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Fractal kinetics analysis of enzymatic hydrolysis of sawdust using cellulase in ethanol production

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Abstract

Sawdust is one of the abundantly lignocellulosic materials in the world. Sawdust is considered promosing for ethanol production, because it contains mainly lignin, hemicellulose, and cellulose. The drying process was applied to pretreat sawdust to make its degradation process easier. Biodegradation of sawdust was conducted by enzymatic hydrolysis using cellulase. The volume of cellulase in the hydrolysis substrate was varied from 5 to 9% v/v. The sugar concentration produced by enzymatic hydrolysis of sawdust every 1 h was recorded as well as its fractal kinetics analysis. Fermentation using yeast in 5 days was also performed to convert sugar hydrolysate to ethanol. Optimal sugar concentration in hydrolysate obtained was about 0.15 mol/L with cellulase volume of 9% v/v and its ethanol concentration was about 0.059% v/v. Fractal kinetics models by Kopelman and Valjamae which can quantitatively describe enzymatic hydrolysis of sawdust using cellulase were used. However, the result of this study indicated that, at high enzyme volume (9% v/v), Valjamae model was more suitable than Kopelman. The fractal exponent value (h) was about 0.667 and the rate constants (k) were about 0.44, 0.53, and 0.58 1/h at the enzyme volume of 5, 7, and 9% v/v. Thus, it can be concluded that enzyme volumes significantly effect rate constants.

INTRODUCTION

Nowadays, fossil based energy sources are decreasing, so it is important to explore an alternative energy like bioethanol which can be used to replace or substitute gasoline as liquid fuel (Fajariah & Hadi, 2014). Bioethanol is an ethanol that is produced from lignocellulosic materials, such as agriculture waste, municipal organic waste, paper waste, etc (Barlianti et al., 2015; Saliu, 2012, Sharma et al., 2012). Chemical and enzymatic processes can be applied to convert lignocellulosic materials into ethanol (Singh et al., 2014; Yu et al., 2014). The main steps in lignocellulosic ethanol production are pretreatment to remove water content and to reduce lignin content, hydrolysis to break polimer bonds of holocellulose into sugar, fermentation to convert sugar into ethanol, and purification to obtain anhydrous ethanol (Itelima et al., 2013; Hikmiyati & Sandrie, 2008; Haryono, 2010).

Sawdust, as one of lignocellulosic materials, can be used as a raw material for ethanol production (Megawati et al., 2015; Irawati et al., 2009; Pelaez et al., 2013). The conversion of holocellulose to sugar is usually catalysed by hydrolytic enzymes, because enzymatic hydrolysis has higher product selectivity than acid-catalysed hydrolysis (Singh et al., 2014; El-Zawawy et al., 2011). Enzymatic hydrolysis is a common process

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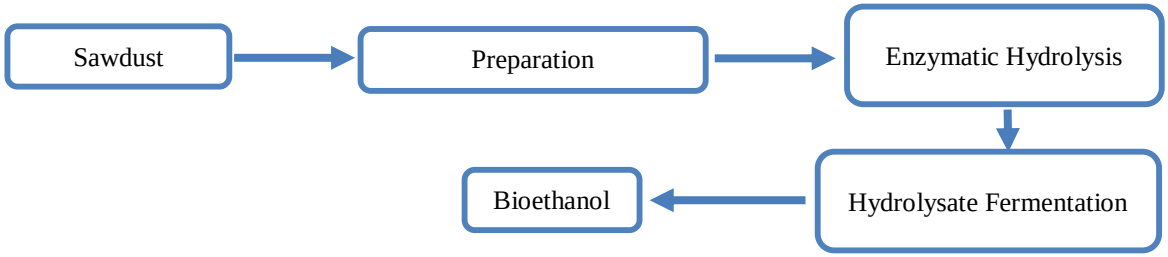

Figure1. Procedure steps of ethanol production from sawdust

for lignocellulosic materials (Stevanie et al., 2017). In addition, hydrolysis of holocellulose using acid as a catalyst will produce another kinds of sugar, like fulfural compounds and acetic acid (Wang et al., 2011). The sugar resulted in hydrolysis is then converted into ethanol by fermentation using yeast (Isroi, 2008). In the present study, sawdust was hydrolysed using cellulase and then fermentated using yeast. Lignocellulosic materials can be converted into fermentable sugar using cellulase and hemicellulase secreted from degrading microorganisms (Gottschalk et al., 2010; Sharma et al., 2012; Singh et al., 2011, Wang & Feng, 2010). Enzymatic hydrolysate can be fermented using *Saccharomyces cerevisiae* (yeast) to produce ethanol (Singh et al., 2014).

The multi-component nature of cellulose substrates makes enzymatic hydrolysis process even more complex. Therefore, homogeneous kinetics model like Michaelis-Menten model is not suitable to express enzymatic hydrolysis of lignocellulosic materials (Wang & Feng, 2010). Meanwhile, fractal kinetic analysis is suitable for a heterogeneous chemical reaction like enzymatic hydrolysis (Kopelman, 1988). According to Valjamae et al. (2003) and Bommarius et al. (2008), a simple fractal kinetic model has also been introduced to fit the data of enzymatic hydrolysis. The fractal kinetic equation of enzymatic hydrolysis of cellulose was also derived by Shen-Agblevor based on Michaelis-Menten equation (Shen & Agblevor, 2011). This paper reported the effect of cellulase volumes on sugar concentration produced by hydrolysis and its fractal kinetics as well as ethanol concentration obtained in fermentation.

RESEARCH METHODOLOGY

Preparation for sawdust and cellulase enzyme

In the present work, the sawdust used was from the waste of a home woodcraft industry in Semarang, Indonesia. Isolated *Aspergillus niger* was obtained from "Biotek, the laboratory of the Faculty of Agriculture Technology, Universitas Gadjah Mada" and dried baker's super yeast was obtained from a Local Chemical Shop. The procedure steps of ethanol production from sawdust can be seen in Figure 1. The preparation steps were sawdust preparation, inoculation for *Aspergillus niger*, and cellulase isolation. The sawdust was prepared as follows: sun-dried for several days, oven-dried at 50°C until a constant weight was obtained, chopped into small size, ground and sieved to get 40 mesh of sawdust. For the *Aspergillus niger* inoculation, the media used was made from 10 g sugar, 5 g jelly powder, and 1 tablet B-complex vitamin in a sterilised test tube. After the isolated *Aspergillus niger* growed on the media, the test tube was sealed and stored in a refrigerator under 4°C.

The initial step to obtain cellulase was making a starter solution to produce *Aspergillus niger* spora suspension. The starter solution was made from suchrose 12.5% (w/v), urea 0.25% (w/v), KH_2PO_4 0.2% (w/v), which were dissolved in 100 mL of aquadest. After that, isolated *Aspergillus niger* was submerged in the starter solution and sealed with aluminium foil and incubated at 30°C for 24 h. *Aspergillus niger* would grow on the surface of the stater and produce *Aspergillus niger* spora suspension. After that, the spora suspension was used to produce cellulase that was performed by dissolving 10 mL of the suspension in a sterilised mixture of 20 g of sawdust, 0.03 g of urea, 0.005 g of $MgSO_4.7H_2O$, 0.0023 g of KH_2PO_4, 80 mL of aquadest, and 1 of mol/L HCl for reaching a pH of 5. The mixture was sealed with aluminium foil and kept at 30°C for 96 h to obtain cellulase. The cellulase produced was extracted using 100 mL of aquadest. The extracted solution was soaked using rotary shaker with a speed of 150 rpm for 1 h, filtered, and the filtrate was stored in a refrigerator.

Hydrolysis and fermentation

The hydrolysis was conducted using 10 g of sawdust powder in 100 mL of HCl solution at a pH of 4-5. The mixture was heated in autoclave at

100°C for 30 min for sterilisation. The enzymatic hydrolysis was performed using various cellulase volumes (5, 7, and 9% v/v) in an Erlenmeyer flask which was sealed by plastic and shaken by a rotary shaker with a speed of 160 rpm for 9 h, and every 1 h, the sample was taken. The sugar concentration analysis was done using Fehling Method. The anaerob fermentation was performed for 5 days and on several experimental conditions (hydrolysate volume = 100 mL, yeast used = 4 g, and pH = 4.6). The adjustable pH was conducted by adding 1 mol/L of a sulfuric acid solution. This fermentation result was filtered by screening paper. The ethanol concentration was analised by GC (Gas Chromatography) with specification GC Clarus 680) (MS Clarus SQ ST).

Fractal kinetics analysis of enzymatic hydrolysis

A time course of enzymatic hydrolysis of lignocellulosic materials is described as Eq. 1 by Kopelman and commonly called as fractal kinetics of cellulose enzymatic hydrolysis, where C_s = residual sugar concentration, C_p = product sugar concentration, C_0 = substrate concentration, k = reaction rate constant, t = time, h = fractal exponent. The fractal exponent is confirmed to $0 \leq h < 1$ (Wang & Feng, 2010).

$$C_S = C_0.exp\left[-k\left(1+\frac{t^{1-h}-1}{1-h}\right)\right] \quad (1)$$

When h = 1, this equation can be expressed as a simple equation as Eq. 2 (Wang et al., 2012).

$$C_s = C_0.exp[-k(1+\ln(t))] \quad (2)$$

The other empirical equation for heterogeneous kinetics model is written in Eq. (3). This equation is similar to a fractal-like kinetic model based on Eq. 1 and was proposed by Valjamae et al (Wang & Feng, 2010).

$$C_p = C_0[1-exp(-k.t^{1-h})] \quad (3)$$

The calculation result of sugar production concentration was fitted with the fractal kinetics model. The model parameters, such as rate constant (k) and fractal exponent (h) were estimated by minimizing the sum of squared errors of prediction (SSE) between the simulated values and the experimental data. The SSE formula is shown in Eq. 4

$$\text{SSE} = \sum_{i=1}^{n}\left(C_{p(ci)} - C_{p(ei)}\right)^2 \quad (4)$$

where $C_{p(ci)}$ = the calculation value of the product concentration, $C_{p(ei)}$ = the experimental value of product concentration and i = the observed time. This simulation was calculated by a curve-fitting method.

RESULTS AND DISCUSSIONS

The effect of enzyme volume and hydrolysis time on sugar concentration

The analysis result indicated that the sawdust used in this present study contained 46% of cellulose and 20% of hemicellulose. Therefore, the sawdust can be hydrolysed into 0.74 g of sugar per 1 g of dry sawdust. With further processes, the sugar can be fermented into 0.38 g of ethanol per 1 g of dry sawdust. It can be concluded that sawdust is one type of biomass that has good prospects for producing bioethanol. Through optimisation of the process variables, optimal conversion will be obtained and the operational cost will also be economical.

In this study, the sugar yields obtained were about 18.9, 21.96 and 26.46% w/w at cellulase volumes of 5, 7 and 9% v/v. Therefore, in order to get more sugar yield, the cellulase volume should be increased. Enzymatic hydrolysis with *Aspergillus niger* culture filtrate from corncobs, peanut shells, and sawdust, respectively, produced sugar yields of 0.63, 0.03 and 0.06 mg/mL (Saliu, 2012). Saha et al. studied enzymatic hydrolysis and lime pretreatment of rice husks and found that the maximum yield of monomer sugar was 154±71 mg/g (32% yield). Thus, in this study, the sugar yield from hydrolysis of sawdust can be increased if the raw material goes under the pretreatment process first.

The sugar production depends on the enzyme volume and hydrolysis time. The data of sugar concentration analysed by Fehling method resulted from enzymatic hydrolysis using cellulase of sawdust was shown in Figure 2. The production of reducing sugar increases as the volume of enzyme increases within the hydrolysis process (up to 9 h). Compared to the hydrolysis level at 7% v/v, there was a slight increase in the sugar concentration when the cellulase volume was 9% (Figure 2). The highest sugar concentration (0.275

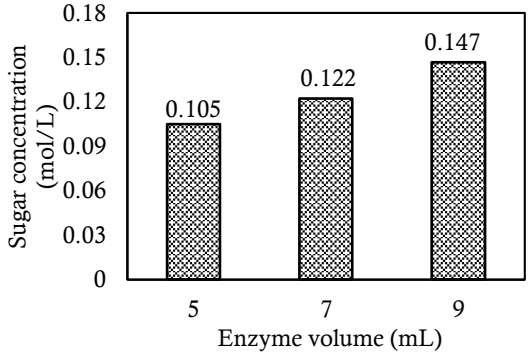

Figure 2. The effect of cellulase volumes on sugar concentrations in enzymatic hydrolysis of sawdust (On the conditions: weight = 10 g, volume = 100 mL, temperature = 30°C, time = 9 h and shaker speed = 160 rpm).

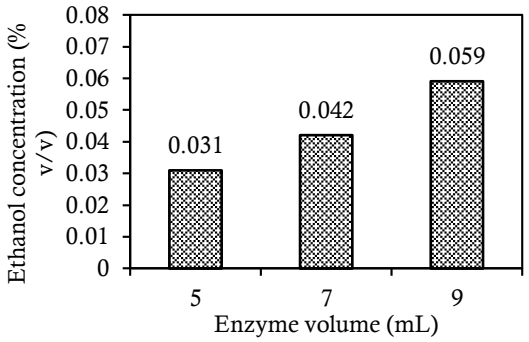

Figure 3. The effect of cellulase volumes on ethanol concentration in sawdust hydrolysis in fermentation (On the conditions: time = 5 days, volume = 100 mL, pH = 4,6, yeast used = 4 g).

mol/L) was obtained when the enzyme volume of 9% v/v was applied. The conversion obtained in this condition was 0.495. According to Sebayang (2006), reaction conversion increases when the enzyme volume increases. Based on the research conducted by Valjamae et al., a high cellulaseconcentration enchances the degree of conversion. However, the increase in the enzyme volume has its limits, at a certain volume, the hydrolysis rate is not significant anymore. In the oil palm empty fruit bunch hydrolysis with cellulase, the sugar concentration and hydrolysis velocity begin to stabilise at the enzyme volume of 5.148% v/v (Barlianti et al., 2015).

The effect of enzyme volume in hydrolysis on sugar concentration from fermentation

The effect of the amount of enzyme used on hydrolysis to ethanol content produced in fermentation is presented in Figure 3. The sample used was the fermentation on the fifth day. Figure 3 indicates that the more the amount of enzyme, the more the ethanol content increases. It can be concluded that the higher the number of enzyme, the higher the sugar concentration will be, so ethanol produced from fermentation will be more numerous (Usmana et al., 2012). This also indicates that the sugar produced from hydrolysis in different enzyme amounts can be fermented well by yeast. Thus, the enzyme volume increase of 5 to 9% v/v has not given rise to an inhibitory compound that may interfere its fermentation. This is in line with Singh et al. 2014, who reported that enzymatic hydrolysis using cellulase did not generate inhibitors and the enzymes were very specific for cellulase. In their research, when the sugar concentration was increased from 10 to 25 g/L, the ethanol concentration increased by 57%.

Hydrolysis kinetics using fractal models

The kinetics of hydrolysis reaction of cellulose into sugar using cellulase as a catalyst was approached with fractal kinetics models proposed by Kopelman and Valjamae. These two models examined the phenomenon of complex mass transfer when an enzyme enters the structure of sawdust. The basic equation used was the model proposed by Michaelis-Menten. The results of the fractal kinetics analysis are presented in Figure 4-6 with various enzyme volumes (5, 7, and 9% v/v). At the enzyme volumes of 5 and 7%, Kopelman model with h = 1 is more suitable to describe the hydrolysis of sawdust using cellulase, compared to Kopelman model with h = 0.68 and Valjamae model. However, Valjamae model is more suitable to describe the hydrolysis of sawdust when the enzyme volume is 9%. These three models are unique. At the enzyme volume of 5%, the sugar concentration increased slowly. At the enzyme volume of 7%, at the beginning of the reaction, the sugar concentration immediately increased, but then it did gradually. The most dramatic increasing number of sugar concentration occurred at the enzyme volume of 9%. Respectively, within 1 h of hydrolysis, the sugar concentrations obtained were 0.08, 0.15, and 0.175 mol/L, at the enzyme volume of 5, 7, and 9%. The kinetics parameters values for these three models are also presented in Table 1.

The rate constants of the reaction speeds increased because the enzyme volumes used were increased. This is in accordance with Bommarius et al. who examined the effect of different cellulase

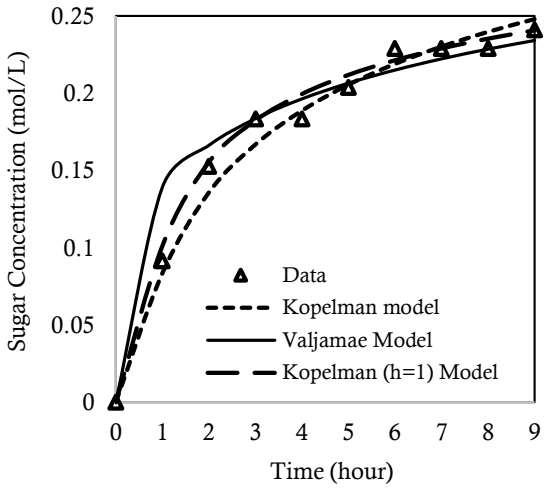

Figure 4. Fractal kinetics analysis of enzymatic hydrolysis of sawdust using 5% v/v of cellulase (On the conditions: weight = 10 g, volume = 100 mL, temperature = 30°C, and shaker speed = 160 rpm).

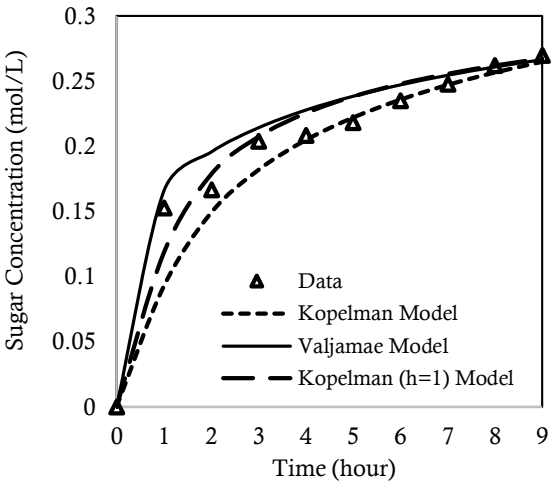

Figure 5. Fractal kinetics analysis of enzymatic hydrolysis of sawdust using 7% v/v of cellulase (On the conditions: weight = 10 g, volume = 100 mL, temperature = 30°C, and shaker speed = 160 rpm)

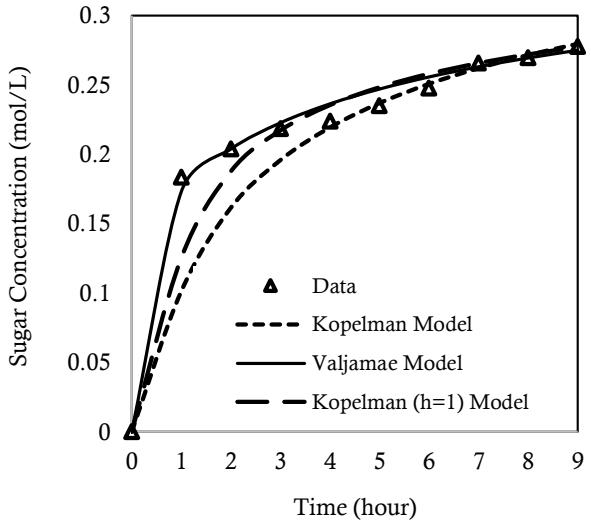

Figure 6. Fractal kinetics analysis of enzymatic hydrolysis of sawdust using 9% v/v of cellulase (On the conditions: weight = 10 g, volume = 100 mL, temperature = 30°C, and shaker speed = 160 rpm)

Table 1. Fractal kinetics parameters of sawdust hydrolysis using cellulase (On the conditions: temperature = 30°C, working volume = 10 g/100 mL, pH = 4-5, and shaker speed = 160 rpm)

	Valjamae Model	Kopelman Model	
Reaction rate constant, k (1/h)			
(5% v/v enzyme)	0.2400	0.3567	0.2995
(7% v/v enzyme)	0.2700	0.4300	0.3600
(9% v/v enzyme)	0.3000	0.4700	0.3864
Fractal exponent, h	0.6672	0.6200	1.0000

volumes on enzyme hydrolysis of Avicel as its substrate. He reported that the rate coefficients and fractal exponents increased as the cellulase volumes increased (Bommarius et al., 2008). Similarly, high cellulase volumes increased the fractal exponents of enzymatic hydrolysis of newsprint waste (Wu and Ju, 1998). Their experiments were conducted in 30 mL of buffer solution (pH = 4.8), using 10g/100 mL

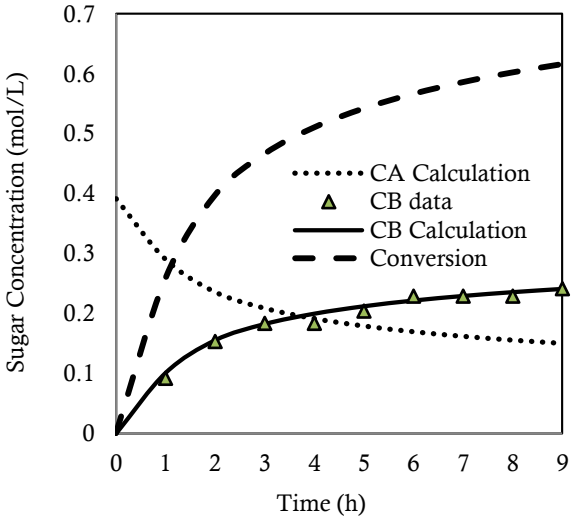

Figure 7. The profile of potential sugar (C_A) and sugar production (C_B) concentrations as well as reaction conversion of enzymatic hydrolysis of sawdust using 5% v/v of cellulase and heterogeneous kinetics model proposed by Kopelman (On the conditions: weight = 10 g, volume = 100 mL, temperature = 30°C, and shaker speed = 160 rpm)

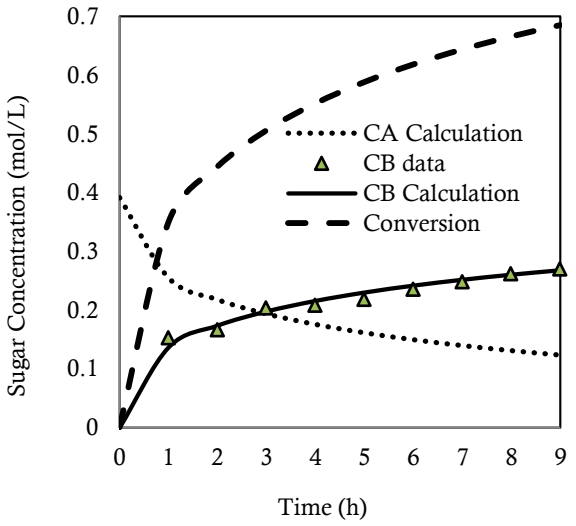

Figure 8. The profile of potential sugar (C_A) and sugar production (C_B) concentrations as well as reaction conversion of enzymatic hydrolysis of sawdust using 7% v/v of cellulase and heterogeneous kinetics model proposed by Kopelman (On the conditions: weight = 10 g, volume = 100 mL, temperature = 30°C, and shaker speed = 160 rpm)

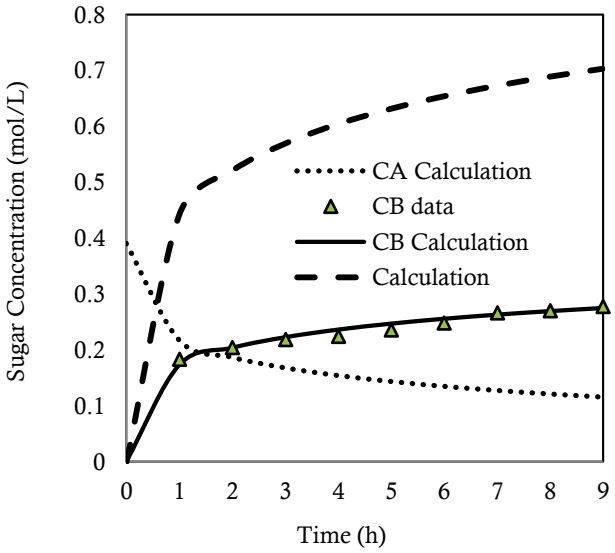

Figure 9. The profile of potential sugar (C_A) and sugar production (C_B) concentrations as well as reaction conversion of enzymatic hydrolysis of sawdust using 9% v/v of cellulase and heterogeneous kinetics model proposed by Valjamae (On the conditions: weight = 10 g, volume = 100 mL, temperature = 30°C, and shaker speed = 160 rpm)

of newsprint waste, at the temperature of 50 °C, and using a shaker with a speed of 170 rpm. The profiles of reduced sugar and reaction result conversion from the calculation of each enzyme volume can be seen in Figure 7-9.

CONCLUSION

The effect of cellulase volume as a catalyst on sugar concentration from sawdust hydrolysis can be obvioulsy seen. From the experiments, the maximum concentration (0.133 mol/L) was

obtained from the addition of 9% enzyme volume. The higher the enzyme volume in the ethanol hydrolysis produced from fermentation, the higher the amount of the sugar hydrolysate will be. The highest ethanol content obtained was 0,059% v/v. Kopelman fractal kinetics model is seen to be more suitable for describing the mechanism of sawdust hydrolysis at low enzyme volumes. However, Valjamae fractal kinetics model is more suitable for describing a mechanism of sawdust hydrolysis at high volumes. From the experiment. It can be concluded that rate constants (k) are strongly influenced by enzyme volumes. It can be seen when at the volume of 5, 7, and 9% v/v, respectively, the values (k) were 0.24, 0.27, and 0.30 1/h.

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