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Kinetics of Enzymatic Hydrolysis of Passion Fruit Peel using Cellulase in Bioethanol Production

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Abstract

This research aims to study the hydrolysis of passion fruit peel using cellulase and its evaluation for ethanol production. Passion fruit peel is a fruit processing waste that has not been utilized properly. Passion fruit peel contains holo-cellulose (64% w/w), which can be converted into ethanol through hydrolysis followed by fermentation. Hydrolysis using cellulase is more efficient and its fermentation using yeast to produce ethanol is common. The hydrolysis is carried out at various enzyme ratios (3, 5, 7, and 9% v/v) and temperature 30 °C, material concentration 5 g/100 mL, pH 4-5, and shaking speed 160 rpm. The kinetics chosen were heterogeneous models; they were the fractal model by Valjamae and Kopelman. Before being hydrolyzed, the essential oil and pectin in passion fruit peel were extracted, because the compositions were quite high; the results were around 16.23 and 11.36% w/w, respectively. The effect of the enzyme ratio on the sugar concentration by hydrolysis is very significant. At 9 h, the glucose concentration reached 45.38, 51.86, 60.50, 66.00 g/L at various enzyme ratios of 3, 5, 7, 9% v/v. During the hydrolysis, the glucose concentration continues to increase and starts to decrease after 9 h. Hydrolyzate solution fermentation obtained from hydrolysis in various enzyme ratios showed consistent results; the higher the enzyme ratio and glucose, and the higher the ethanol will be (5.6, 6.8, 7.6, and 8.9% v/v). The kinetics model by Valjamae is more appropriate to describe the enzymatic hydrolysis mechanism of passion fruit peel than Kopelman. The fractal exponent values obtained from the Valjamae and Kopelman models were 0.28 and 0.27. In Valjamae model, the enzyme ratio rises, from 3 to 9% v/v, the rate constant rises from 0.22 to 0.53 1/h. In the Kopelman model, the rate constant rises too, from 0.21 to 0.51 1/h.

Keywords: *bio-ethanol; cellulase; enzymatic hydrolysis; fractal kinetic; passion fruit peel*

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INTRODUCTION

In the beginning, bio-ethanol or ethanol from plants was produced from sugar extracted from sugar cane, corn, cassava, etc. The sugar can be directly fermented using yeast into ethanol which is called as the first generation (Hattori and Morita, 2010). During the fossil energy crisis in the second decade, the second generation of ethanol called as the cellulosic ethanol

was invented. This ethanol is from lignocellulosic biomass which consists mainly of lignin, cellulose, hemicellulose, and small amounts of extractive compounds. Bioethanol as a substitute for fossil fuels will help curb CO_2 emissions (Cardona *et.al*., 2010). Since it is produced from biomass, it can avoid the accumulation of CO_2 emissions (Liu *et.al*., 2010). Tropical countries have abundant lignocellulosic materials in agricultural waste, such as rice husk, corn cob, passion fruit peel, and banana peel, that contain high holo-cellulose (hemicellulose and cellulose) (Megawati *et.al*., 2015). These materials are also abundantly in the world. In fact, these materials are less utilized, so they can be threats to the environment (Walker, 2010).

Passion fruit peel is an agricultural waste that contains holo-cellulose in the form of reducing the sugar by 26.3% w/w. This sugar type can be easily converted into ethanol using yeast fermentation. Thus, the potential for passion fruit peel to become ethanol is 0.29 L/kg of raw material. Moreover, passion fruit peel also contains protein (4.3% w/w), pectin (13-20% w/w), and essential oil (0.2% w/w) (de Oliveira *et.al*., 2016; Seixas *et.al*., 2014). Passion fruit pectin through biological processes can also be used for the production of polyhydroxyalkanoates (PHAs), a type of biodegradable plastic (Locatelli *et.al*., 2019) and antibiotic drugs similar to penicillin (de Almeida *et.al*., 2014).

Lignocellulosic biomass consists of lignin, cellulose, hemicellulose, and a small amount of extractive compounds. Cellulose is a polysaccharide consisting of several hundred linear chains to more than ten thousand D-glucose units connected to C-beta, while the starch bonds in C-alpha (Wertheim and Jeskey, 2956). Hemicellulose is a heterogeneous glucose, mannose, galactose, xylose and arabinose polymer. Mannose and glucose are the main elements of hemicellulose in softwood, while xylose is abundantly found in hardwood. Through chemical hydrolysis, hemicellulose's chemical structure is broken down more easily than cellulose's (Palmqvist and Hagerdal, 2000). Lignin is a complex chemical compound that functions as a plant binding (hemicellulose and cellulose). Lignin is formed from aromatic compounds which are interconnected by aliphatic chains.

Theoretically, lignocellulosic material can be converted into ethanol using main steps i.e. hydrolysis to convert sugar polymer into sugar monomer, fermentation to convert sugar monomer into ethanol, distillation to remove water in the ethanol-water solution, and ethanol purification to obtain pure ethanol (99.96%). The first important step is hydrolysis. Fermentation and distillation are two established processes in ethanol industry. Hydrolysis of lignocellulosic biomass can be conducted using two methods i.e. acid and enzymatic hydrolysis. Acid hydrolysis can be performed using dilute or concentrated acid or combination of dilute and concentrated acid hydrolysis. According to Badger, enzymatic hydrolysis is more preferable because the usage of an acid solution may cause corrosion (Badger, 2002). Cellulase is an enzyme which is easy to produce and effective to use to convert holo-cellulose into sugars (Cutrim *et.al*., 2019). Meanwhile, *Saccharomyces cerevisiae* or yeast has been very commonly used to ferment sugar into ethanol (Walker and Stewart, 2016).

Accordingly, biomass based on lignocellulosic material does not only contain holo-cellulose, but also contains protein compound (4.3-20% w/w) (de Oliveira *et.al*., 2016; Seixas *et.al*., 2014). When the biomass is converted into ethanol, it is very important to extract the protein before being hydrolyzed. The study of protein extraction before biomass conversion into ethanol is rarely conducted, so this research topic should be developed. Several biomass materials that contain high protein content are alfalfa grass, soya leaf, nuts waste, and wheat straw (Chiesa and Gnansounou, 2011). This principle is also be applied to lignocellulosic materials which have chemical composition other than holo-cellulose, such as fruits peel and flowers (Balinese orange peel, passion fruit peel, cocoa peel, mahula flower, etc). Therefore, before the raw material is processed into bioethanol, the material should be initially extracted into chemicals products - essential oil, pectin, silica powder, xylitol, etc. This concept is adapted from biorefinery in which one material can be converted into several chemical products, just not into bio-ethanol (Vanthoor-Koopmans *et.al*., 2013). In this research, passion fruit peel was extracted into essential oil before converted into the second-generation bioethanol.

Meanwhile, accurate kinetic models of hydrolysis are important for designing and optimizing the processes (Megawati *et.al*., 2010). Holo-cellulose hydrolysis in solid materials is a heterogeneous reaction (Kostylev and Wilson, 2013). Homogeneous kinetics such as the Michaelis-Menten model is inappropriate (Wang and Feng, 2010). The simplest heterogeneous kinetics models were explored by Valjamae and Kopelman. These models are developed to deal with heterogeneous reactions of cellulose fibers hydrolysis (Barlianti *et.al*. 2015). In this research, the two models were chosen to express the passion fruit hydrolysis kinetics with cellulase as enzymes. The more complicated equation was explored by Shen-Agblevor. This equation considers the enzyme deactivation phase which follows the first and second orders reaction (Barlianti *et.al*. 2015). The Shen-Agblevor model was simplified by Valjamae and Kopelman. The enzyme deactivation phase and its equilibrium are represented by the fractal parameter, which is the fractal exponent. A time course of enzymatic hydrolysis of cellulose using cellulase is described by Valjamae as Eq. (1), where C_p = product glucose concentration (g/L), C_0 = initial glucose concentration (g/L), t = time (h), k = reaction rate constant, and h = fractal exponent. The fractal exponent is confirmed to $0 \leq h < 1$ (Barlianti *et.al*. 2015). The other empirical equation for heterogeneous kinetics

model was explored by Kopelman, as in Eq. (2) (Valjamae *et.al*., 2003). This equation is different from Valjamae which derives its kinetic equation based on the concentration of the sugar concentration produced. In the Kopelman model, the kinetic equation is derived from the residual sugar concentration (C_s (g/L)).

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

$$C_S = C_0.exp\left[-k\left(1 + \frac{t^{1-h}-1}{1-h}\right)\right] \quad (2)$$

MATERIALS AND METHODS

Preparation of Passion Fruit Peel

Passion fruits used in this research were obtained from Rembang, Jawa Tengah, Indonesia. The passion fruit was peeled and dried under the sunlight for several days, until the moisture content reaches around 7% w/w dry material. The dried peel was then grounded and sieved in order to obtain 40 mesh-sized particles. *Aspergillus niger* strain YD17 was supplied by Biotek Laboratory, Faculty of Agriculture Technology, Universitas Gadjah Mada. All of the chemicals used in this study were analytical grade. A pretreatment process was carried out by extracting the essential oil from passion fruit peel using n-hexane as a solvent. The process of extraction was done for 20 cycles in a Soxhlet extraction apparatus.

Preparation of Cellulase Enzyme

10 g sugar, 5 g jelly powder, and 1 tablet B-complex vitamin were put in a sterilized test tube in order to make a media for *Aspergillus niger* inoculation. *Aspergillus niger* was put in the prepared media and after it was grown up, the test tube was tightly sealed and stored in a refrigerator under 4 ^oC. In order to obtain a cellulase enzyme, a starter solution to produce *Aspergillus niger* spore suspension was made by dissolving 12.5% (w/v) sucrose, 0.25% (w/v) urea, and 0.2% (w/v) KH_2PO_4 in 100 mL of distilled water. After that, the previously isolated *Aspergillus niger* was submerged in the starter solution and sealed with aluminum foil and incubated at 30 ^oC for 24 h. After *Aspergillus niger* was grown on the surface of the starter and produced *Aspergillus niger* spore suspension. The spores that grow a lot on the surface indicate a very good quality. The spore suspension was used to obtain cellulase enzyme by dissolving 10 mL of the suspension in a sterilized mixture of 20 g passion fruit peel, 0.03 g urea, 0.005 g $MgSO_4.7H_2O$, 0.0023 g KH_2PO_4, 80 mL distilled water, and 1 mol/L HCl (in order to make the acidity level pH 5). The mixture was sealed with an aluminum foil and kept at 30 ^oC for 96 h. The cellulase enzyme was then extracted using 100 mL distilled water as a solvent in a rotary shaker with a speed of 150 rpm for 1 h. After that, the solution was filtered, and the filtrate was stored in a refrigerator. The enzyme activity produced was not tested for quality but was directly used for the hydrolysis of passion fruit peel. The enzyme quality can be evaluated from the hydrolysis kinetics parameter.

Enzymatic Hydrolysis of Passion Fruit Peel with Cellulase

The enzymatic hydrolysis of passion fruit peel using cellulase was carried out under an acid condition (pH 4-5) in a 250 mL erlenmeyer flask. Prior to the hydrolysis, substrate and buffer solution were sterilized in an autoclave (TOMY Autoclave, ES-315) at 100 ^oC for 30 min. Cellulase enzyme with certain numbers of volumes (3, 5, 7, and 9% v/v) was loaded into the flask in order to initiate the hydrolysis. The process was performed in a shaking incubator set under the speed of 160 rpm at ambient temperature for 9 h. A small amount of solution was taken as a sample in every 1 h. In order to deactivate the enzyme before measuring the sugar concentration, each sample was always put in boiling water for 2 min. The glucose concentration in the sample was analysed using the Fehling method and all experiments were duplicated. Meanwhile, the initial glucose concentration was calculated theoretically from both hemicellulose (18% w/w) and cellulose (48% w/w) content in the passion fruit peel from the literature (Yapo and Koffi, 2008).

The initial glucose concentration (C_0) and glucose produced concentration (C_p) every 1 h (t) are used to calculate the hydrolysis kinetics. The residual glucose concentration (C_s) is the reduction result of initial glucose and glucose produced. The glucose yield is calculated as the ratio of the glucose produced weight (w_p) every initial glucose weight (w_0), as in Eq. (3).

$$Yield(\%) = \frac{w_p}{w_0} x100\% \quad (3)$$

Fermentation of Passion Fruit Peel Hydrolyzate with Yeast

Before being fermented, the hydrolyzate is neutralized by adding 1N $Ca(OH)_2$. This neutralization will produce salt. A sedimentation was carried out to separate the maximum deposition of salt using 16.65 g/L alum, which was then filtered. The solution fermentations were prepared using 150 ml of neutralized hydrolysate and supplementing minerals such as 0.15 g Urea ($(NH_2)_2CO$) and 0.045 g N–P–K Fertilizer and adjustable pH by adding 1 mol/L sulphuric acid solution until the pH reaches 4.6. The solution was then sterilized in an autoclave (TOMY Autoclave, ES-315) with a temperature of 100 ^oC for 4 h and cooled down for 24 h. After that, the medium was fermented with 1.1% w/v Baker's yeast for 5 days in an anaerobe condition. This fermentation result was filtered by screening papers to separate the remaining biomass of passion fruit peel and yeast. This filtering was carried out in a cold cabinet, so that ethanol does not evaporate much. The ethanol concentration was analysed by GC (Gas Chromatography) with the specification of GC Clarus 680 (MS Clarus SQ ST).

Kinetics of Enzymatic Hydrolysis of Passion Fruit Peel

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. In the Valjamae model, the concentration of glucose produced (C_p) is calculated using Eq. (2). The SSE formula is shown in Eq. (4), where $C_{p(Ci)}$ = the calculation value of the product concentration, $C_{p(Di)}$ = the experimental value of product concentration, n = the amount of data, and i = the observed time. In the Kopelman model, the kinetic calculation is not based on the concentration of the glucose produced, but the residual glucose concentration (C_s) and the SSE equation are expressed as Eq. (5). This simulation was calculated by a curve-fitting method. The initial glucose concentration (C_0) value was calculated theoretically from both hemicellulose and cellulose contents in passion fruit peel, and the result was 0.39 mol/L. Accordingly, the rate constant increased as the cellulase loading did too, but the fractal exponent was constant at cellulase loading variations (Kostylev and Wilson, 2013). In this work, the correlation of enzyme volume ratio with constant reaction rate can be expressed by the empirical equation.

$$SSE = \sum_1^i \left| C_{p(Di)} - C_{p(Ci)} \right|^2 \quad (4)$$

$$SSE = \sum_1^i \left| C_{s(Di)} - C_{s(Ci)} \right|^2 \quad (5)$$

RESULTS AND DISCUSSION

The Effect of Enzyme Volume Ratio of Hydrolysis on Product Glucose Concentration

In this research, the results showed that the essential oil yield of passion fruit peel obtained was about 16.23% w/w. Accordingly, the composition of essential oil in passion fruit peel is quite high (de Oliveira *et.al*., 2013), such as methyl butanoate, methyl (E)-2-butenoate, ethyl butanoate, ethyl (e)-2-butenoate, methyl 2-hexanoate, and ethyl-2-hexanoate (Mamede *et.al*., 2017). Therefore, before being used as a raw material for producing bioethanol, extraction of passion fruit peel essential oil should be done first. After extracting the essential oil, the pectin should also be extracted. The extraction using nitric and acetic acid results respectively showed that the passion fruit peel contains around 13 and 12.9% w/w (de Oliveira *et.al*., 2016; Seixas *et.al*., 2014). Therefore, the concept of biorefinery can be applied for maximizing passion fruit peel utilization; they are essential oil extraction, pectin extraction, conversion to bioethanol. This concept can be adopted for the orange peel waste material that has been done and can be developed further (Lopez *et.al*., 2010). In addition, the passion fruit peel hydrolysis results showed that the influences of the enzyme volume ratio on hydrolysis were significant.

Table 1. Glucose concentration (g/L) of passion fruit peel enzymatic hydrolysis using cellulase with various enzyme ratio (under condition: temperature 30 °C, working volume 5 g/100 mL, pH 4-5, and shaking speed 160 rpm)

Time (h)	Enzyme ratio (% v/v)			
	3	5	7	9
1	13.44	17.71	21.35	33.00
2	22.69	25.93	33.00	40.33
3	25.93	33.00	38.21	45.38
4	30.25	36.30	42.71	48.40
5	36.30	40.33	45.38	51.86
6	40.33	45.38	49.73	55.85
7	42.71	48.40	55.85	60.50
8	43.73	51.86	55.85	63.68
9	45.38	51.86	60.50	66.00

The glucose concentration tends to increase in line with the enzyme volume ratio. On hydrolysis for 9 h, glucose concentrations rose from 45.38, 51.86, 60.50, and 66 g/L because the enzyme ratio was higher than 3, 5, 7, and 9% v/v enzyme ratio, respectively (see Table 1).

The glucose concentration during the hydrolysis was converted into glucose weight. The glucose yield is calculated based on the product glucose weight ratio to theoretical glucose weight, such as Eq. (3) and the results can be seen in Fig. 1. The effect of enzyme amount has also been studied for fresh passion fruit hydrolysis using the proteinase-cellulase mixture as an enzyme to make passion fruit juice. The results showed that the higher the dose of the enzyme, the higher the juice yield; at an enzyme dose of 0.02 to 0.2%, the juice yield increases from 85 to 92% on hydrolysis for 5 h (Yang *et.al*., 2017). In this study, at hydrolysis time of 5 h and enzyme ratio of 3 to 9%, glucose yield increased from 52 to 79%. Holo-cellulose hydrolysis is certainly more difficult than fresh fruit hydrolysis.

The Effect of Time of Hydrolysis on Product Glucose Concentration

On top of that, for the effect of time, it was observed that during hydrolysis, at 3% v/v enzyme ratio, glucose

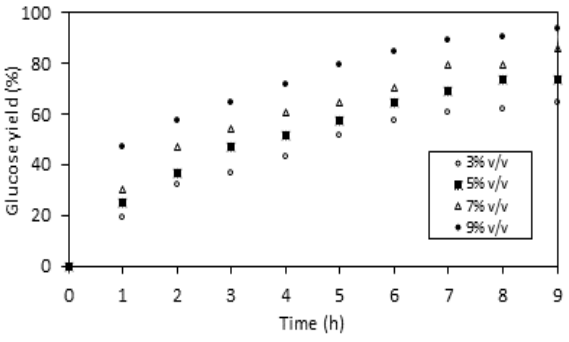

Figure 1. Glucose yield of passion fruit peel enzymatic hydrolysis using cellulase with various enzyme ratio

(under condition: temperature 30 ºC, working volume 5 g/100 mL, pH 4-5, and shaking speed 160 rpm) concentration increased steadily, from 21.35 to 60.50 g/L. This shows that the glucose concentration continued to rise during the hydrolysis. This is an advantage of hydrolysis using an enzyme in which the glucose formed does not degrade into a byproduct. Therefore, the glucose concentration does not decrease. If the enzyme activity is finished, the glucose concentration will be stable. This contrasts with acid hydrolysis where the sugar formed can be degraded into a byproduct which can interfere hydrolysate fermentation into ethanol (Karimi *et.al*., 2006). In this study, from Table 1, the highest glucose concentrations obtained in the operating conditions of 9% v/v enzyme ratio, the glucose concentration increased from 33.00 to 66.00 g/L. These results are similar to the literature's reports. It has been reported that sugar cane bagasse hydrolysis with *A. flavus* produced reducing sugar which continued to increase and became stable after 72 h; its concentration reached 2.3 g/L, without decreasing (Siqueira *et.al*., 2010). Theoretically, enzymes as catalyst for a reaction are indeed very selective in forming certain products, so the possibility of the products number going down during the reaction is rare. This is an advantage of the process of using enzymes (Hedstrom, 2010). The optimum hydrolysis time is seen at around 6 h, after which the sugar concentration slowly increases.

The Effect of Enzyme Volume Ratio of Hydrolysis on Ethanol Concentration of Fermentation

The data presented in Table 2 displays the ethanol amount produced by fermentation from a different quantity of enzymes used in each hydrolysis process. The increased amount of enzyme leads to more ethanol obtained. Therefore, due to a lot of enzymes used in the hydrolysis, it can be concluded that the higher the glucose concentration, the higher the product's ethanol will be (Usmana *et.al*., 2012). The phenomenon occurred also indicates that the sugar produced from hydrolysis using various enzyme volume ratios could be fermented well by yeast. Thus, the enzyme ratio increase of 3 to 9% v/v does not give rise to an inhibitory compound that may interfere the fermentation process.

Table 2. Ethanol concentration of passion fruit peel hydrolysate fermentation using yeast with various cellulase enzyme volume of hydrolysis (under condition: hydrolysate volume 100 mL, yeast 4 g, pH 4.6, and time 5 days)

Enzyme ratio of hydrolysis (% v/v)	Ethanol concentration of fermentation (% v/v)
3	5.6
5	6.8
7	7.6
9	8.9

Similarly reported by Singh *et.al*., enzymatic hydrolysis using cellulase will not generate any inhibitor and the enzymes were very specific for cellulose. In their research, the ethanol concentration was increased by 57% when the glucose concentration was increased from 10 to 25 g/L (Singh *et.al*., 2014).

Kinetics of Enzymatic Hydrolysis of Passion Fruit Peel using Cellulase

The experimental data followed the fractal kinetics model in Figures 2-3. Each kinetic parameter of the calculation results can also be seen in Table 3. The two models can describe the mechanism of hydrolysis with their respective accuracy. Accordingly, holo-cellulose is a hard material, so it is difficult to be penetrated by enzymes, so heterogeneous models are more suitable than homogeneous ones (Valjamae *et.al*., 2003; Megawati *et.al*., 2018). In addition, the most important information is that cellulase must be absorbed on the substrate surface and then spread on to the reactive site before the enzymatic hydrolysis occurs. Valjamae model was explored to study the pure cellulose hydrolysis with α-glucosidase type cellulase. The results show that the constant rate ranged from 0.033 to 0.14 1/h and the fractal exponent from 0.32 to 0.6. Meanwhile, for corn stover hydrolysis, the rate constant and fractal exponent ranged from 0.06 to 0.41 1/h and 0.666 to 1, respectively. Before being used, the corn stover was hydrolyzed using Avicel and ARP (ammonia recycled percolation) (Wang *et.al*., 2010). These two previous studies have shown that the Valjamae model is suitable for enzymatic hydrolysis using cellulase.

In this study, Valjamae models is better than Kopelman model to describe passion fruit peel enzymatic hydrolysis using cellulase. Valjamae's models derive the kinetic equation from the product changes formed during the reaction. This will be more corresponding to the results than the reactant changes, because the initial reactants number is difficult to investigate.

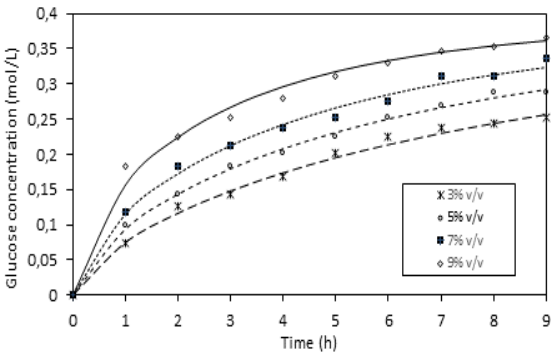

Figure 2. Fractal kinetics analysis of passion fruit peel enzymatic hydrolysis using cellulase based Valjamae Model

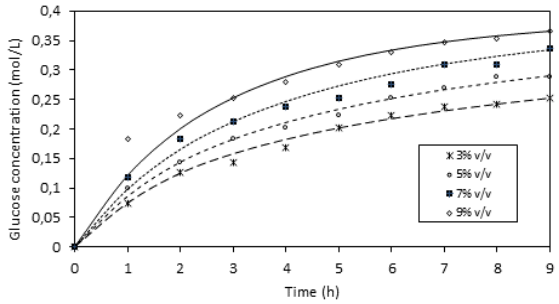

Figure 3. Fractal kinetics analysis of passion fruit peel enzymatic hydrolysis using cellulose based on Kopelman Model

Table 3. Fractal kinetics parameter of enzymatic hydrolysis of passion fruit peel using cellulose

Enzyme (mL)	Valjamae		Kopelman	
	Rate constant (1/h)	Fractal exponent	Rate constant (1/h)	Fractal exponent
3	0.21	0.27	0.31	0.28
5	0.28	0.27	0.37	0.28
7	0.35	0.27	0.51	0.28
9	0.51	0.27	0.75	0.28
Average error	4.95%		5.01%	

The initial glucose content in passion fruit peel is theoretically analyzed from the results of the analysis of holo-cellulose levels, so it becomes less accurate. Recently, the fractal exponent value (h, 1/h) for cellulose material ranges from 0.2-0.4 (Valjamae *et.al*., 2003). In this study, fractal exponents for the passion fruit peel hydrolysis ranged from 0.27 and 0.28 for the Kopelman and Valjamae model, respectively. Therefore, the calculation results in this study are in accordance with the literature (Wang *et.al*., 2010). The fractal exponent value is strongly influenced by the pore size and hardness of the materials.

In addition, the rate constant and fractal exponents also appear to be strongly influenced by the enzyme volume ratio. The higher the enzyme volume ratio, the higher the rate constant and fractal exponents (Wang and Feng, 2010). In the Valjamae model, the rate constant rises too, from 0.21 to 0.51 1/h. Moreover, in the Kopelman model, the rate constant rises from 0.31 to 0.75 1/h, respectively. In this study, the fractal exponent value is made constant, based on the simulation results on each enzyme volume ratio, then averaged and re-simulated. An increase in fractal exponent due to an increase in enzyme loading was illustrated from the previous studies (Wang *et.al*., 2010).

The calculation results suggest that the reaction rate constant is highly influenced by the enzyme volume ratio. The enzyme volume ratio is parallel with the reaction rate constant. Therefore, if hydrolysis is carried out at a high enzyme volume ratio, then the hydrolysis will be faster, and the sugar produced increases.

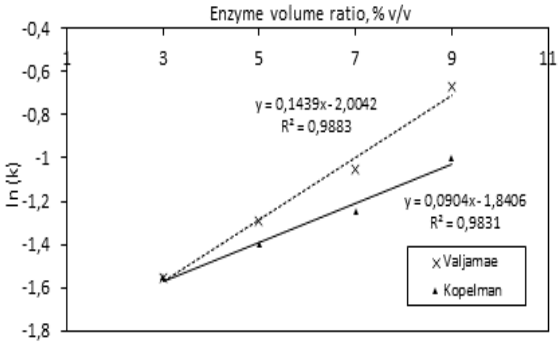

Figure 4. Linear fitting plot of the natural logarithm of the fitting apparent rate constant ln (k) versus the enzyme ratio (% v/v) of enzymatic hydrolysis of passion fruit peel using cellulose

The correlation of enzyme volume ratio with reaction rate constant can be expressed by empirical equations, Eq. (6) and (7) for the Valjamae and Kopelman model, respectively, where E = enzyme ratio (% v/v). The constants in the empirical equations were obtained by linearization from the plot of ln (k) versus % v/v of enzyme ratio (see Fig. 4).

$$\ln(k) = 0.1439E - 2.004 \quad (6)$$

$$\ln(k) = 0.0904E - 1.181 \quad (7)$$

CONCLUSION

Passion fruit peel is very promising to produce the second generation of bio-ethanol. The initial main process of bioethanol production is hydrolysis. The hydrolysis of passion fruit peel can be performed using enzymatic hydrolysis. Cellulase enzyme can be chosen to hydrolyse passion fruit peel to produce fermentable sugars. The ratio of enzyme volume and time greatly affected the passion fruit peel hydrolysis. At 8 h, the glucose concentration increased, from 43.73, 51.86, 55.85, and 63.68 g/L, because the enzyme ratio increased, from 3, 5, 7, and 9% v/v, respectively. During the hydrolysis, the glucose concentration continues to rise, the most significant increase occurs up to 6 h, then increase slowly. At the highest enzyme ratio (9% v/v), the glucose yield reached 83% w/w after 6 h, then reached 93% w/w after 9 h. The ratio of enzymes used in hydrolysis is higher (3 to 9% v/v), the concentration of glucose produced by hydrolysis increases (45.38 to 66.00 g/L) and the ethanol concentration of fermented products also increases (5.6 to 8.9% v/v). The fractal kinetic by Valjamae is better than Kopelman to express enzymatic kinetics hydrolysis of passion fruit peel using cellulase. The fractal exponents obtained from Kopelman and Valjamae models were 0.27 and 0.28, respectively. The rate constant is highly influenced by the enzyme volume ratio. The enzyme volume ratio rises, so the rate constant also does. In the Valjamae model, the hydrolysis velocity constant increases from 0.22 to 0.53

1/h, because the enzyme ratio increases from 3 to 9% v/v.

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