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A Kinetic Study in Fermentation of Cocoa Pod Husk using Zymomonas Mobilis

by Nove Kartika Erliyanti

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A Kinetic Study in Fermentation of Cocoa Pod Husk using *Zymomonas Mobilis*

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A Kinetic Study in Fermentation of Cocoa Pod Husk using *Zymomonas Mobilis*

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Abstract. In many years, bioethanol research has been an outstanding topic because of renewable energy issue. The aim of this research is focused on utilization of cocoa pod husk as a feedstock for bioethanol production through fermentation using *Z. mobilis*. Moreover, the kinetic model of cocoa pod husk fermentation using *Z. mobilis* as a microorganism was also preformed in this study. Fermentation of cocoa pod husk was carried out at room temperature for 0, 2, 4, 6, and 8 days of reaction. The bacteria were also varied with 8%, 10%, 12%, 14%, and 16% (v/v) of concentration towards the substrate mixture. The kinetic model was conducted by Michaelis – Menten equation followed by Eadie-Hofstee plot and batch kinetic data plot for its fitting parameter technique. The result of this study indicates that the slow reaction has occurred regarding the low value of conversion and small value of maximum reaction velocity. The highest conversion was achieved on the 8th day with 14% (v/v) of bacteria concentration that is 10.65%. The maximum velocity of reaction for 16% (v/v) of bacteria addition was obtained at approximately 0.0017 mol/L.hour.

1. Introduction

Biofuels have been a promising research topic in this decade due to the depletion of petroleum reserves as main source of fossil fuel. This kind of renewable energy was developed because of not only about the energy crisis but also an environmental issue like global warming and air pollution [1]. One of the most common and potential compounds among many types of biofuel is bioethanol. Bioethanol usually was synthesized by fermentation of sugar component from plantation crops like cassava, corn, and even cellulosic biomass [2]. Bioethanol has been used as a substitute engine fuel and fuel additive in many countries like Brazil and US. Ethanol basically has suitable properties for spark ignition IC engines because its motor octane number (MON) value achieves 90, compared to 91 for gasoline [3].

Many studies have been conducted relating to bioethanol synthesis from various crops like sugar cane, sorghum, cassava, and other organic waste [4]–[6]. Recently, the lignocellulosic biomass also can be converted into bioethanol via the developed advanced technology including delignification, saccharification, and strong acid hydrolysis. It made many kinds of biomass like trees, grasses, and other biomass wastes like fruit peel are available for bioethanol production feedstock [6], [7]. Cocoa pod husk is one of the most abundant biomass in Indonesia because this country has large the cocoa plantation [8]. Moreover, cocoa pod husk contains around 35% of cellulose and 32% of carbohydrates as a main raw material for bioethanol production [9]. On the other hand, various types of microorganisms have been reported for the fermentation process in biodiesel production. The most

common microorganism for fermentation is *Saccharomyces cerevisiae* or usually called yeast that belongs to fungi group [2]. But it has found that *Zymomonas mobilis* can metabolize glucose from cellulosic biomass effectively and yields more ethanol than that of *S. cerevisiae*. This bacterium processes glucose through the Entner-Doudoroff pathway and gives many advantages like less ATP generated, and also its high temperature tolerance and low pH resistance [10].

Synthesis of ethanol from cocoa pod husk had been reported by Samah et.al. using *S.cerevisiae* and could produce ethanol until 17.3% v/v after 26 hours of fermentation [8]. Unfortunately, there are not many investigations that use *Zymomonas mobilis* for converting cocoa pod husk which contains lignocellulose into bioethanol. This study was focused on utilization of cocoa pod husk as a feedstock for bioethanol production through fermentation using *Z. mobilis*. Furthermore, a kinetic study was performed in this investigation using enzymatic reaction model. The objective of this research is to develop the kinetic model of cocoa pod husk fermentation using *Z. mobilis* as a microorganism. Certainly, this study would be beneficial for the scale-up of bioethanol production from cocoa pod husk using *Z.mobilis*.

Methodology

2.1 Materials
The cocoa pod husk with moisture content approximately 60 – 80% was gained from the cocoa plantation at Kademangan, Blitar, Indonesia. *Zymomonas mobilis* bacteria as a source of enzymes was used in this study including with the nutrient broth as its growth media. Other supporting materials utilized for experimental process were 97% concentrated H_2SO_4, NaOH, and glucose and obtained from local market. Otherwise, a set of experimental apparatus containing hydrolysis and fermentation reactor unit, and also a set of distillation equipment were utilized in this study. Furthermore, many supporting tools like thermometer, oven, analytical balance, autoclave, incubator, and other related laboratory tools were used in this experimental section.

2.2 Experimental Section
Bioethanol was synthesized from cocoa pod husk by four processes which are delignification and hydrolysis in order to convert cellulose contained in cocoa pod husk into glucose, fermentation process to produce bioethanol, and purification by distillation method. 100 grams of the cocoa pod husk had been processed in this experimental step. Delignification of the cocoa pod husk was carried out using 10% of NaOH solution to dissolve its lignin compound. Then, the cocoa pod husk was hydrolyzed by 10% (v/v) of H_2SO_4 solution at 100^0C for 3 hours. This hydrolysis process aims to break down the complex and long chain of cellulose into simple compound like glucose and fructose. After that, the fermentation process was conducted at room temperature for several days that are 0, 2, 4, 6, and 8 days. This process used a starter from inoculated *Z. mobilis* bacteria on the media that are incubated at 30^0C for 1 day. Then, this starter was added into the reactor with the variables performed of 8, 10, 12, 14, and 16% (v/v). The fermented product was purified using distillation method to obtain high purity bioethanol product. Bioethanol content was analyzed by simple technique using density and specific gravity measurement. Its specific gravity, then was compared by ethanol content standard from AOAC (Association of Official Agricultural Chemists) table and its ethanol content value could be calculated regarding by the table [11].

2.3 Kinetic Model
Basically, the fermentation of glucose is an enzymatic reaction which the chemical equation is given below [3],

$$C_2H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \qquad (1)$$

Or, it could be written in simple way with,

$$A \rightarrow 2B + C \tag{2}$$

The kinetic model of a single-substrate enzymatic reaction was well known as Michaelis – Menten kinetic equation [12][13]. This kinetic equation was expressed by,

$$(-r_A) = -\frac{dC_A}{dt} = \frac{V_M C_A}{K_M + C_A} \tag{3}$$

Where, $(-r_A)$ is a kinetic rate of A reactant depletion and can be changed by the decreased of reactant A concentration over time or $-dC_A/dt$. Then, V_M is the maximum forward velocity of the reaction and K_M is the Michaelis – Menten constant.

The kinetic model was developed by fitting data parameter method. Firstly, the equation (3) was converted to linear equation by two method that are Eadie-Hofstee plot and batch kinetic data plot [13].

Eadie-Hofstee plot technique was carried out by changing the equation (3) into this linear equation,

$$(-r_A) = V_M - K_M \frac{(-r_A)}{C_A} \tag{4}$$

The kinetic rate of $(-r_A)$ was obtained from the slope of $-dC_A/dt$ using the same differential technique for batch kinetic data [12]. Then, $(-r_A)$ vs $(-r_A)/C_A$ was plotted resulting a linear regression with slope $-K_M$ and V_M as y-axis intercept. The other method used is batch kinetic data plot which is an integration of equation (3) into linear equation [13].

$$V_M t = C_{A0} - C_A + K_M \ln \frac{C_{A0}}{C_A} \tag{5}$$

or,

$$V_M - \frac{C_{A0} - C_A}{t} = \frac{K_M}{t} \ln \frac{C_{A0}}{C_A} \tag{6}$$

The linear plotting of $1/t \ln (C_{A0}/C_A)$ vs $(C_{A0} - C_A)/t$ was conducted yielding a line of slope $-1/K_M$ and V_M/K_M as intercept. From these two methods, the maximum velocity of reaction and the Michaelis-Menten constant was obtained.

3. Results and Discussion

Cocoa pod husk has a high carbohydrate and cellulose content, so that these compound should be converted first into simple sugar namely glucose by hydrolysis process [9]. Sulphuric acid solution with 10% (v/v) of concentration used in this hydrolysis and producing glucose with 9.89% (v/v) of concentration. The resulting glucose product is quite smaller than Nazir et al. that used 1.5% (w/v) of sulphuric acid solution yielding glucose content until 15.59% as reducing sugar [14]. This glucose compound, then being a reactant for the fermentation process and the conversion (%) of this reaction is shown at figure 1 and figure 2.

Glucose levels is not only the most appropriate factor that influences the reaction kinetics but also other factors like temperature, pH, microorganism, and reaction time [3]. The temperature condition in this study was set at room temperature, while the pH level was maintained around 5 which is the most suitable environment for the growth of *Z. mobilis* [15]. Based on figure 2, it can be seen that the conversion of reaction (%) is still low under 12% after 192 hours of fermentation. Generally, more enzymes were added into the reaction which is expressed on bacteria concentration (% v/v), would make the reaction kinetics more faster resulting the higher conversion of reaction.
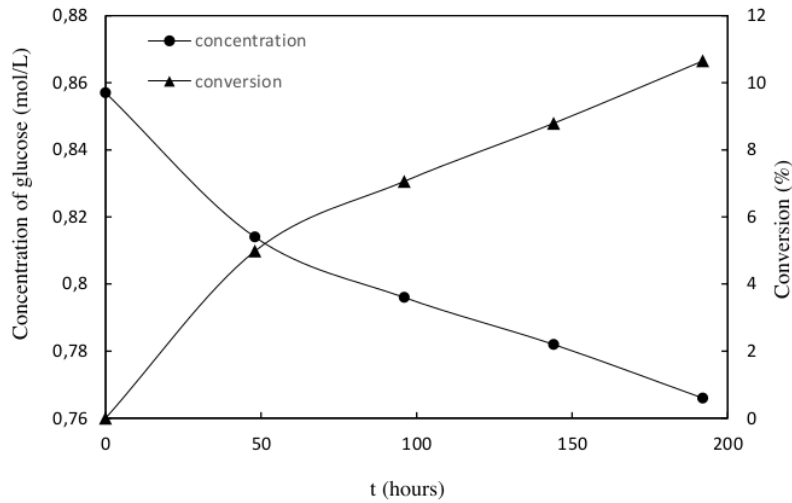
Figure 1. The decreasing of glucose concentration (mol/L) along with the rise of conversion (%) respect to time
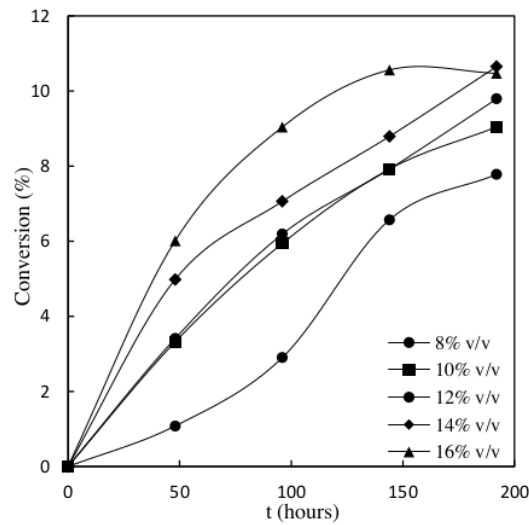

Figure 2. Plot conversion (%) vs time (hours) at various bacteria concentration.
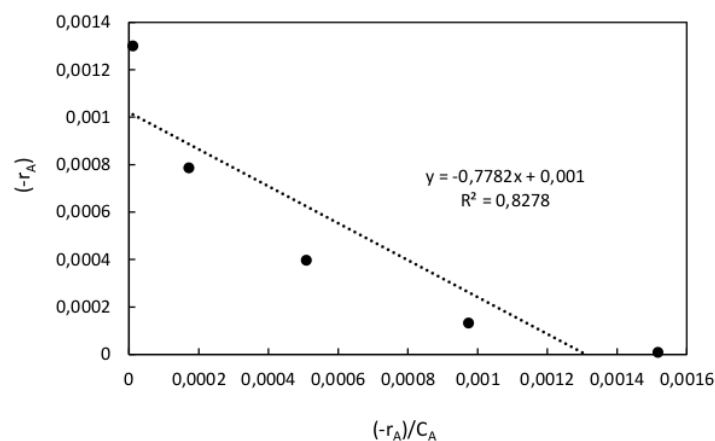
Figure 3. Eadie-Hofstee plot of fermentation with 16% (v/v) of bacteria concentration.

But there is an anomaly while bacteria concentration rises until 16% (v/v) showing lower conversion after 150 hours reaction. It could be happened because ethanol or other side product compound accumulates in the mixture inhibiting the *Z. mobilis* cell growth [15]. Furthermore, figure 1 shows that the concentration of glucose was reduced slowly below 0.1 mol/L along with conversion rise to around 10%. It might be concluded that the reaction rate was very slow regarding with lower conversion reaction and the depletion of glucose was quite insignificant.

Glucose concentration data was used for determination of reaction rate ($-r_A$) via slope $-dC_A/dt$. Then, this data was plotted using Eadie-Hofstee method as per shown in figure 3. From Eadie-Hofstee plot, the linear equation was obtained by linear regression that is y = -0.7782 x + 0.001 where -0.7782 of slope is $-K_M$ and 0.001 of intercept is V_M. It can be concluded that from Eadie-Hofstee plot resulting K_M value of 0.7782 and 0.001 of the maximum velocity (V_M). Otherwise, batch kinetic data plot shows different result yet more accurate regression than Eadie-Hofstee plot. Batch kinetic data plot gives linear equation that is y = -1.1544 x + 0.0017 with root means square deviation (R^2) reach 0.9672. From this equation, K_M and V_M can be calculated by its slope and y-axis intercept yielding 0.866 of K_M and 0.00147 of V_M. Batch kinetic data gives the Michaelis – Menten kinetic parameter value higher and more accurately than that of the Eadie-Hofstee plot. It might be happened since Eadie-Hofstee plot equation contains ($-r_A$) at both coordinates so that yields more error [13]. Moreover, the kinetic model gives a brief reason for the low conversion reaction indicating from the small value of maximum reaction velocity.

4. Conclusion

Synthesis of bioethanol from cocoa pod husk via fermentation using *Zymomonas mobilis* was successfully done. Unfortunately, the conversion of reaction and the reaction rate is still low. From Eadie-Hofstee plot and batch kinetic data plot, it can be concluded that the rate of fermentation is very slow based on the small value of maximum reaction velocity (V_M).
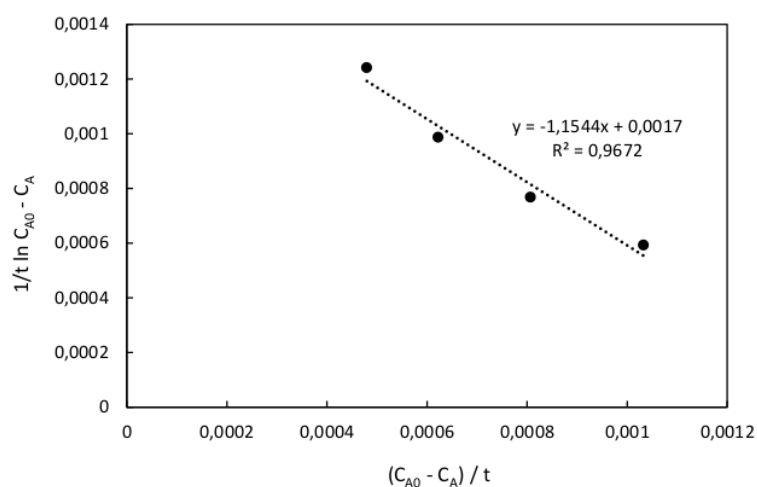
Figure 4. Batch kinetic data plot of fermentation with 16% (v/v) of bacteria concentration.

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