

Design of Stirred Tank Reactor for Cellulase Enzyme Rumen Liquid Based Bioethanol Production from Banana Rod

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Research

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Design of Stirred Tank Reactor for Cellulase Enzyme Rumen Liquid Based Bioethanol Production from Banana Rod

Qurrotun A'yuni Khoirun Nisa^{*1}, Vita Paramita^{*1}

Abstract

Due to pandemic, supply of alcohol as disinfectant really needed for nowadays. This research aims to determine the levels of bioethanol produced from banana rods by the hydrolysis process. The target to be achieved with the reactor design is to achieve high ethanol purity using a first order reaction kinetics approach. The study was carried out on various variables including fermentation time (6 - 30 hours), hydrolysis temperature (80 - 90 °C), and substrate enzyme ratio (1:1 - 1:3). While the parameters to be tested are glucose levels and bioethanol levels. The optimal effect of the variable on alcohol content is obtained when the alcohol content value is more than 17.2% with an enzyme ratio of 3 - 3.5 at a temperature of 94 - 96 °C for 35 - 40 hours. From the research, it was found that it is possible to enlarge the reactor up to 200 times by using a ratio to obtain the ideal stirred tank reactor dimensions with a conversion of 97.15%.

Keywords: Banana Rod, Bioethanol, Fermentation, Hydrolysis

Introduction

The pandemic, which now almost spread worldwide caused by the corona virus (COVID-19), become one of the big concerns. One of the things affected is a change in health behavior, including the consumption of alcohol. Alcohol 70% as a disinfectant is increasingly needed, ethanol can kill bacteria by rapid denaturation of proteins and membranes, resulting in subsequent interference with metabolism and cell lysis (Strunk et al, 2011). Bioethanol is kind of alcohol that can be produced from lignocellulose such as agricultural, wood and plant waste containing starch or carbohydrates, and then converted into water-soluble glucose (Chittibabu, 2011). After obtaining glucose, then it is processed into ethanol by a fermentation process. Ethanol fermentation is the activity of breaking down sugars (carbohydrates) into ethanol compounds by releasing CO₂ gas. One of the alternative biomass for

bioethanol is banana rod. So far, banana rods are not widely used and will only become waste. Banana tree waste is one of the potential sources of ethanol with a method that can be developed continuously and with low production costs (Bello et al, 2014). This study aims to determine the level of ethanol produced in banana rod fermentation using the *Saccharomyces cereviceiae* bacteria in a stirred tank reactor. Another objective is to obtain the ideal reactor design from reaction kinetics. Currently, studies on the subject of bioethanol production from biomass have been carried out such as banana weevil fermentation with *Saccharomyces cereviceae* can also produce bioethanol (Ingale et al, 2011) Banana tree waste is one of the potential sources of ethanol with a method that can be developed continuously and with low production costs. This research was conducted to determine the potential of banana rods as an alternative biomass for producing bioethanol.

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Materials and methods

The research materials were banana rod, distilled water, *Saccharomyces cerevisiae*, cellulase enzymes and urea. Banana rod were obtained from the banana plantation park of Diponegoro University, while for the preparation of cellulase enzymes obtained from the rumen of cows in the slaughter house of Semarang, Central Java and isolated in the Biology Laboratory of the State University of Semarang by filtration under cold condition. The raw material preparation process starts with the isolation of cellulose from the banana rod. Then, the hydrolysis process is carried out biologically using cellulase enzymes from the cow's rumen. The hydrolyzed solution was then fermented using the help of *Saccharomyces cerevisiae*. At this stage of the analysis includes total glucose conversion and alcohol content. The process schematic is depicted in Figure 1 using the Aspen Hysys application version 8.8.

Optimization of the process of making bioethanol using a stirred tank reactor with banana rod samples. The variables used in the practicum are independent variables and dependent variables. independent variables include temperature, time, and enzyme ratio. While the dependent variable includes total alcohol and glucose levels.

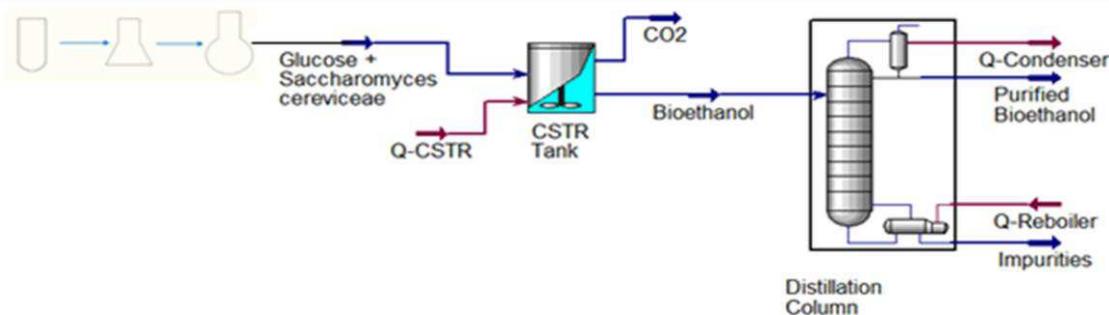


Fig 1. Bioethanol production process scheme

Determination of variables using central composite design (Yulianto et al, 2018) on Response Surface Methodology (RSM) standard design factor/block/run: 3/1/16. The first step is to determine a summary for variable consisting of temperature with the lowest value, namely 80 °C; the middle value is 85 °C and the highest value is 90 °C. The second step determines the time with the lowest value of 10 hours; the middle value is 20 hours and the highest value is 30 hours. The third step is to determine the enzyme ratio with the lowest value, namely 1. The middle value is 2 and the highest value is 3. In this experiment, the alpha for rotatability calculation is 1,6818. After the variables from the RSM method are obtained, proceed with the process. Before the hydrolysis and fermentation processes, previously carried out the isolation process of cellulase from the rumen of cows and cellulose from banana rods.

Results and discussions

Growth of *Saccharomyces cerevisiae*

Saccharomyces cerevisiae used as an inoculum with a total of 10^6 CFU / ml. *Saccharomyces cerevisiae* were cultured using the Total Plate Count (TPC) method with a 10^{-2} dilution using physiological salts.

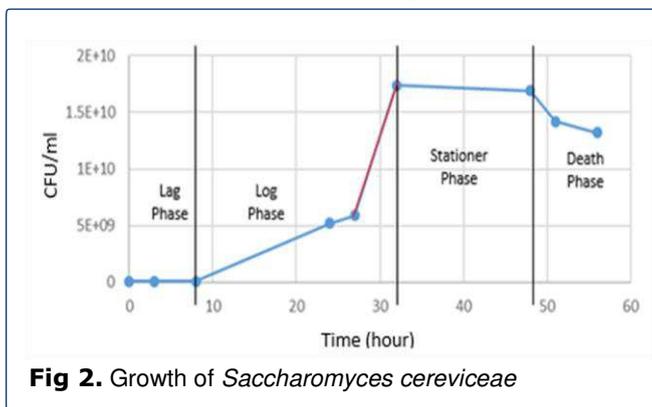


Fig 2. Growth of *Saccharomyces cerevisiae*

From figure 2, the value of reaction rate constant (k) = 0.8681 is obtained. The data is taken by determining the highest slope (hour 27 - 32) in order to obtain the number of splits per unit time is 0.31206/hour with the splitting of 2.496481 times. The average doubling time was 2.002819 hours.

Saccharomyces cerevisiae have doubling time approximately 90 min (Friedman, 2011. Herskowitz, 1988. Kaeberlein, 2005. Kaeberlein, 2010), in 30 °C or 86 °F their doubling time is 1.25–2 hours (Boekhout, 2003).



Fig 3. Growth of *Saccharomyces cerevisiae* significant in hour 27 - 32

Glucose content

Testing the total glucose content begins with the absorbance test of the bioethanol solution using the Genesys 20 Spectrophotometer in 540 nm. Cellulase enzyme that isolated from rumen liquid under cold condition shows a high activity of enzyme (Paramita et al, 2015) that use in hydrolisis process. From the standard curve is obtained it can be obtained average of glucose content is around 1.494 ± 0.007 . Glucose is phosphorylated in two stages. Two ATPs are used to produce fructose 1,6 dysphosphate, which is then broken down by the enzyme aldolase to form two 3-carbon triose phosphates. Inorganic phosphate is assimilated into two triose diphosphates from which four H atoms are accepted by the two oxidized NAD molecules. Finally, four ATPs are formed by the transfer of phosphate from triose diphosphate to ADP which results in the formation of two pyruvic acid molecules. Some pyruvic acid and other intermediates are used by yeast cells via various metabolic pathways as building blocks for new yeast cells and some glycerol is made from an intermediate material, dihydroxyacetone. However, most of the pyruvic acid is immediately converted to ethanol and carbon dioxide (Jacques et al, 1999).

Alcohol content

Research to determine alcohol content using the specific gravity of the sample using a ratio between the density of the substance and water at a temperature of 60°F, then calculating the alcohol content using the content table of the mixture of C₂H₅OH and water in tables 2 – 112 (Perry et al, 2008).

From the experimental results, a response fitted surface can be created in Figure 4 (a). Figure 4 (a) shows the relationship between alcohol content, time and enzyme ratio. By applying multiple regression

analysis to experimental data, a second level polynomial equation is obtained to represent the total glucose content obtained as follows:

$$Z(x,y) = 20.492626362494 - 0.063412438753933*x - 0.00040367857425189*x^2 - 1.1333289756657*y + 0.072786180860938*y^2 + 0.00092797515045627*84.62925*x + 0.0092797515045633*84.62925*y - 0.0056202679091437*x*y - 3.2438715$$

In Figure 4 (a) it is known that the optimum alcohol content value of more than 17.2% is achieved at an enzyme ratio of 3 – 3.5 with a length of time between 35 – 40 hours. Figure 4 (b) shows the relationship between total glucose content, temperature and enzyme ratio. By applying multiple regression analysis to experimental data, a second level polynomial equation is obtained:

$$Z(x,y) = 20.492626362494 - 0.076526586975219*x + 0.00045133572925656*x^2 - 1.1333289756657*y + 0.072786180860938*y^2 + 0.00092797515045627*20.0*x + 0.0092797515045633*x*y - 0.0056202679091437*20.0*y - 1.4297202$$

In Figure 4 (b) it is known that the optimum alcohol content value reaches more than 17.2% at a temperature of 80 - 85 °C with an enzyme ratio of 3 – 3.5. Figure 4 (c) shows the relationship between alcohol content, temperature and time. By applying multiple regression analysis to experimental data, a second level polynomial equation is obtained to represent the total glucose content obtained as follows:

$$Z(x,y) = 20.492626362494 - 0.076526586975219*x + 0.00045133572925656*x^2 - 0.063412438753*y + 0.00040367857425189*y^2 + 0.00092797515045627*x*y + 0.00927975150*1.999375*x - 0.0056202679091437*1.999375*y - 1.9749868$$

In Figure 4 (c) it is known that the optimum alcohol content reaches more than 17.2% at temperatures 80 -

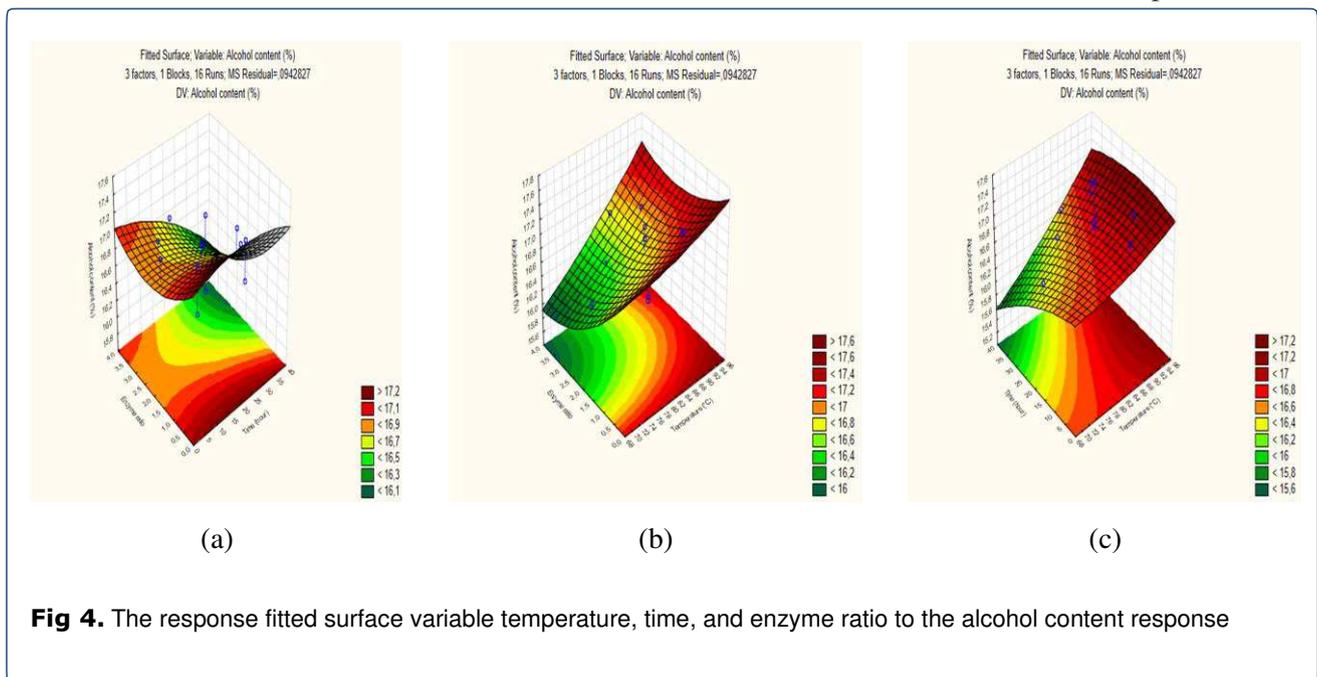


Fig 4. The response fitted surface variable temperature, time, and enzyme ratio to the alcohol content response

85 °C regarding to the length of time in the range of 35 until 40 hours. Thus, it can be concluded that the optimum conditions for variable influence on alcohol content are obtained when the alcohol content value is more than 17.2% with an enzyme ratio of 3 – 3.5 at a temperature of 80 - 85 °C for 35 - 40 hours.

Process optimization for the production of bioethanol from banana rod was carried out through 16 experiments using temperature, time, and enzyme ratio dependent variables. The t test value indicates a value greater than the p value. The accuracy of this model can be determined from the coefficient of determination (R^2). The R^2 value represents a measure of how much variability in the observed response values can be explained by the experimental variables and their interactions. From the R^2 value, it can be concluded that the estimated value by the model is close to the value obtained from the experimental results. The value of R^2 is always between 0 and 1. The closer the R^2 value is to 1, indicating that the model is good at predicting responses, it is stated that R^2 higher than 0.90 is considered as the indication of the model high correlation (Wang et al, 2015). In this case, the coefficient of determination ($R^2 = 0.94314$) indicates that 94.31% of the variability in response can be explained by the model (Table 1).

Table 1 Summary effect estimates

Factor	Effect	Standar error
(1) Temperature (°C) (L)	0.42711	0.107660
Temperature (°C) (Q)	0.02119	0.007403
(2) Time (hour) (L)	0.29396	0.093875
Time (hour) (Q)	0.21013	0.113363
(3) Enzymes ratio (L)	0.58346	0.093803
Enzymes ratio (Q)	0.17361	0.112953
1L by 2L	-0.09457	0.122654
1L by 3L	-0.01182	0.122654
2L by 3L	0.24448	0.122654
Average	0.20528	0.099669
R^2	0.94314	

The proximity of the estimated values to the model is close to the values obtained from the experimental results shown in Figure 5. The plot values in the graph show a satisfactory correlation between the experimental and estimated values, because the

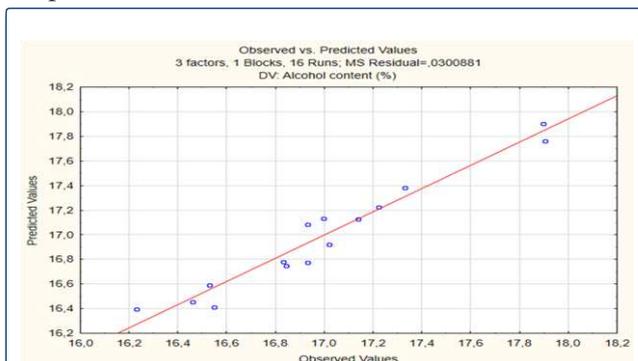


Fig 5. Comparison of experimental data and estimated alcohol content

deviation between the experimental and estimated values is close to the linear line. The regression

coefficient can be clarified by means of the Pareto diagram (Figure 6) for each variable.

From the block diagram, it appears that the dependent variable that has the most influence in the process of making bioethanol from banana rod is the enzyme ratio indicated by higher value of independent parameter value than 0.05 as the p value (Paramita et al, 2019).

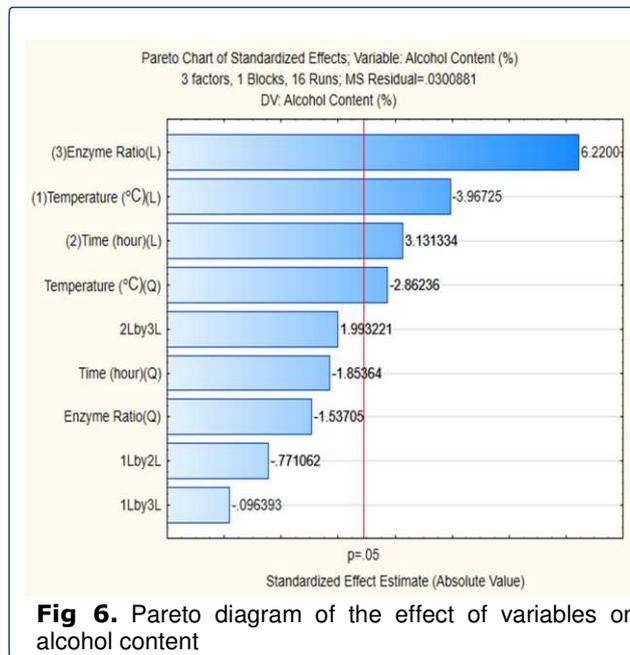


Fig 6. Pareto diagram of the effect of variables on alcohol content

The results of the response surface model are in the form of Analysis of Variance (ANOVA) which is given in Table 8. ANOVA is needed to test the significance and adequacy of the model. The Fisher ratio of variance, the value of $F (= S^2_r / S^2_e)$, is a statistically valid measure of how well the factors explain variations in the mean data and the effect of the predicted real factors. The greater the F value, the more uniformity it is. The ANOVA of the regression model shows that this model shows a correlation, as evidenced by the F value of the Fisher test ($F_{model} = 82.801$).

Table 2 Analysis of variants of the polynomial equation model for the production of banana rod bioethanol

Factor	SS	df	MS	F
(1) Temperature (°C) (L)	0.473	1	0.473	15.739
Temperature (°C) (Q)	0.246	1	0.246	8.1930
(2) Time (hour) (L)	0.295	1	0.295	9.8050
Time (hour) (Q)	0.103	1	0.103	3.4350
(3) Enzyme ratio (L)	1.164	1	1.164	38.689
Enzyme ratio (Q)	0.071	1	0.071	2.3620
1L by 2L	0.017	1	0.017	0.5940
1L by 3L	0.000	1	0.000	0.0090
2L by 3L	0.119	1	0.119	3.9720
Error	0.180	6		
Total	3.174	15		82.801

Optimization parameters of alcohol content in bioethanol from the saddlepoint banana rod against temperature, time, and enzyme ratio were determined by a critical value. Thus, the critical value for optimization of alcohol content is achieved at a temperature of 84.47908 °C for 20.39485 hours with an enzyme ratio of 2.56383.

Table 3 Predicted values for optimum alcohol content at critical values of temperature, time & enzyme ratio

Factor	Observed minimum value	Critical value	Observed maximum value
Temperature (°C)	70.65910	84.47908	93.40890
Time (hour)	3.18207	20.39485	36.81793
Enzyme ratio	0.31	2.56383	3.68000
Estimated value of alcohol content (%)	16.70644 (with the solution: saddlepoint)		

Scale up continuous stirred tank reactor

The scale-up process for this reactor algorithm is as follows:

Integration of yield equation (Fogler et al, 1999), where C_A = concentration of glucose; C_{A0} = concentration of glucose at $t = 0$; C_B = concentration of bioethanol

$$\ln \frac{C_{A0}}{C_A} = kt \quad (1)$$

For the formation of bioethanol (B), the equation becomes (Fogler et al, 1999):

$$\ln \frac{C_{A0}-C_B}{C_{A0}} = kt \quad (2)$$

Table 4 Bioethanol conversion by the time

Time (hour)	Bioethanol concentration (mol/L)	$\frac{C_{A0}-C_B}{C_{A0}}$
0	0	1
3	0.003173913	0.993652
6	0.005826087	0.988348
21	0.013891304	0.972217
25	0.015478261	0.969043
27	0.01823913	0.963522
30	0.01880435	0.971552

Finding the value of the reaction rate constant (k) using semi logarithmic graph analysis. The plot of $\ln \frac{C_{A0}-C_B}{C_{A0}}$ as a function of time (t) will form a straight line with a slope of $-k$. Then plot the value $\frac{C_{A0}-C_B}{C_{A0}}$ against time (t) on a semi-logarithmic scale in the reaction rate analysis, where the slope value of this

linear equation will be equal to $-k$.

From Figure 7, it is obtained a straight line equation $y = -0.0009x + 0.9983$ so that it can be seen that the slope ($-k$) of the graph is -0.0009 . The value of the reaction rate constant (k) is 0.0009 , the reaction rate for the formation of bioethanol becomes: $r_B = (0.0009 \text{ hour}^{-1}) C_B$. The calculation of the scale up to a pilot plant scale algorithm:

Calculating the scale up ratio (R). Determining laboratory scale volume using equation for residence time (τ) = $\frac{C_B - C_{B0}}{-r}$ (3)

obtained $V_1 = 1,111.111 \text{ ml}^3 (= 0.000001 \text{ m}^3)$, $N_1 = 700 \text{ rpm}$ and $D_{T1} = 0.00618 \text{ m}$ regarding to $H/D = 2$ (Tipasri & Wongwuttanasatian, 2019).

$$V_1 = \left(\frac{\pi D_{T1}^2}{4}\right) (H_1) = \left(\frac{\pi D_{T1}^3}{2}\right) \quad (4)$$

Volume ratio:

$$\frac{V_2}{V_1} = \frac{\pi D_{T2}^3/2}{\pi D_{T1}^3/2} = \frac{D_{T2}^3}{D_{T1}^3} \quad (5)$$

$$R = \left(\frac{V_2}{V_1}\right)^{1/3} = \frac{D_{T2}}{D_{T1}} \quad (6)$$

Using R values for the magnification (Himmelblau & Riggs, 2012): $D_{a2} = R D_{a1}$. Determine the stirring speed for the new geometry (N_2) from the small-scale stirring results (N_1)

$$N_2 = N_1 \left(\frac{1}{R}\right)^n = N_1 \left(\frac{D_{T1}}{D_{T2}}\right)^n \quad (7)$$

$n = 1$; for the same fluid movement

Table 5 Calculation on scale up pilot plant for CSTR

Magnification (times)	V_2 (m ³)	D_{T2} (m)	N_2
1	0.000001	0.00618	700
10	0.001111	0.06178	70.0
100	1.111111	0.61779	7.00
200	8.888889	1.23557	3.50

Applying 200 times magnification from the laboratory scale up to industrial scale (8,888.89 L), the inside diameter was 1.23557 m, the cylindrical height and ellipsoidal head height were 2.47114 m and 0.61778 m so, the total tank height is 3.70671 m.

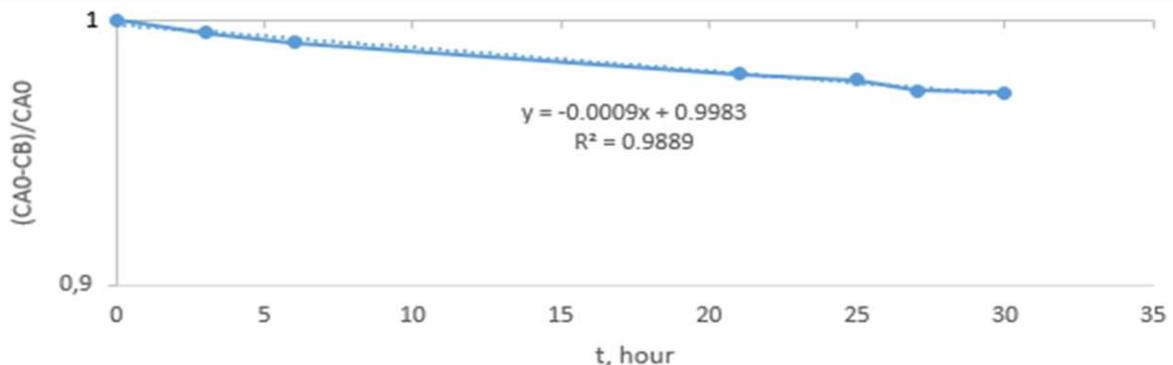


Fig 7. Figure 7. Semilogarithmic graph of the relationship between value $\frac{C_{A0}-C_B}{C_{A0}}$ against time

Conclusion

Optimization of the process of making bioethanol using a stirred tank reactor with banana rod samples. The variables used in the practicum are independent variables and dependent variables. independent variables include temperature, time, and enzyme ratio. While the dependent variable includes total alcohol and glucose levels. The optimum conditions for variable influence on alcohol content are obtained when the alcohol content value is more than 17.2% with an enzyme ratio of 3 – 3.5 at a temperature of 80 - 85 °C for 35 - 40 hours. Optimization parameters of alcohol content in bioethanol from the saddlepoint banana rod against temperature, time, and enzyme ratio were determined by a critical value. Thus, the critical value for optimization of alcohol content is achieved at a temperature of 84.47908°C for 20.39485 hours with an enzyme ratio of 2.56383. The calculation of the scale up to a pilot plant scale found that it is possible to enlarge reactor up to 200 times by using a ratio to obtain the ideal stirred tank reactor dimensions with a conversion of 97.15%.

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Authors contributions

This experimental work, data collection, data analysis, illustrations, and manuscript preparation were completed by Qurrotun A'yuni Khoirun Nisa'.

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Availability of data and materials

The datasets generated for this study are available on request to the author.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agreed with this publication.

Competing interests

There are not competing interests

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Figures

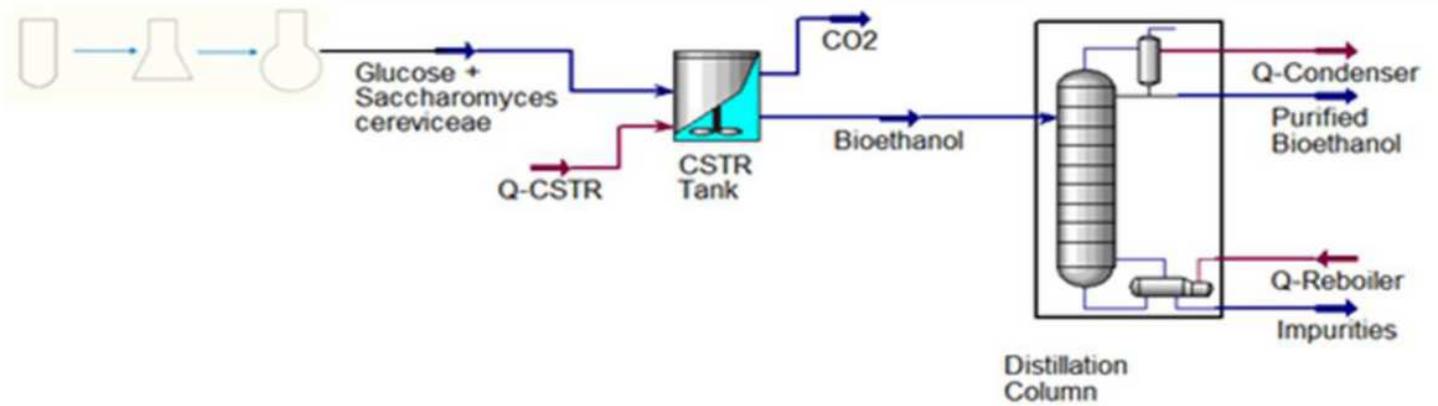


Figure 1

Bioethanol production process scheme

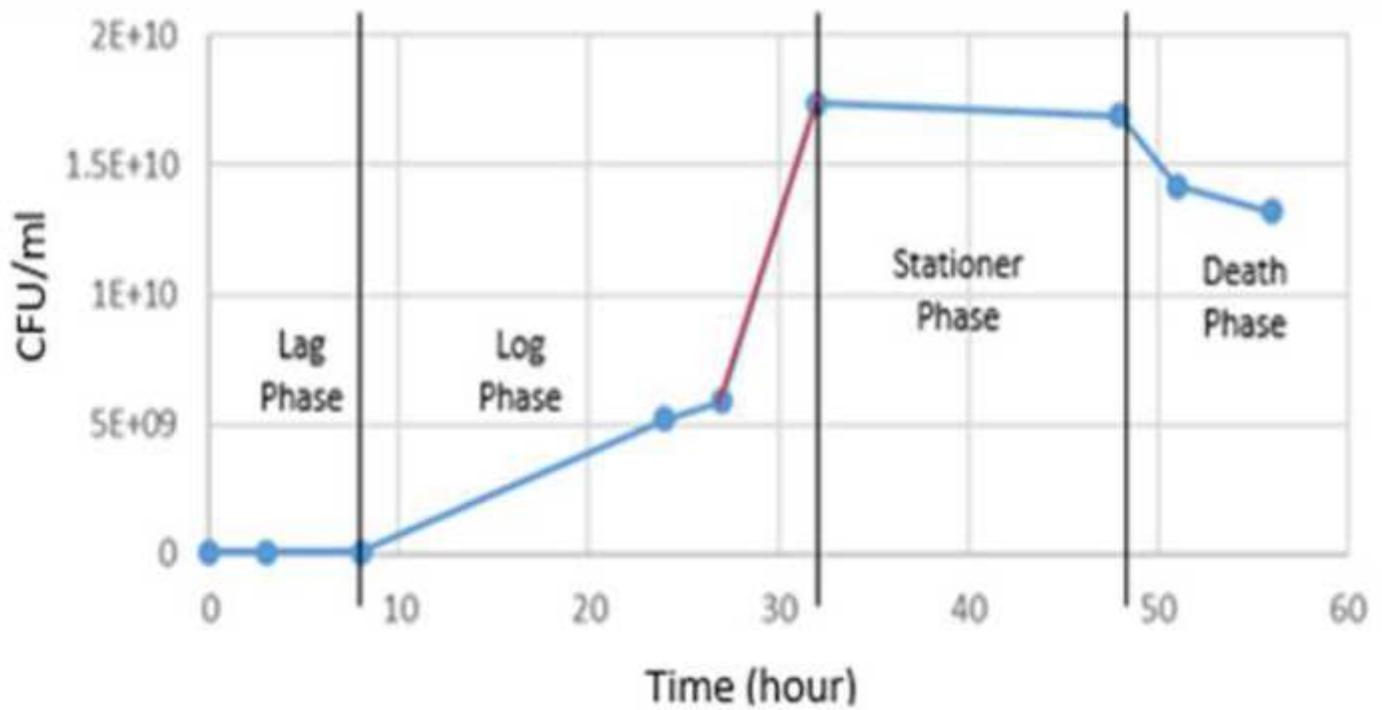


Figure 2

Growth of *Saccharomyces cereviceae*

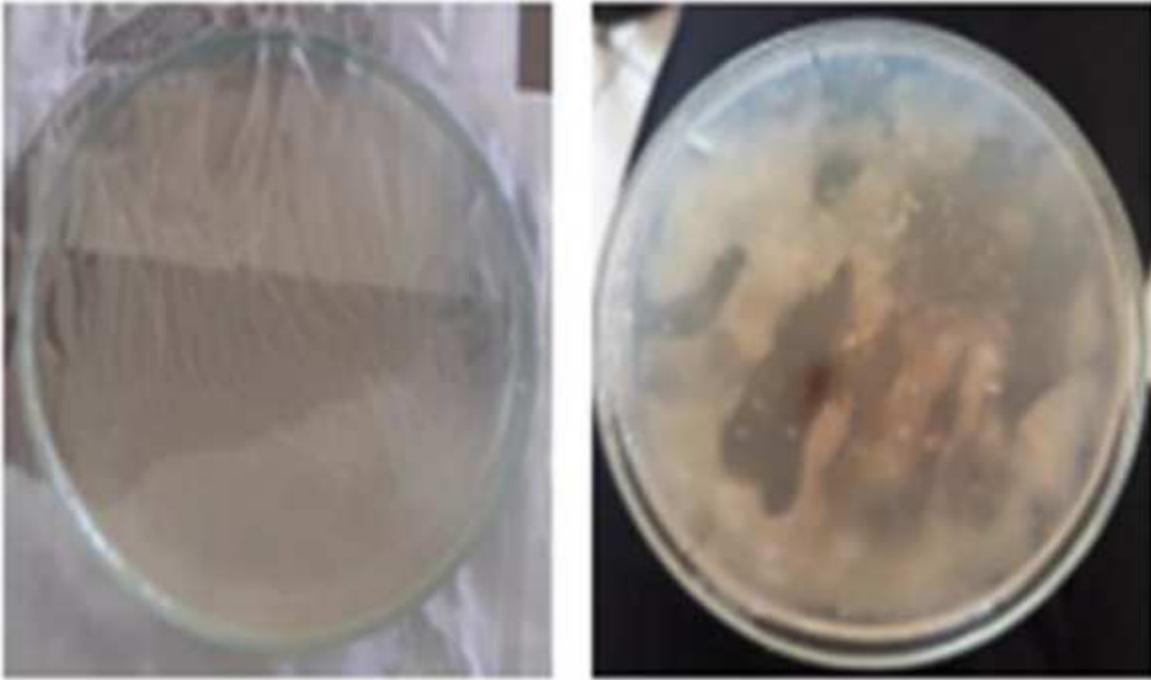


Figure 3

Growth of *Saccharomyces cereviceae* significant in hour 27 32

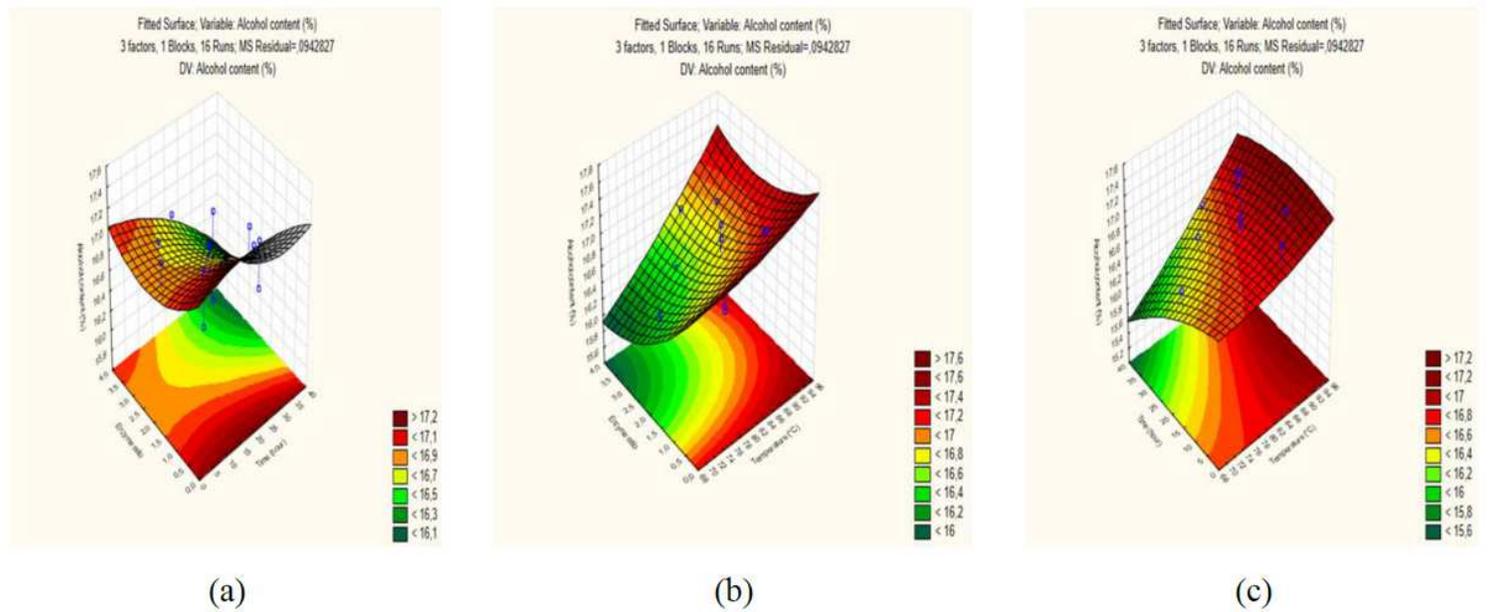


Figure 4

The response fitted surface variable temperature, time, and enzyme ratio to the alcohol content response

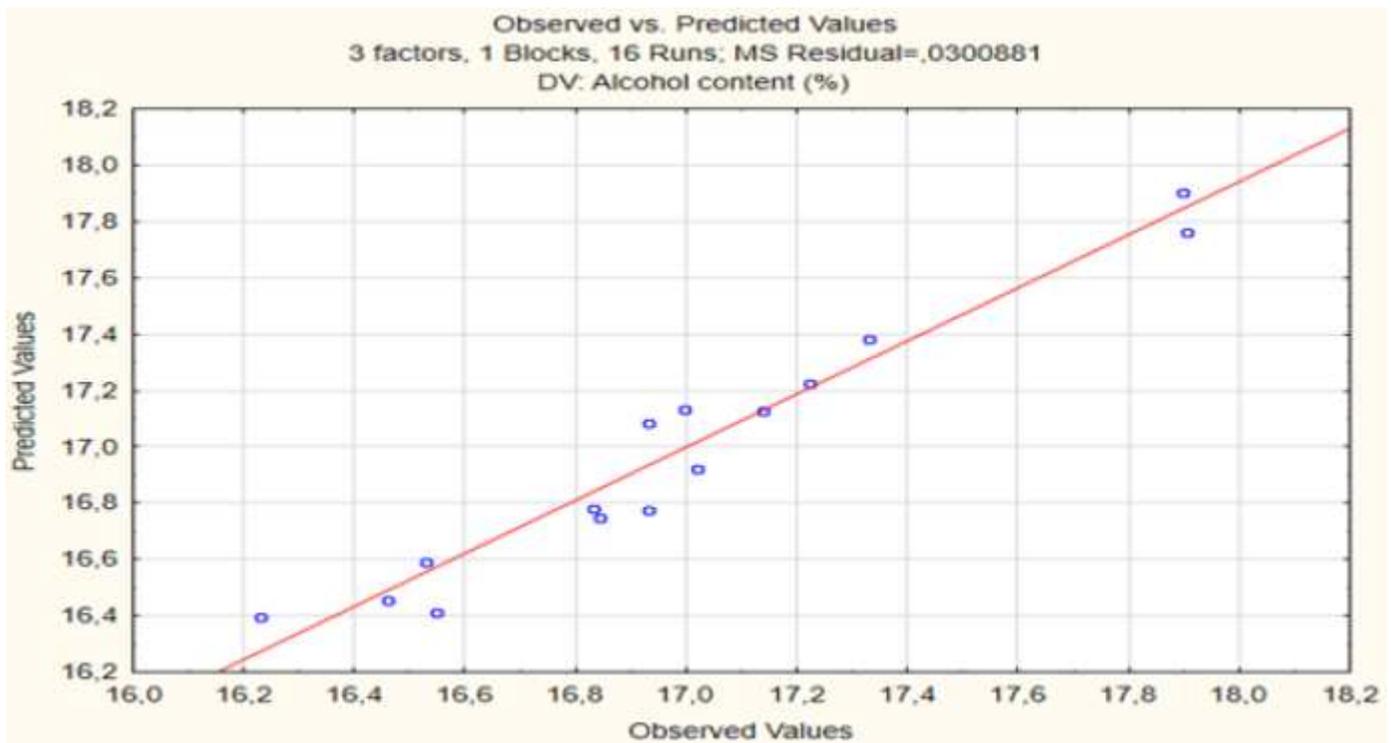


Figure 5

Comparison of experimental data and estimated alcohol content

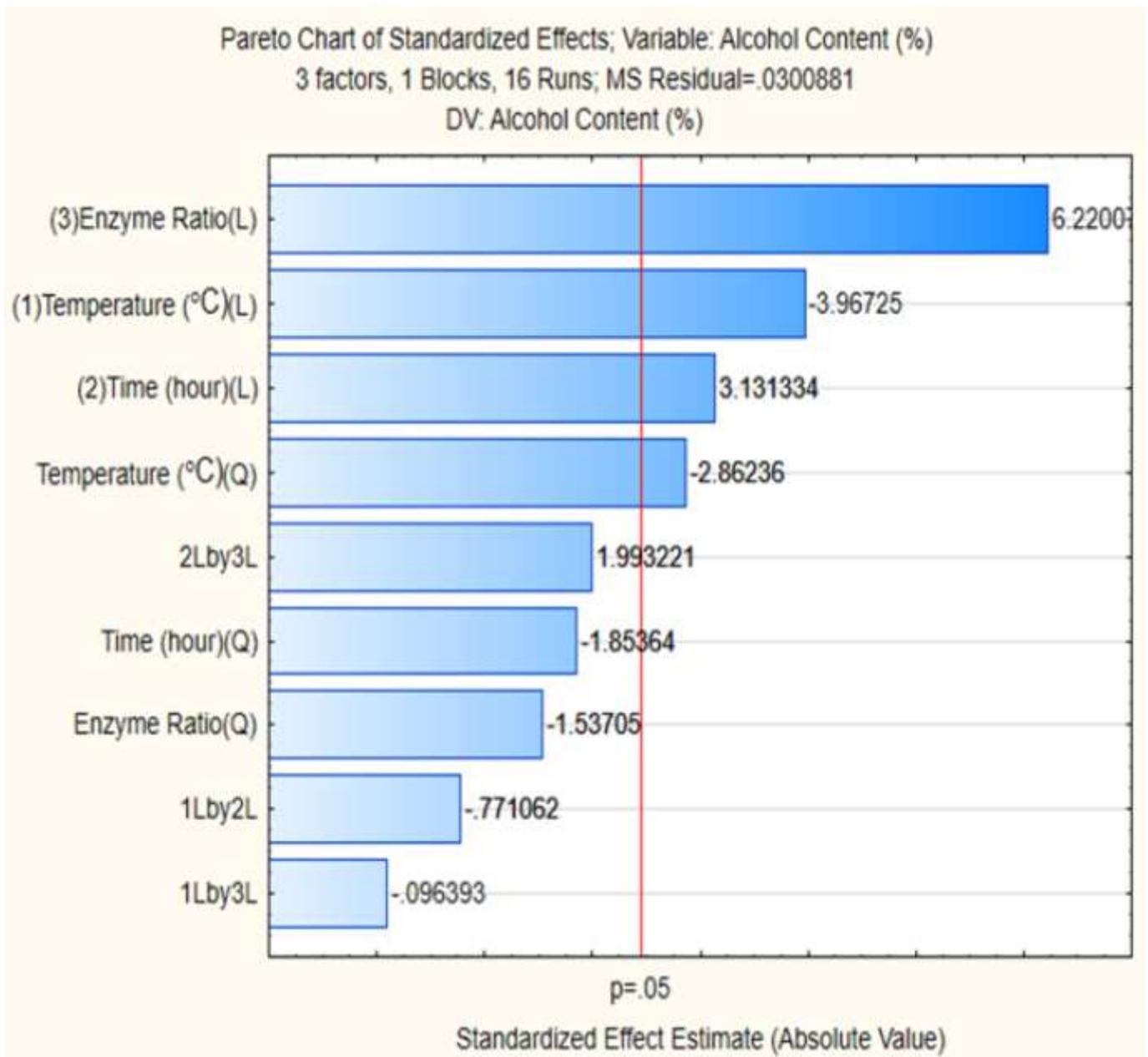


Figure 6

Pareto diagram of the effect of variables on alcohol content

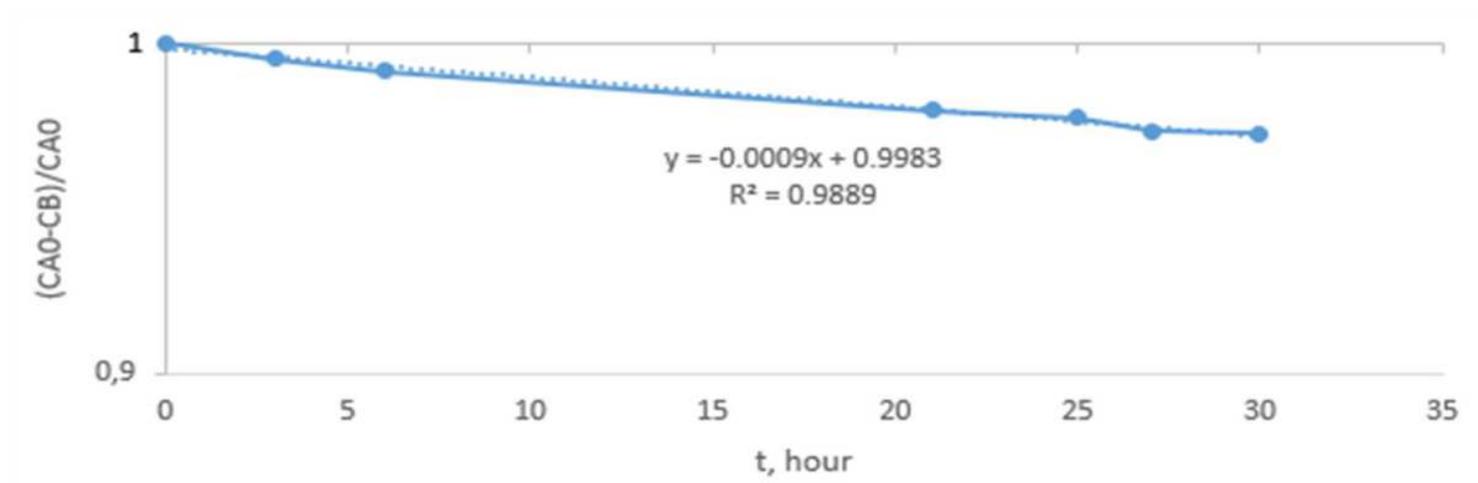


Figure 7

Figure 7. Semilogarithmic graph of the relationship between value $(CA_0 - C_B) / CA_0$ against time

Supplementary Files

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