

# Kinetics study of enzymatic hydrolysis of Tetraselmis chuii using Valjamae model

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## Kinetics study of enzymatic hydrolysis of *Tetraselmis chuii* using Valjamae model

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**Abstract.** The *Tetraselmis chuii* microalgae have a potential as an alternative raw material for producing bioethanol, because of its low lignin and high carbohydrate content. The purpose of this research is to study the kinetics of enzymatic hydrolysis of *Tetraselmis chuii* using Valjamae model. This experiment was carried out with a variety of enzymes (cellulase, xylanase, and cellulase-xylanase mixture, enzyme concentrations (10, 20, and 30% w/w), times (10, 20, 30, and 40 min), and temperatures (40 and 45 °C). The results show that the activation energy of hydrolysis using cellulase-xylanase mixture is 27,253 J/mol, xylanase 116,121J/mol, and cellulase 140,914 J/mol. At 45 °C, the concentration of the cellulase-xylanase mixture increased (10, 20, and 30% w/w), the reaction rate constants (0.028, 0.110, and 0.171 1/min) as well as the fractal exponents (0.4, 0.6, and 0.7). Thus, the glucose production rate is higher, where at 40 minutes produces higher yields (23.5, 25.5, 28.5, and 32.5%). In this study, the constant rate of *Tetraselmis chuii* hydrolysis using cellulase-xylanase mixture at 45 °C is 0.1714 1/min; higher than the one conducted at 40°C which is 0.1454 1/min. The kinetic parameters of the enzymatic hydrolysis are expected to help in bioreactor design.

### 1. Introduction

Energy necessities continue to increase along with the increasing population and technological advances. Meanwhile, the current energies are obtained from fossils which will eventually run out, so an energy crisis will occur. Therefore, an alternative energy is needed, including bioethanol (Illukpitiya et al., 2016; Megawati et al., 2015). The development of ethanol manufacturing technology starts from food (the first generation bioethanol), biomass (the second generation bioethanol), and microalgae (the third generation bioethanol) (El Harchi et al., 2018). The microalgae have the potential as a raw material for producing bioethanol, because they are rich in carbohydrates (Prabandono & Amin, 2015). In addition, they can be easily cultivated, because they can grow with or without soil; they can also grow in wastewater (Vahabisani et al., 2015).

The microalgae types can be used for producing bioethanol are *Glacilaria*, *Gelidium*, *Kappapycus*, *Sargassum*, and *Laminaria* (El Harchi et al., 2018). The *Tetraselmis chuii* microalgae contain carbohydrates (starch 19.62, cellulose 10.20, and hemicellulose 49.54%) and without lignin (Padil et al., 2016). It can be concluded that converting them into monosaccharides is easier compared to lignocellulosic materials. The main process of bioethanol production is hydrolysis (Chen et al., 2013). The glucose polymer contents (cellulase, starch or glycogen) in microalgae must be converted as efficiently as possible converted to obtain sugar, which can then be fermented into bioethanol (de Farias



Silva et al., 2018). The enzymatic hydrolysis of microalgae is superior to that of acids, because it consumes low energy, converts lots of sugar, and does not cause corrosion (Shokrkar et al., 2018). The enzymes that are often used are cellulases and xylanases.

The enzymatic hydrolysis of cellulose to glucose conversion goes through two important stages. The first is converting cellulose into cellobiose by breaking the glycosidic bonds within cellulose using  $\beta$ -1,4-glucanase. The second one is converting cellobiose into glucose by breaking the  $\beta$ -1,4-glucosidic bonds within cellobiose. Cellulase consists of exo cellulases or cellobiohydrolases, endo cellulase or endo- $\beta$ -1,4-glucanase and  $\beta$ -1,4-glucosidase or cellobiose. Xylanase is an extracellular enzyme that consists of  $\beta$ -xylosidase, exo xylanases, and endo xylanases. The  $\beta$ -xylosidase can hydrolyze xylooligosaccharides to xylose. Exo xylanases can break xylanase into xylose and oligosaccharides. Meanwhile, endo xylanases can break the  $\beta$  1-4 bonds inside the xylan chain regularly. The bond broken is determined based on the substrate chain's length, the degree of branching, the substitution groups' presence or absence, and the hydrolase enzyme's breaking pattern.

The substrate's complex nature in the enzymatic hydrolysis of cellulose makes homogeneous kinetics models by Michaelis-Menten less suitable (Megawati et al., 2018). Before the hydrolysis occurs, cellulase must be adsorbed on the substrate's surface and then diffuse to the reactive site. The fractal kinetics model is a heterogeneous model used to find out the complex reaction mechanisms and to develop large-scale process models. According to Megawati et.al (2018), fractal kinetics using the Valjamae model gave better results than the Kopelman model on passion fruit peels hydrolysis using cellulase. In this research, the kinetics of the Valjamae model were chosen to study the hydrolysis of *Tetraselmis chuii* using variations in cellulase, xylanase, and cellulase-xylanase mixture, enzyme concentrations, and temperatures.

## 2. Methods

### 2.1. Data

The glucose yield data from the enzymatic hydrolysis of *Tetraselmis chuii* was obtained from a research conducted by Padil et al (Padil et al, 2016). The enzymes used were cellulase, xylanase, and cellulase-xylanase mixture. The hydrolysis was performed at variations of temperatures, enzyme concentrations, and times. The hydrolysis was done by mixing 500 mg of *Tetraselmis chuii* in a 250 mL Erlenmeyer mixed with a 100 mL buffer solution (sulfuric acid solution pH 4.5). After that, the microalgae solution was put into a shaker batch, which is set at 40°C. After the solution's temperature reached 40°C, the cellulase enzyme of 10% (w/w) was put into the Erlenmeyer. The hydrolysis was carried out for 60 min and samples were taken every 10 min. After the hydrolysis, solids were separated from the hydrolysis solution using a centrifugation technique. The hydrolysis solution was then heated at 90°C for 15 min using a water bath, then stored in a freezer at -30°C to stop the enzyme activity. The above procedure was repeated for variations of enzyme types, enzyme concentrations, and temperatures. For the cellulase-xylanase mixture, the hydrolysis was done using cellulase enzyme, then xylanase enzyme was added, so it was carried out simultaneously. The glucose levels were analyzed using Nelson Somogy and the glucose yields are presented in tables 1-3.

### 2.2 Calculate the kinetics.

The yield data was converted into glucose product yields' concentrations using equation (1), with  $C_p$  = glucose product yield's concentration (g/L),  $C_o$  = glucose concentration in the substrate (g/L). Meanwhile, the initial glucose concentration in the substrate was calculated using carbohydrate composition data in *Tetraselmis chuii* (79.36% w/w), microalgae concentration of 5 g, and a volume of 100 mL. The result shows that the initial glucose concentration in the substrate is 3.968 g/L.

$$C_p = C_o \cdot yield \quad (1)$$

An empirical equation for heterogeneous reaction kinetics using the Valmaja model is written in equation (2), with  $k$  = reaction rate constant (1/h),  $t$  = time (min), and  $h$  = exponent fractal. The value of  $h$  is set to  $0 \leq h < 1$  (Megawati et al., 2018).

**Table 1.** Glucose product yields from the *Tetraselmis chuii* hydrolysis with various enzyme concentrations (microalgae concentration 5 g/L, volume 100 mL, temperature 45°C, and pH 4.5).

Time (min)	Enzyme concentration (w/w)		
	10%	20%	30%
Cellulase			
10	0.80	1.70	2.53
20	1.20	1.75	2.73
30	1.25	1.80	2.80
40	1.30	1.85	3.05
Xylanase			
10	2.60	3.65	4.05
20	2.65	3.80	4.35
30	3.00	4.05	5.18
40	3.10	5.18	5.35
Cellulase-xylanase mixture			
10	10.50	18.50	23.50
20	13.50	21.00	25.50
30	16.80	24.00	28.50
40	22.50	28.00	32.50

(Padil et al., 2016)

**Table 2.** Glucose product yields from the *Tetraselmis chuii* hydrolysis at various temperatures (microalgae concentration 5 g/L, volume 150 mL, temperature 45°C, and enzyme concentration 30% (w/w)).

Time (min)	Temperature (°C)	
	40	45
Cellulase		
10	1.65	2.53
20	1.95	2.73
30	2.05	2.80
40	2.25	3.05
Xylanase		
10	1.65	4.05
20	1.93	4.35
30	2.05	5.20
40	2.23	5.35
Cellulase-xylanase mixture		
10	21.00	23.50
20	23.60	25.50
30	27.50	28.50
40	30.00	32.50

(Padil et al., 2016)

$$C_p = C_0 \left[ 1 - \exp(-k \cdot t^{1-h}) \right] \quad (2)$$

The data on the relationship between glucose concentration and time at various temperatures and various enzyme concentrations were used to determine the reaction kinetics parameters. The value of each parameter was tested, so that the calculation results approached the experimental data. For the more systematic procedure, the differences in the calculation results and experimental data were calculated based on the minimum sum of squared error (SSE) values, such as equation (3), where  $C_{p(cal)}$  = product concentration calculated result,  $C_{p(d)}$  = product concentration data, and  $i$  = trial time.

$$SSE = \sum_{i=1}^n (C_{p(cal)} - C_{p(d)})^2 \quad (3)$$

**Table 3.** Glucose yields of *Tetraselmis chuii* hydrolysis on various enzyme types (microalgae concentration 5 g/L, volume 150 mL, temperature 40°C, and enzyme concentration 30% (w/w)).

Time (min)	Enzyme		
	Cellulase	Xylanase	Cellulase-xylanase mixture
10	2.50	4.15	22.50
20	2.55	4.20	25.60
30	2.58	5.00	28.00
40	2.60	5.08	32.00

(Padil et al., 2016)

### 3. Results and Discussion

#### 3.1. The Effect of Enzyme Type on Hydrolysis Rate of *Tetraselmis chuii*

The analysis result of *Tetraselmis chuii*'s composition is presented in table 4. It can be seen that the carbohydrate content is about 79.36% w/w, so the initial glucose concentration contained before being hydrolysed was about 3.968 g/L. This shows that the *Tetraselmis chuii*'s compositions are relatively high, making them prospective to be a raw material in producing bioethanol. According to Mantecon et al (2019), the *Tetraselmis chuii* contained 30-35% carbohydrates. Meanwhile, several microalgae that also contained very high carbohydrates are *Scenedesmus dimorphus* (21-52%), *Spirogyra sp.* (33-64%), and *Porphyridium cruentum* (40-57%).

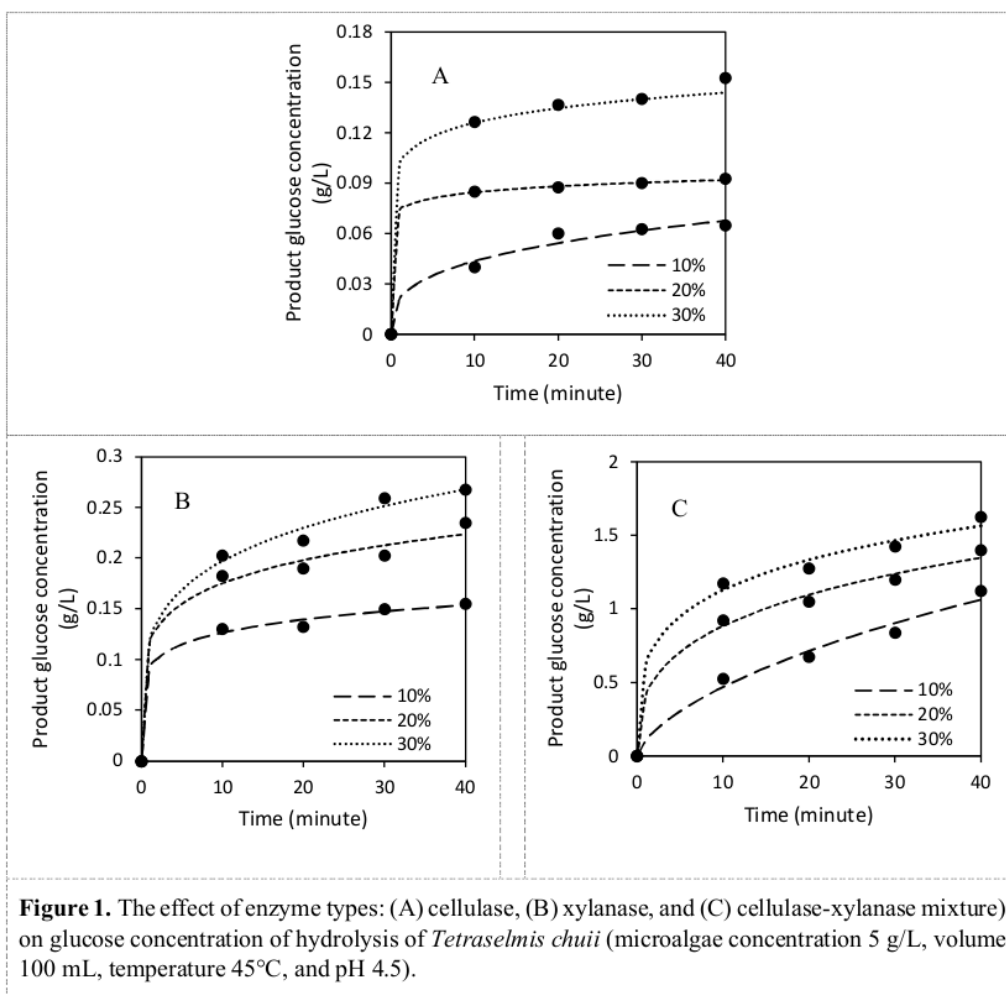
**Table 4.** Composition of *Tetraselmis chuii*.

Component	Composition (% w/w)
Carbohydrates	79.36
Hemicellulose	49.54
Cellulose	10.20
Starch	19.62
Protein	19.57
Fat	01.07

The optimization results of the Valjamae Model fractal kinetics parameters at 45°C can be seen in table 5 and figure 1. It can be seen that the enzyme types greatly influence the hydrolysis kinetics. The cellulose-xylanase mixture can accelerate the hydrolysis more than xylanase and cellulase added separately. At an enzyme concentration of 30%, the reaction rate constants are 0.171, 0.030, and 0.026 1/min and fractal exponents 0.7, 0.8, and 0.9, respectively for the cellulose-xylanase mixture, xylanase, and cellulase. Thus, it can be concluded that the cellulase-xylanase mixture can accelerate the hydrolysis of *Tetraselmis chuii* 5.68 times than the two enzymes added separately.

**Table 5.** Kinetic parameters of *Tetraselmis chuii* enzymatic hydrolysis with various enzyme types (microalgae concentration 5 g/L, volume 100 mL, temperature 45°C, and pH 4.5).

Enzyme	Enzyme Concentration (% w/w)	Rate Constant $k$ (1/min)	Fractal Exponent, $h$	Average Error
Cellulase	10	0.0053	0.6813	1.32E-05
	20	0.0187	0.9393	2.72E-07
	30	0.0258	0.9026	1.92E-05
Xylanase	10	0.0281	0.8585	1.70E-05
	20	0.0299	0.8200	8.71E-05
	30	0.0302	0.7728	5.99E-05
Cellulase-xylanase mixture	10	0.0281	0.3467	0.0031
	20	0.1103	0.6404	0.0020
	30	0.1714	0.7088	0.0027



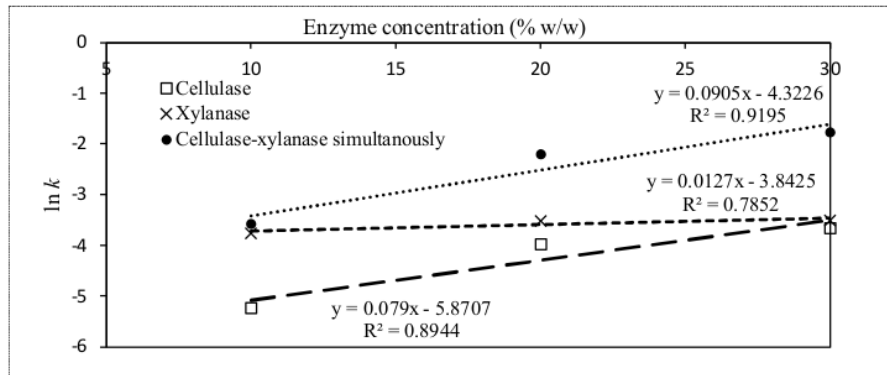
**Figure 1.** The effect of enzyme types: (A) cellulase, (B) xylanase, and (C) cellulase-xylanase mixture) on glucose concentration of hydrolysis of *Tetraselmis chuii* (microalgae concentration 5 g/L, volume 100 mL, temperature 45°C, and pH 4.5).

The hydrolysis using the xylanase enzymes functions to convert xylan (hemicellulose) into xylooligosaccharides and xylose, whereas cellulase enzymes to change the bonds (1-4) of glucosides

from cellulose to produce cellobiose which is then converted to glucose monomers (Setyoko & Utami, 2016). The *Tetraselmis chuii*, however, have more hemicellulose content than cellulose (Padil et al., 2016), so the hydrolysis rate constant using cellulase is higher. However, using cellulase-xylanase mixture is more effective, because it can hydrolyze two contents of *Tetraselmis chuii* at once; they are cellulose and hemicellulose.

### 3.2. The Effect of Enzyme Concentration on the *Tetraselmis chuii* Hydrolysis Kinetics

The enzyme concentration is very influential in the *Tetraselmis chuii* hydrolysis kinetics (figure 2). At pH 4.5 and temperature 45°C, the higher the enzyme concentration (10, 20, and 30%), the faster the hydrolysis using cellulase-xylanase mixture will be. Respectively, the hydrolysis rate constants are 0.0281, 0.1103, and 0.1714 1/min. This is caused by the ability of more enzymes which can break down cellulose and hemicellulose, so they can easily bind the substrate and convert it into glucose (Stevanie, 2016). The greater the value of rate constant ( $k$ ), the faster the reaction will be (Levenspiel, 1972). According to Megawati et al (2018), in the passion fruit peel hydrolysis, the higher the concentration of cellulase enzymes (5, 7, and 9% v/v), the faster hydrolysis (0.24, 0.27, and 0.3 1/h) will be. The reaction rate constant will increase along with the enzyme concentration, so the reaction will be faster. Likewise, accordingly, in the hydrolysis of *Eucheuma cottoni* the more kappa-caragenase enzymes used (0, 2.5, and 5% w/v), the higher the sugar concentrations were (2, 3, and 3.25%). The reaction rate constant will increase along with the enzyme concentration, so the reaction will be faster.



**Figure 2.** The correlation between reaction rate constants and enzyme concentrations of *Tetraselmis chuii* enzymatic hydrolysis (microalgae concentration 5 g/L, volume 100 mL, temperature 45°C, and pH 4.5).

When the enzyme concentration is higher, the reaction speed will increase to a certain glucose concentration limit. However, the glucose concentration will be constant, even though the enzyme concentrations increase; this is caused by the ineffectiveness of enzyme addition (Reed, 1975). The empirical correlations between the enzyme concentration and reaction rate constants using cellulase, xylanase, and cellulase-xylanase mixture are explained in equations (4)-(6). The empirical equations were obtained through a linear regression graph between  $\ln(k)$  and enzyme concentration ( $C_E$ ) presented in figure 2.

$$\ln(k) = 0.0790 \cdot C_E - 5.8707 \quad (4)$$

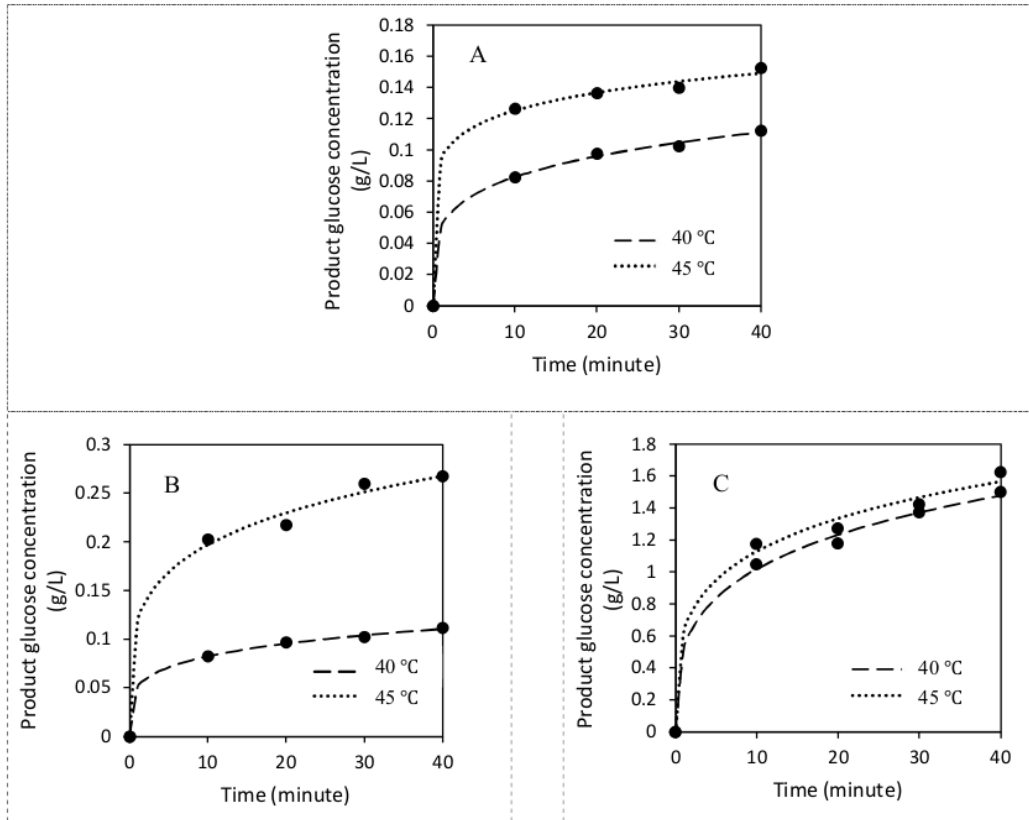
$$\ln(k) = 0.0127 \cdot C_E - 3.8425 \quad (5)$$

$$\ln(k) = 0.0905 \cdot C_E - 4.3226 \quad (6)$$



### 3.3. The Effect of Temperature on the *Tetraselmis chuii* Hydrolysis Kinetics

Temperature is one of the factors that can affect the reaction rate. The effect of temperature on the *Tetraselmis chuii* hydrolysis using cellulase, xylanase, and cellulase-xylanase mixture is presented in figure 3. The optimal temperature for hydrolysis using cellulase is 50°C (He et al., 2018) and xylanase 45°C (Qiu et al., 2016). An increase in temperature results in faster reactions, because it speeds up the enzymes' motions, so the collisions with the substrates will occur more frequently (Levenspiel, 1999). The optimization results of the hydrolysis rate constants and fractal exponents are shown in table 6.



**Figure 3.** The effect of temperature on glucose concentration of the *Tetraselmis chuii* hydrolysis using A-cellulase, B-xylanase, and C-cellulase and xylanase simultaneously (microalgae concentration 5 g/L, volume 100 mL, temperature 45°C, and pH 4.5).

The correlations between hydrolysis rate constants and temperature using the Arrhenius law are explained in equations (7)-(9), respectively for hydrolysis using cellulase, xylanase, and cellulase-xylanase mixture. The equations were obtained from the linear regression graph between  $\ln(k)$  and  $1/T$ . The equations show the value of slope ( $-E/R$ ) and intercept ( $\ln A$ ), where  $T$  is the temperature (K),  $E$  is the activation energy (J/mol),  $R$  is the ideal gas constant (8.314 J/mol.K), and  $A$  is the frequency factor (Ashokkumar, 2017).

$$k = 3.00 \times 10^{17} e^{-116,121/T} \quad (7)$$

$$k = 4.14 \times 10^{21} e^{-140,913/T} \quad (8)$$

$$k = 5.11 \times 10^3 e^{-27,253/T} \quad (9)$$

**Table 6.** The kinetic parameters in the *Tetraselmis chuii* enzymatic hydrolysis at temperature variations (microalgae concentration 5 g/L, volume 100 mL, temperature 45°C, and pH 4.5).

Enzyme	Rate Constant, k (1/min)	Fractal Exponent	Average Error
Cellulase			
a. 40 °C	0.0128	0.7826	2.12E-06
b. 45 °C	0.0258	0.9026	1.92E-05
Xylanase			
a. 40 °C	0.0129	0.7863	1.06E-06
b. 45 °C	0.0302	0.7728	5.99E-05
Cellulase-xylanase mixture			
a. 40 °C	0.1454	0.6749	0.0010
b. 45 °C	0.1714	0.7088	0.0027

The activation energy amount shows the minimum energy amount that must be possessed by a reactant to be able to react (Ashokkumar, 2017). Thus, the smaller the activation energy, the faster the hydrolysis process will be. In this case, the smallest activation energy is obtained by using the cellulase-xylanase mixture (27,253 J/mol).

#### 4. Conclusion

The heterogeneous kinetics model of Valjamae can quantitatively describe the enzymatic hydrolysis mechanism of *Tetraselmis chuii* well. The activation energy in the *Tetraselmis chuii* enzymatic hydrolysis by cellulase-xylanase mixture is lower (27,253) than xylanase (116,121) and cellulase (140,914 J/mol). Therefore, enzymatic hydrolysis by cellulase-xylanase mixture is the fastest. The higher the concentration of the enzyme used, the faster the hydrolysis is, so that the concentration of sugar obtained also increases. Likewise, with the influence of temperature, the enzymatic hydrolysis of *Tetraselmis chuii* at 45°C is faster than 40°C.

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