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Hydrolysis of *S. platensis* Using Sulfuric Acid for Ethanol Production

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Abstract. *S. platensis* is a microalga that contains carbohydrate composition of 30.21% which makes it potential to be used as raw material for ethanol production. Hydrolysis of *S. platensis* is the first step for converting its carbohydrates into monosaccharides. The second step is fermentation of monosaccharides into ethanol. This research aims to study the effect of temperature and microalgae concentration on the hydrolysis of *S. platensis* using sulfuric acid as catalyst. This research was conducted using 300 mL sulfuric acid of 2 mol/L, hydrolysis temperatures of 70, 80 and 90 °C, and microalgae concentrations of 20, 26.7, and 33.3 g/L. The effect of temperature is significant in the hydrolysis of *S. platensis* using sulfuric acid. At microalgae concentration of 20 g/L and hydrolysis time of 35 minutes, the higher the temperatures (70, 80, and 90 °C), the more the glucose yields would be (8.9, 13.5, and 22.9%). This temperature effect got stronger when the hydrolysis was running for 15 minutes. Every time the hydrolysis temperature increased by 10 °C, the glucose yield increased by 13.0% at microalgae concentration of 33.3 g/L. At temperature of 90 °C and time of 35 minutes, the higher the microalgae concentrations (20, 26.7, and 33.3 g/L), the higher the glucose yields would be (25.5, 27.7, and 28.2%). The highest glucose concentration obtained was 2.82 g/L at microalgae concentration of 33.3 g/L, temperature of 90 °C, and time of 35 minutes.

Introduction

Microalgae is one of natural resources that has many benefits, especially as food or dietary supplements, cosmetic ingredients, and biofuels [1-4]. One of microalgae types that multiplies well in Indonesia is *S. platensis*, because it can be cultivated in both freshwater and sea water. This microalgae type contains 30.21 of carbohydrate, 13.30 of protein, and 48.36% of lipid. These compounds have the potential to be used as bio-based raw materials to produce cosmetics, pharmaceuticals, biopolymers, bioethanol, and biodiesel [5-11]. Accordingly, microalgal biomass is very potential when converted into monosaccharides through hydrolysis (Eq. 1), then processed into ethanol through fermentation using yeast (Eq. 2) [12-14]. The conversion of microalgae to ethanol is very promising [15,16] as well as 10 g/L of lipid-extracted microalgae debris can be maximum converted into 3.83 g/L of ethanol [17].

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6 \quad (1)$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (2)$$

Based on the description above, the initial stage of making ethanol from *S. platensis* is hydrolysis, which is to break the sugar polymer chain, so that it becomes a sugar monomer. This stage should be carried out after extracting the protein and lipids, so that they can be used as intermediate raw materials in the cosmetic manufactures, food supplements, and biodiesel [3].

Hydrolysis with alkaline solution is preferred to free the lignin in the biomass, so hydrolysis with acid solution is more suitable because there is relatively little lignin in the microalgae [18,19]. In addition, hydrolysis with acid solution can reduce production costs [19,20]. The use of acid solution can produce glucose around 70-95%, as well as the sulfuric acid as a catalyst will be stronger in accelerating hydrolysis, so that the yield obtained is increased and efficient compared to using enzymes. In addition, the sulfuric acid concentration of 1 mol/L could help hydrolysis, and at a temperature of 100 ºC and 60 minutes, the yield achieved was quite high (around 22.82%) [12].

The concentration of raw materials on the solvent volume affects the glucose yield obtained [21]. This is because the balance will shift if the reagent is made in excess with optimal results [21,22]. Moreover, temperature also greatly affects hydrolysis. According to the Arrhenius law, the higher the temperature, the higher the reaction rate constant will be. Based on the reasons above, this study aims to study the effect of temperature, time, and microalgae concentration on sugar yield in *S. platensis* hydrolysis with sulfuric acid as catalyst.

Materials and Methods

S. platensis powder was obtained from CV Ugo Plankton-shop, Purworejo, sulfuric acid H_2SO_4 (Merck, 1.00731.2500) was used as a catalyst, Nelson-Somogyi reagents, and arsenomolybdate solution were obtained from a local shop. Before being used, the *S. platensis* powder was sieved using an 80 mesh (Endecotts, S/Steel) so that the size was identical which was then stored in a container. The analysis of *S. platensis* composition was done using several methods; they are total hydrolysis method using acid for carbohydrates, micro-Kjeldahl method for protein, Soxhlet method for protein, and Chesson-datta method for hemicellulose, cellulose, and lignin.

The hydrolysis of *S. platensis* was done in a 500 mL three-neck flask equipped with a spiral condenser. The sulfuric acid solution (2 mol/L) used was as much as 300 mL and the concentration of *S. platensis* was varied at 20, 26.7, and 33.3 g/L. The heating was done using a hotplate magnetic stirrer (NESCO MS H 280 pro) with the temperature kept constant at variations of 70, 80, and 90 ºC and a magnetic stirrer speed of 400 rpm. The hydrolysis operated for 35 minutes with 5 minutes interval sampling. The analysis of glucose content was done using the Nelson-Somogyi method. Meanwhile, the glucose yield is calculated according to Eq. (3). After the hydrolysis was complete, the hydrolyzate was filtered using a filter paper which was then stored in a refrigerator.

$$\text{Yield (\%)} = \frac{glucose\ mass\ (g)}{microalgae\ mass\ (g)} x100\% \quad (3)$$

Results and Discussion

The composition of *S. platensis* used in this study contained carbohydrates, proteins, and lipids of 9.49, 58.24, and 0.62% (w/w) (Table 1). Accordingly, *S. platensis* contains carbohydrates (30.21), protein (13.3), and lipids (48.36% (w/w)) [3]. The largest content of microalgae (*Chlorella*, *Dunaliela*, *Chlyamadomonas Scenedesmus,* and *S. platensis*) are starch and glucose (> 50% w/w), which are useful as raw materials for making ethanol [26]. The composition of microalgae varies because it is influenced by plant age, growing media, and growing conditions. The composition of microalgae is very important to determine the operational process of its utilization. It means that lipid extraction needs to be done before microalgae hydrolysis. The main compounds used for bioethanol production are carbohydrates [19]. Carbohydrates accumulate on the outer and inner cell walls, which contain cellulose and hemicellulose, and can be used to produce sugar monomers in the form of glucose, through acid/alkaline or enzymatic hydrolysis [9,12-15,23,24].

A biorefinery is a natural material processing concept adopted from the term crude oil refinery. Each oil fraction is used for fuel-based products [11]. Biorefinery emphasizes the conversion of compounds in a natural material into various useful products. Therefore, microalgae biorefinery is a material processing through extraction of bioactive proteins, carbohydrates, lipids, pigments, and all

the metabolism produced by microalgae. The waste can be used as fuel [18,25]. So, the biorefinery of *S. platensis* can be done by utilizing its protein and lipids, then converted into ethanol [7,17].

Table 1. The Composition of *S. platensis*

Composition (% w/w dry)	*S. platensis* (This study)	*S. platensis* [3]	*S. platensis* [26]
Protein	58.24	13.30	71.00
Carbohydrate	9.49	30.21	16.90
Lipid	0.62	48.36	7.00
Hemicellulose	3.28	-	-
Cellulose	19.56	-	-
Lignin	11.37	-	-

Table 2. Glucose yield (%) of hydrolysis of *S. platensis* (operation condition: sulfuric acid concentration 2 mol/L and volume 300 mL)

Microalgae (g/L)	20.0			26.7			33.3		
Temperature (°C)	70	80	90	70	80	90	70	80	90
Time (min) 5	4.2	4.1	9.7	6.0	9.5	10.0	6.2	10.8	13.2
10	5.7	9.8	12.5	6.3	9.9	12.9	7.8	11.4	16.4
15	6.0	10.3	15.3	6.6	12.8	19.0	8.0	11.7	20.0
20	6.5	11.8	17.0	6.8	13.6	20.3	8.7	15.0	22.3
25	6.8	13.2	18.2	8.5	15.8	21.3	9.0	16.6	22.6
30	8.6	13.9	20.9	8.8	16.0	21.9	9.6	18.2	23.1
35	8.9	14.3	25.2	9.2	16.4	27.7	10.4	18.2	28.2

Besides catalyst types, hydrolysis is also influenced by temperature [19,25]. Table 1 and Fig. 1-3 show the effect of temperature on the glucose yield; the higher the temperature, the higher the glucose yield. This occurs because temperature affects the ability of acids to break down sugar polymers [22,28]. Recently, hydrolysis is an endothermic reaction that requires heat to react. In this study, hydrolysis of *S. platensis* with 2 mol/L sulfuric acid at microalgae concentration of 20 g/L and temperatures of 70, 80, and 90 °C resulted in glucose yields of 8.9, 13.5, and 22.9%, respectively. Meanwhile, at microalgae concentration of 26.7 g/L the glucose yields were 9.2, 16.4, and 34.2% and at microalgae concentration of 33.3 g/L the glucose yields were 8.9, 16.5, and 28.2%, respectively.

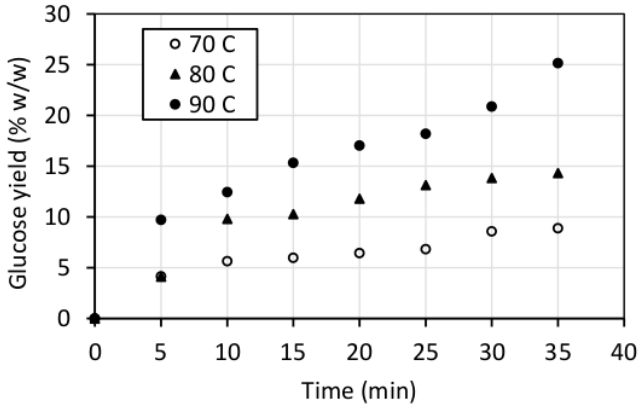

Figure 1. The temperature effect on hydrolysis of *S. platensis* (operation condition: sulfuric acid concentration 2 mol/L, microalgae concentration 20 g/L, volume 300 mL)

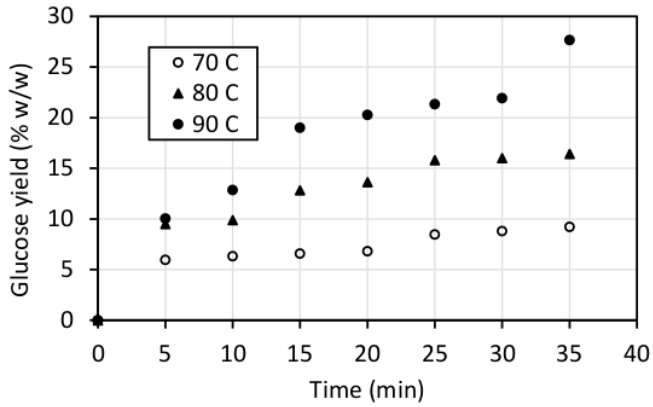

Figure 2. The temperature effect on hydrolysis of *S. platensis* (operation condition: sulfuric acid concentration 2 mol/L, microalgae concentration 26.7 g/L, volume 300 mL)

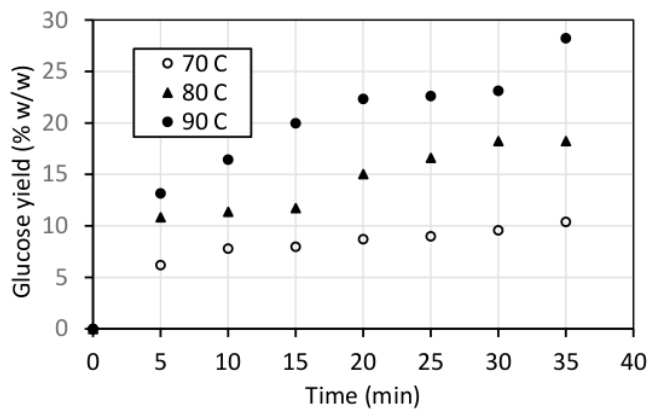

Figure 3. The temperature effect on hydrolysis of *S. platensis* (operation condition: sulfuric acid concentration 2 mol/L, microalgae concentration 33.3 g/L, volume 300 mL).

In addition, a hydrolysis of *Tetraselmis chuii* using sulfuric acid 1% (v/v) at temperatures of 60 and 70 °C, the highest sugar concentration was obtained at 70 °C which was 18.15 g/L. Whereas, the hydrolysis at 100 °C for 30 minutes resulted in a sugar concentration of 7.27 g/L [27]. This indicates that there was sugar degradation. The sugar degradation can be occurred due to high temperature and catalyst concentration [19]. In the recent study, at microalgae concentration of 33.3 g/L and temperatures of 60, 70, and 80 °C, for 30 minutes, the resulting glucose concentrations were 0.80, 1.49, and 2.20 g/L, respectively (see Table 3).

In addition, hydrolysis time also affects the glucose yield. The longer the reaction time (0 to 35 minutes), the higher the glucose yield produced. As reported previously, hydrolysis *Tetraselmis chuii* took place optimally at 1 N sulfuric acid, temperature of 100 °C, and time of 60 minutes. Moreover, the hydrolysis of *Tetraselmis chuii* using 0.2 M sulfuric acid, at temperature of 121 °C, and time of 30 minutes resulted in a sugar concentration of 4 g/L [28]. In the recent study, at microalgae concentration of 26.7 g/L and temperature of 70 °C, the glucose yields produced were 0.48, 0.58, 0.68, 0.73, 0.74, 0.78, and 0.81 g/L, respectively for 5, 10, 15, 20, 25, 30, and 35 minutes. On top of that, during the hydrolysis (0 to 35 minutes) and at all temperature variations, the glucose yields continued to increase. This means that there is no sugar degradation. At temperature of 90 °C and microalgae concentration of 33.3 g/L, the sugar yield increased to 8.9%.

Table 3. Glucose concentration (g/L) of hydrolysis of *S. platensis* (operation condition: sulfuric acid concentration 2 mol/L and volume 300 mL)

Microalgae (g/L)		20.0			26.7			33.3	
Temperature (°C)	70	80	90	70	80	90	70	80	90
Time (min) 5	0.42	0.41	0.97	0.60	0.95	1.00	0.62	1.08	1.31
10	0.57	0.98	1.24	0.63	0.99	1.29	0.78	1.14	1.64
15	0.60	1.03	1.53	0.66	1.28	1.90	0.80	1.17	2.00
20	0.65	1.18	1.70	0.68	1.36	2.03	0.87	1.50	2.23
25	0.68	1.32	1.82	0.85	1.58	2.13	0.90	1.66	2.26
30	0.86	1.39	2.09	0.88	1.60	2.19	0.96	1.82	2.31
35	0.89	1.43	2.52	0.92	1.64	2.77	1.04	1.82	2.82

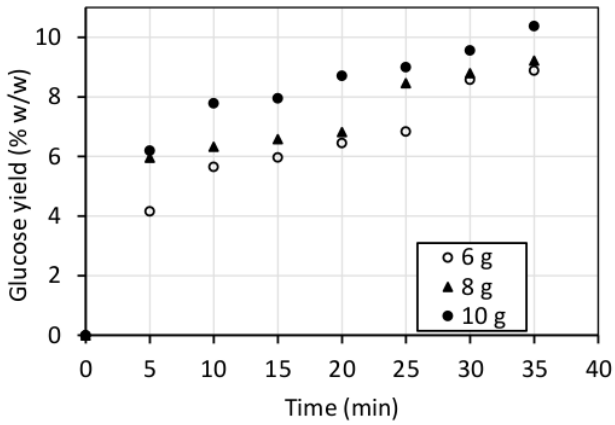

Figure 4. The microalgae concentration effect on hydrolysis of *S. platensis* (operation condition: sulfuric acid concentration 2 mol/L, temperature 70 °C, volume 300 mL)

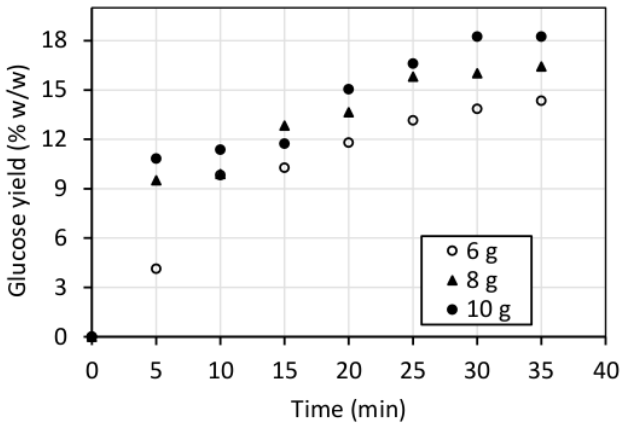

Figure 5. The microalgae concentration effect of hydrolysis on *S. platensis* (operation condition: sulfuric acid concentration 2 mol/L, temperature 80 °C, volume 300 mL)

Several factors that affect acid hydrolysis are mass/volume ratio, type of acid, acid concentration, time, and temperature [29]. In Fig. 4-6 it can be seen that at temperature of 70 °C and time of 35 minutes, the higher the microalgae concentrations (20, 26.7, and 33.3 g/L) the higher the

glucose yields (8.8, 9.2, and 8.9%). Likewise, at temperature of 80 ºC and time of 35 minutes, the glucose yields increased by 13.5, 16.4, and 16.5%, respectively. In certain cases, if the material concentration is too high the yield will decrease, because the catalyst access to the material decreases. The ingredient ratio that is too large results in less glucose formed. During the hydrolysis, at all variations in the microalgae concentration and temperature, the yields continued to increase, for example at temperature of 90 ºC and time of 35 minutes, the glucose concentrations were 22.9, 32.4, and 28.2 g/L, respectively at microalgae concentrations of 20, 26.7, and 33.3 g/L.

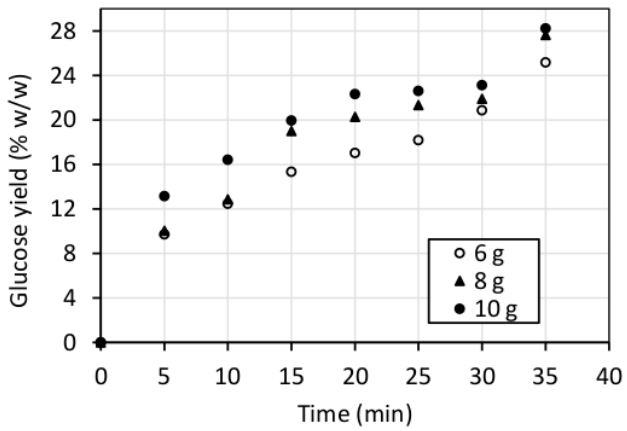

Figure 6. The microalgae concentration effect on hydrolysis of *S. platensis* (operation condition: sulfuric acid concentration 2 mol/L, temperature 90 ºC, volume 300 mL)

Conclusion

The hydrolysis of *S. platensis* using 2 mol/L sulfuric acid at various microalgae concentrations of 20, 26.7, and 33.3 g/L and temperatures of 70, 80, and 90 ºC ran well for 35 minutes; the resulting glucose concentrations continued to increase. The higher the temperature, the higher the glucose concentration. At microalgae concentration of 20 g/L, for 20 minutes, the glucose concentrations increased from 0.39, 0.71, and 1.02 g/L, respectively at temperatures of 70, 80, and 90 ºC. The effect of microalgae concentration is similar, the higher the microalgae concentration, the higher the glucose concentration. At temperature of 80 ºC and time of 35 minutes, the glucose concentrations increased from 1.43, 1.64, and 1.82 g/L due to the higher microalgae concentrations (20, 26.7, and 33.3 g/L). This study resulted in the highest glucose yield of 28.2% under several conditions (microalgae concentration of 33.3 g / L, temperature of 90 ºC, and time of 35 minutes). This means, *S. platensis* can be used as a raw material for producing sustainable ethanol.

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