A.

results (Figure 7). By using Equation (3), the following values are calculated according to Equation (7).

$$R^2 = 1 - \frac{SS_{Res}}{SS_T} = 1 - \frac{0.0065}{0.0404} = 0.8405 \quad (7)$$

The R^2 value in the regression of the Monod kinetic evaluation chart is 0.8405. The outcome of linear regression depends on the similarity between the simulation and experimental model. The coefficient of determination (R^2) can be used to measure the level of similarity between the simulation and experimental model [22]. R^2 value lies from 0 to 1. As the R^2 value approaches one, the result of the linear regression model is getting better. This implies the variability in an independent variable can be accounted in a regression model. The R^2 value in Figure 7 starts to approach 1, which suggests that most of the data on caffeine decline in experimental findings in robusta coffee fermentation using the independent variable in the form of fermentation time can be explained by the Monod kinetic model [28].

The experimental C_A value (experiment data) may be compared with the simulated C_A. The C_A value of the simulation results is obtained using the values of $k = -0.0085$ and $C_M = -25.4989$ from the calculation results in Figure 8, which are substituted in Equation (6).

The C_A simulation and experiment pairs are also used in C_A vs. t in Figure 8 to examine the discrepancy between the C_A experiment and simulation. The graph in Figure 8 illustrates the C_A difference between the experimental data and the simulation results. Both data demonstrated that the C_A value fell concomitantly with the increase in fermentation time. The decrease in caffeine concentration (C_A) in fermentation is due to the activity of *S. cerevisiae*, which causes caffeine to turn into simpler compounds (e.g., xanthine); consequently, caffeine levels drop when the fermentation process occurs [21]. Moreover, Figure 8 shows that there is compatibility between the C_A data from the simulation results and experimental data for the fermentation time from 24–63 h. At a fermentation time of greater than 63 h, the simulation data differed from the experiment data.

When it comes to fermentation kinetics, Monod kinetics is highly dependent on the use of the substrate. If the number of substrates in the fermentation media decreases, the microbial performance declines as well. When this occurs, the Monod kinetic model will cause deviations, which result in Monod kinetics being only applicable to a limited area simulation [29]. As a result, in Figure 8, the Monod kinetic simulation data in the form of C_M and k may only be used in a time period of 24–63 h. After 63 h, the simulation data of Monod fermentation kinetics are incompatible to be applied due to the deviation becoming greater.

The findings of the data comparison in Figure 8 present that the 72 h fermentation did not produce evidence of variations in caffeine content consistent with the Monod kinetic model. Thus, the fermentation data were eliminated for the 72 h time variable. The elimination of data for the 72 h time variable is based on several factors, namely, the results of the comparison of simulation data with experimental data show deviations in the 72 h time variable. Based on the theory, *S. cerevisiae* could be in the stationary phase and the number of microbial deaths increased due to the decreasing amount of substrate. Such occurrence resulted in the decreasing performance of *S. cerevisiae*; therefore, the caffeine content in the 72 h fermentation was not significantly reduced [30].

Re-simulation was run after the removal of the 72 h fermentation data. The re-simulation procedure is the same as the previous one, starting with making a Monod kinetic evaluation graph using 24–60 h fermentation data, determining the new k and C_M values, and analyzing and re-validating using the coefficient of determination (R^2) value test.

Figure 9 shows the equation $y = 101.42\ x + 5.9101$, with a value of $R^2 = 0.9701$. Hence, the slope value acquired was 101.42 and the intercept was 5.9101. By using the same procedure in the initial simulation to calculate C_M and k, the parameter

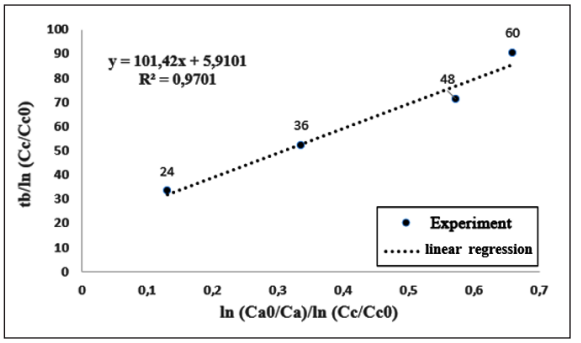

Figure 9: Monod kinetic evaluation graph after fitting data.

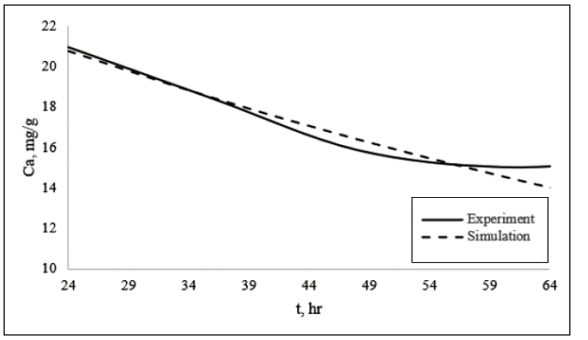

Figure 10: Comparison between simulation and experimental C_A after re-simulation.

values obtained based on the re-simulation were C_M = −26.1476 and k = −0.0107.

Figure 9 was then retested using the coefficient of determination to measure the level of compatibility of the Monod kinetic model used. Using Equation (3), the value of R^2 was calculated as follows:

$$R^2 = 1 - \frac{SS_{Res}}{SS_T} = 1 - \frac{0.00088}{0.02971} = 0.9701 \quad (8)$$

The value of R^2 in the second simulation was 0.9701. This value is greater and closer to value one compared to the outcomes of the first simulation. This shows that the linear regression model of Monod kinetics in the re-simulation is better and more in line with the experiment data [28]. With better regression results, C_M and k values in the re-simulation were more accurate than the outcomes of the initial simulation. Using these C_M and k values, it is possible to determine the C_A values of the simulation results for 24, 36, 48, and 60 h of fermentation.

A comparison of C_A data from simulation results with experiments is shown in Figure 10. Thereafter, an LSD test may be carried out to validate secondary data pairs and simulated data. The LSD method is performed to validate the experiment C_A and C_A simulation. The LSD calculates the smallest significance between the two means. The LSD technique explains that if the difference between the two data exceeds the LSD, those data are different significantly [28]. The C_A numbers are valid when the difference between the simulation and experiment C_A is lesser than the LSD value. The results of the LSD test results can be seen in the following table.

Table 2: LSD testing method

C_A Simulation	C_A Experiment	(Simulation–Experiment)	LSD
20.7771	20.9372	0.1601	0.7066
18.4586	18.3954	0.0633	0.7066
16.3989	15.8535	0.5454	0.7066
14.5689	15.0063	0.4374	0.7066

The C_A data from the re-simulation in Figure 10 is more in line with the experimental data when compared to using the C_A results from the first simulation (Figure 8). According to the LSD test results in Table 2, the difference between the C_A data from the simulation results and the C_A from the experimental findings is lower than the least significant difference (LSD). If the difference in the data pair has a smaller value than the LSD, then the data pair is considered not significantly different (close to) [28]. Thus, the results of the LSD test demonstrate that the re-simulation data are valid because the value is close to the experimental data.

The results of the comparison in Figure 10 and the LSD test in Table 2 display the suitability of the Monod model with experimental data for fermentation with a time of 24 to 60 h. This demonstrates that in this study, the kinetics of Monod fermentation will be appropriate for replicating the fermentation of robusta coffee using *S. cerevisiae* with a fermentation duration of 24–60 h.

In the results of the comparison of secondary data with data from simulation results, there are deviations in the simulation data for the 72 h time variable. Experimental data from T = 60 h to t = 72 h showed an increase in caffeine content. Increased levels of caffeine occur due to the biosynthesis of caffeine from

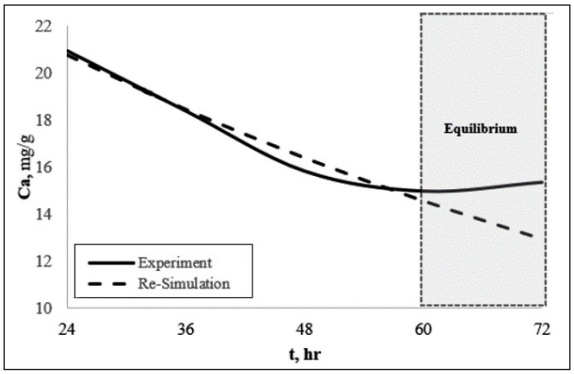

Figure 11: Deviation between the experimental data and simulation data.

xanthine, which may occur during the stationary phase of *S. cerevisiae* [31]. This demonstrates that there is a reversible reaction in the process of robusta coffee fermentation with the following notation:

$$\text{Caffeine} \overset{\textit{S cerevisiae.}}{\Longleftrightarrow} \textit{S. cerevisiae} + \text{xanthine}$$

The reversible reaction will reach a position/condition where the numbers of products and reactants do not change (in this study, the amount of caffeine and xanthine does not change). Such a state may be called equilibrium [32]. In Figure 11, the experimental data for $t = 60$ h are predicted to reach an equilibrium condition.

Monod kinetics explains the increase in growth rate with the use of the substrate by the microorganism *S. cerevisiae*, which is elaborated through the forward reaction notation and the following Equation (9):

$$\text{Caffeine} \xrightarrow[\textit{Saccharomyces c.}]{} \textit{S. cerevisiae} + \textit{Xanthine}$$

$$-r_a = -\frac{dC_a}{dt} = \left[\frac{C}{A}\right] k \frac{C_c C_A}{C_A + C_M} \quad (9)$$

According to Equation (8), the kinetics of Monod fermentation can explain the phenomenon of decreased caffeine content due to the activity of *S. cerevisiae* in the fermentation process [20]. From the findings of the two simulations, the Monod kinetics is appropriate for the time variables of 24, 36, 48, and 60 h. This is because during these time spans, the decrease in caffeine content may still be explained through the kinetics of the Monod reaction. Meanwhile, over a variable period of 72 h, the fermentation reaction moved toward the equilibrium zone, which could not be explained by the Monod reaction kinetics. Based on the results of the analysis and validation, the valid values of k and C_M are −0.0107 and −26.1476, respectively [33].

4 Conclusions

Monod kinetics is suitable for simulating the fermentation of Dampit Robusta coffee using *S. cerevisiae* with a fermentation time of 24–60 h. The simulation of robusta coffee fermentation results in a reaction rate constant (k) is −0.0107 and Monod constant (C_M) is −26.1476 with R^2 at 0.9701. The simulation data are valid and could be applied to the fermentation time range from 24–60 h before the equilibrium phase of fermentation. Future studies are needed to review the decaffeination process before 24 h to ensure the capability of Monod kinetics to simulate a coffee beans fermentation process below 24 h.

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Author Contributions

A.H.S.: conceptualization, investigation, writting an original draf, reviewing and editing; V.N.: conceptualization, methodology, supervision; N. H. F. N. D.: research design, data analysis, writting an original draf, editing; F.N.A.: research design, experiment, data collection; A.F.N: research design, investigation, experiment; S.D.A.R: experiment, data analysis. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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