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Hydrolysis Pretreatment of *Tetraselmis chuii* into Glucose by Using Diluted Sulfiric Acid

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Abstract. Microalga has emerged as third generation in fuel based biomass which converted into several products and its content can be also utilized as model compound. Microalga comprises cellulose, hemicellulose and lignin. These cellulose and hemicellulose can be converted into glucose, as model compound for fuel and chemical production. This study focused on *Tetraselmis chuii* as feedstock and the hydrolysis pretreatment into glucose conversion by employing diluted sulfuric solution in varied temperature and concentrations. *Tetraselmis chuii* was used as microalga feedstock since they are distributed in Indonesia's ocean. In order to obtain fuel or chemical product, microalga need to be pretreated first. This process was conducted with focused on breakdown the linkage between cellulose, hemicellulose or lignin. Glucose was generated from this hydrolysis process. Thus, it would apply as feedstock for bioethanol or furan production which utilized as intermediate compound/building block compound. This study aimed into the significant parameters for Tetraselmiss chuii hydrolysis and their biochemical composition. Experimental result was assayed in quantitative method by using High Performance Liquid Chromatography (HPLC). It was found out that Tetraselmiss chuii contain alpha cellulose and hemicelluloses without lignin present. Moreover, temperature has significant influenced for hydrolysis process than sulfuric acid concentration. The highest glucose temperature was 61 g/L which obtained at 100°C by using 1.25%(v/v) diluted sulfuric acid concentration.

Keywords: *diluted sulfuric acid; glucose; hydrolysis; temperature; Tetraselmis chuii.*

1 Introduction

Indonesia is a maritime country which has biodiversity, including the diversity of marine microalgae. Microalgae or also called phytoplankton is a microscopic plant that has body size 3-30 m and has no roots, stems, and leaves. Microalgae has a eukaryotic cell and different pigments, such as green pigment (chlorophyll), brown (phycosantine), turquoise (phycobilline), and red (phycoeritrine) [1]. Microalgae is classified as plant because it has chlorophyll and has a network of cells resemble higher plants. Microalgae diversity is very high, it has been estimated that about 200.000-800.000 microalgae species exist on earth which 35.000 species have been identified. Microalgae cells grow in aqueous media, so that it has a higher level of efficiency in terms of water, carbon dioxide, and nutrients when compared to other higher plants [2]. The growth of microalgae itself consists of three main phases, namely the lag phase, exponential phase, and stationary phase [3].

Recently, biomass utilization as fossil fuel replacement feedstock has significantly increased as well for chemicals. Since, biomass is considered as a renewable and efficient source for covering people need over fuel and chemicals. Researcher developed technology and route pathway process in order to obtain renewable product. For instance, domestic waste and cattle dung for biogas production, palm oil for biodiesel and lignocellulosic material (e.g. crop plant, hard wood, etc) for bioethanol. However, it was found out that lignocellulosic material has some problems. Lignocellulosic material, such

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as corn, starch, sugar cane, is also consumed as food. Competition between lignocellulosic material as food consumption and fuel source would cause instability in economic. Prolonged harvest time is also becoming disadvantage of lignocellulosic material especially for hard wood material.

Tetraselmis chuii is green microalgae which has single cells and size 7-12 μm. *Tetraselmis chuii* has an elliptical form and has 4 pieces of feather whip (flagella) on the front end that serves as an active locomotor [4]. Chlorophyll is a dominant pigment and it is composed of two kinds, carotenes and xantofile [5]. This species can be found in sea water, and already widely cultivated, especially in shrimp hatchery. Breeding took place rapidly through cell division. Vegetative cell of protoplasm in *Tetraselmis chuii* repeatedly performed a cleavage from a single stem cell into 2-16 new cells [6].

Tetraselmis chuii can grow at temperature around 23-25ºC. At temperatures of 25-35ºC, *Tetraselmis chuii* still be alive, but not growing. Salinity for *Tetraselmis chuii* is very crucial to maintain the osmotic pressure of the protoplasm with water in the environment. *Tetraselmis chuii* can grow at salinity 0-35 ppt but the good conditions for growth of *Tetraselmis chuii* is 25-35 ppt [7]. The optimal pH range for growth is 8 to 9.5 [8].

Glucose is a simple monosaccharide that has the molecular formula C6H12O6. It's also called as dextrose, D-glucose, or sugar fruit. Glucose is a simple sugar that is produced through the process of hydrolysis [9]. The rupture of the crystal structure of cellulose and hemicellulose will break down into simple sugars such as glucose, galactose, mannose, xylose, and arabinose [10]. Concentrated acid was first applied in the process of hydrolysis and proven as an effective liquid to degrade cellulose and hemicellulose into simple monomers. The use of concentrated acid is usually carried out at low temperatures [11]. However, concentrated acid hydrolysis causes a high cost of investment and maintenance [12]. Ho et al. [13] used Chlorella vulgaris as a raw material to produce glucose. Microalgae powder with weight variation was hydrolyzed with a solution of sulphuric acid concentration at 0.1%, 0.2%, 0.5%, 1%, and 5% (v/v). Temperature and time were fixed variables. The result of this study showed that the highest glucose yield was 46.5% and it was achieved at 1% (v/v) sulfuric acid concentration.

Harun et al. [14] used *Chlorococcum infusionum* as raw material to be hydrolyzed with NaOH solution. Time and the concentration of NaOH were varied. The highest glucose yield was 35%, achieved at a temperature of 120ºC, concentration of NaOH was 0.75% (w/v) for 30 minutes. This study utilized *Tetraselmis chuii* as a raw material to be converted into glucose by using varied temperatures and diluted sulphuric acid concentrations. The objectives of this study were to look for the significant effect of temperature hydrolysis and diluted sulfuric acid and also to find the biochemical's composition of *Tetraselmis chuii*.

2 Methodology

2.1 Material

Tetraselmis chuii powder was procured from Balai Besar Perikanan Budidaya Laut Lampung. Sulphuric acid (98%, Merck), D-glucose (Merck) and distilled water were used in this study.

2.2 Analysis of Microalga Composition

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A gram of *Tetraselmis chuii* was mixed with 150 ml sulphuric acid. The mixture was refluxed at 100°C for 2 hours. This process was performed in order to determine the composition of cellulose, hemicellulose and lignin within the microalgae. This experiment was conducted by using the Chesson gravimetric method [15].

2.3 Hydrolysis Process

Tetraselmis chuii powder was dissolved about 3 grams in diluted sulphuric acid in certain concentration. The mixture was stirred around 250 rpm in a three-neck boiling flask that connected to condenser for 30 minutes. Hydrolysis process was performed in varied temperature.

The hydrolysis apparatus was designed as can be seen in Figure 1. Hydrolysis products were analyzed by using High Performance Liquid Chromatography (HPLC) with C18 (YMT-ODS) coloumn, in 250 mm x 4.6 mm sized. Volume injection was 5 µm, flow rate 1 mL/min, mobile phase was aquabidest 100% and using Refracto Index Detector (RID) as detector. This analysis was conducted in the UPT Laboratory of Diponegoro University.

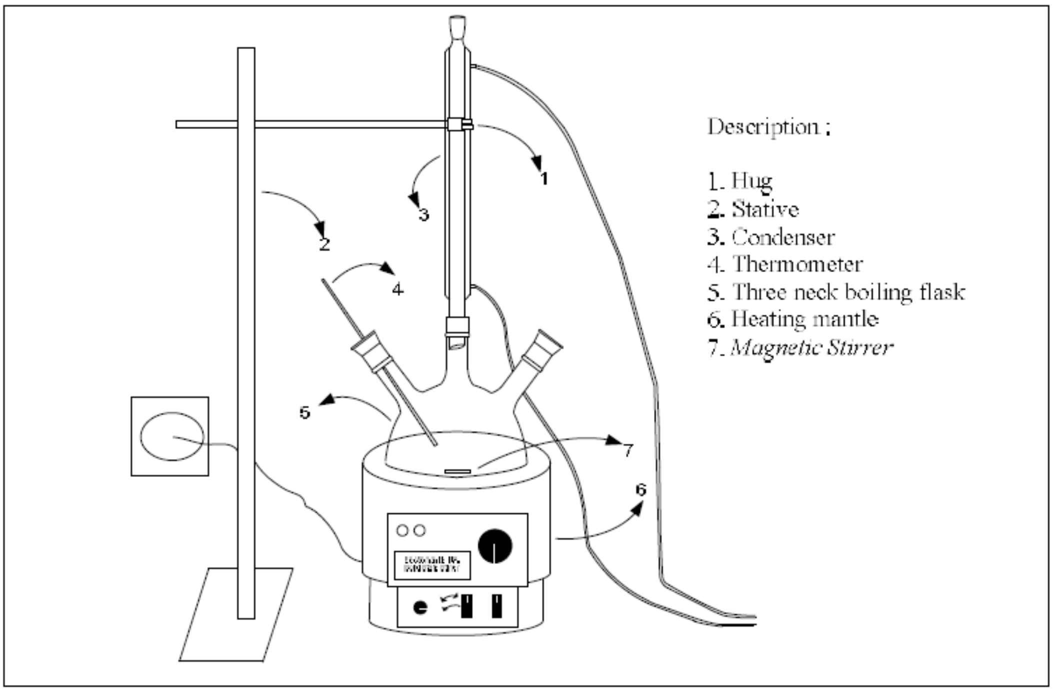

Figure 1 Hydrolysis Apparatus.

3 Results and Discussion

3.1 *Tetraselmis chuii* compositions

Composition analysis results was shown that *Tetraselmis chuii* has 47.2% of alpha - cellulose, 35.8% of hemicellulose and balance hot water soluble (HWS). This result was illustrated in Figure 2. It was clearly shown that there is a lignin absence within *Tetraselmis chuii*. The similar result was reported by Demirbas [16]. Lignin existances which is linked cellulose and hemicellulose will be barried the accessibility of enzymes or fermentation processes. It also decreases hydrolysis rate of carbohydrate [17].

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These cellulose and hemicellulose are compiled within inner cell wall layer of microalgae as reported by Yamada and Sakaguchi in Chen et al. [18] and linked by 1,4-β-glycosides. The higher cellulose and hemicellulose content within *Tetraselmis chuii* indicates its potencies for biomass feedstock. Especially, there is no lignin content in *Tetraselmis chuii*.

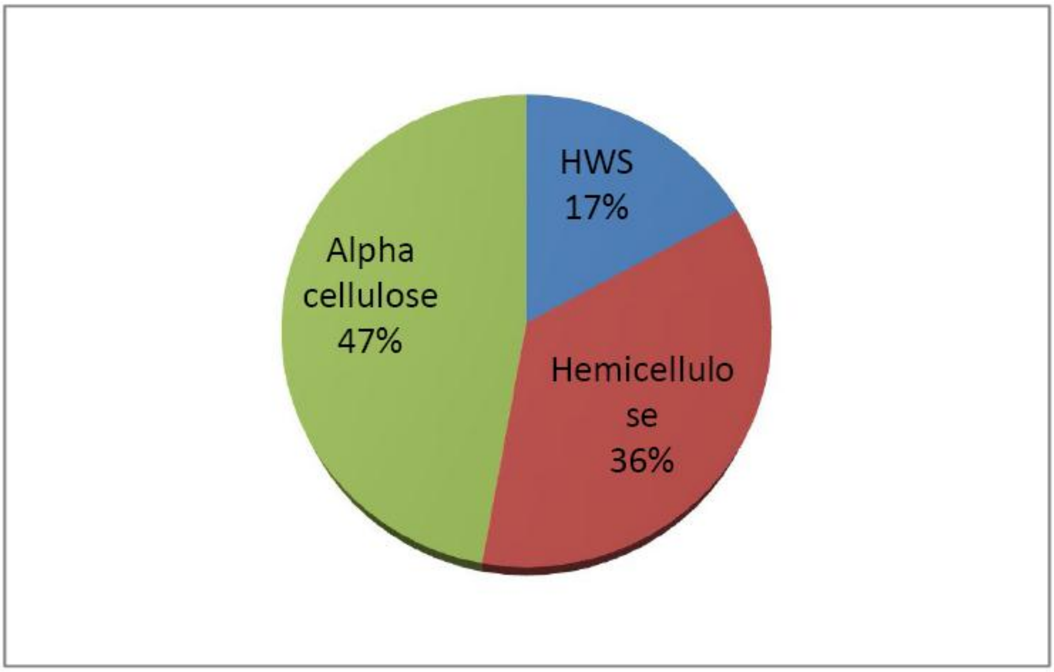

Figure 2 Biochemical's composition of *Tetraselmis chuii.*

3.2 Effect of Diluted Sulfuric Acid Concentration

This experiments were carried out by using varied diluted sulfuric acid concentration. They were 0.25, 0.75, 1.25, 1.75, and 2.25% (v/v). It was found out that higher glucose concentration was achieved at concentration 2.25% (v/v) as can be seen in Figure 3. It shows that glucose production tends to increase while the sulfuric acid was added. Eventhough, there was no significant glucose for each concentration in this experiments, Jeong et al. [19] already proved that sulfuric acid is more effective for acid hydrolysis process in order to convert biomass to glucose. Jeong et al. [19] used sulfuric acid, hydrochloric acid, formic acid, and nitric acid at low concentrations as a catalyst for *Gelidium amansii* hydrolysis and obtained the optimum yield glucose about 26.08% by using sulfuric acid.

Another research was done by Ho et al.[13] regarding diluted sulfuric acid concentration. They varied the concentration from condition 0.1, 0.2, 0.5, 1 and 5% (v/v) and got the highest glucose yield around 96.% when the diluted sulfuric acid 1% (v/v). It will be benign environmentally and low operating cost.

According to Xiang et al. [20], the acidic solution with a concentration of 1-2% (v/v) and a maximum temperature of 100°C are ideal conditions to degrade biomass into glucose monomers. While at temperatures above 100 °C and the medium concentration exceeds 2% (v/v), sulphuric acid will faster degrade the glucose compounds. Glucose degradation preceded by the formation of 5-hidroxymethylfurfural. The high concentration of sulphuric acid will produce levulinic acid and formic acid [21].

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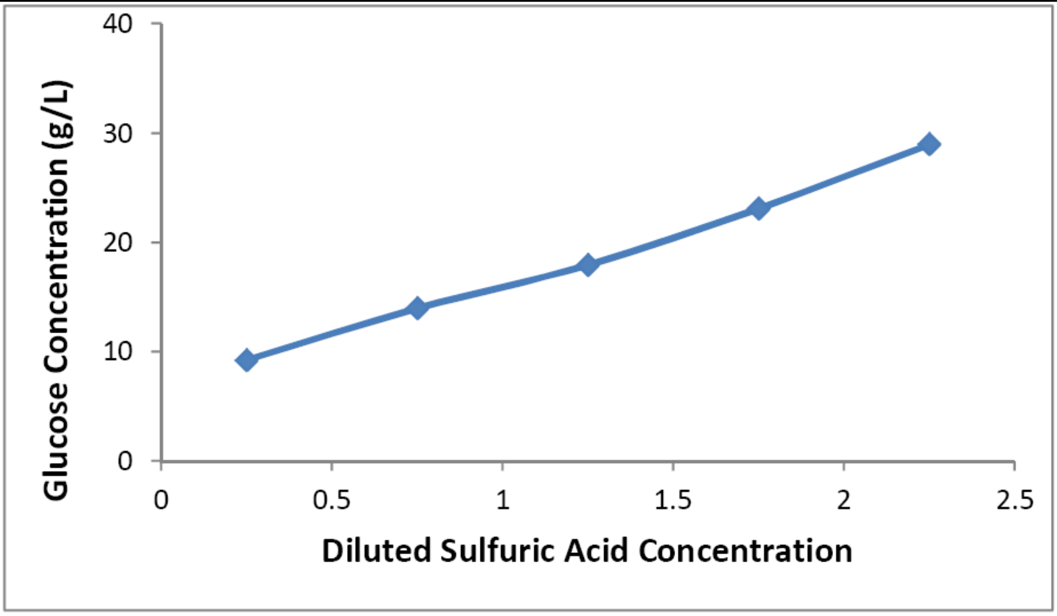

Figure 3 The influence of varied diluted sulfuric acid concentrations to glucose production.

3.3 Effect of Temperature Hydrolysis for Glucose Production

Temperature has the significant impact for biomass hydrolysis. It is believed that adjacent temperature with cellulose's melting point would able to decompose cellulose as it reported by Hsu et al. [22]. Related with it, varied hydrolysis temperatures were applied in this experiment. Figure 4 shows the relation between generated glucose and temperature. It displays fluctuated tendency of glucose concentration over temperature. Highest glucose concentration was obtained at 100ºC with 1.25%(v/v) diluted sulfuric acid. It was around 61 g/L. However, there was an increased glucose concentration at 70ºC around 60.8 g/L. It seemed that cellulose and hemicellulose bonding started to disrupt at 60ºC and breakdown the carbon chain into glucose.

Hemicellulose chains began to break down at temperature of 55-65°C with acid hydrolysis [23]. Hemicellulose chain is more easily broken than cellulose due to an amorphous structure and short chain. In green microalgae, a major constituent of hemicellulose components are galactoglucomannan. Galactoglucomannan consists of galactose, glucose, and mannose, a hexose group which has six carbon atoms in the monomer [24]. Addition of acid concentration can increase the quantity of H + ions which will cut off the glycosides bond contained in cellulose [25]. Cellulose is a long chain fibers in which monomers bind to each other through the bond of 1,4-β-glycosides, has a low flexibility due to the strong intermolecular force [26]. Glucopyranose ring structure also makes it difficult to rotate the molecule. Cellulose can be broken down into glucose units by dissolving it with a chemical acid. This reaction through three stages. First, the process of hydrolysis will cause swelling of cellulose fibers. After that, the solution will go into the crystalline structure of cellulose. As a result, the cellulose fibers will be degraded [27].

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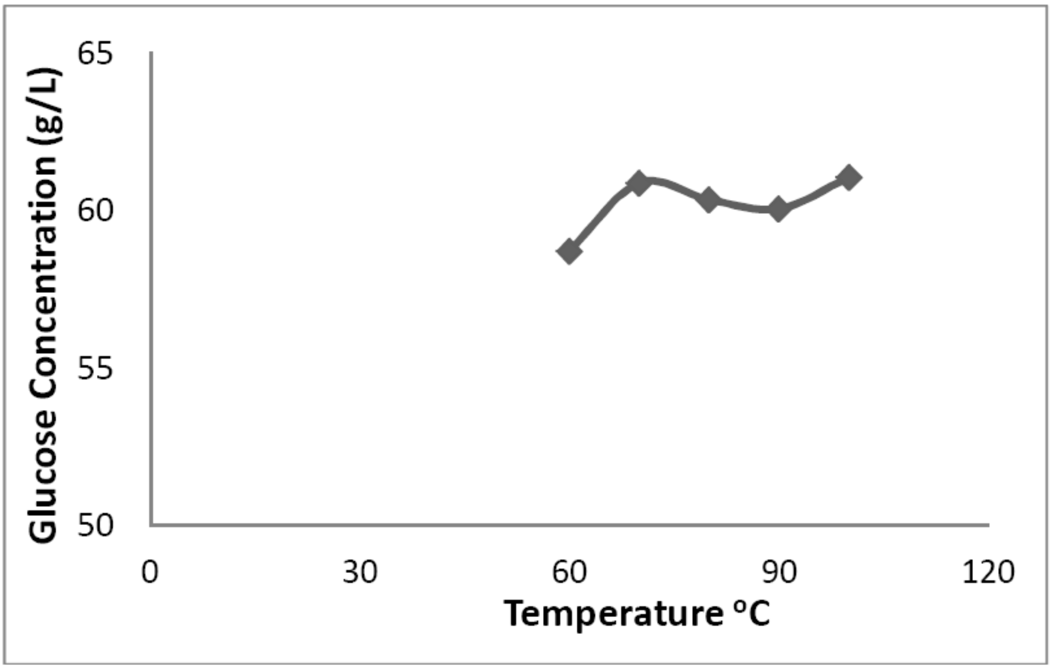

Figure 4 The influence of varied hydrolysis temperature to glucose productions

4 Conclusions

From the experiment, it is clearly shown that *Tetraselmis chuii* consist of 47.2% cellulose, 35.8% hemicellulose and 17% other components. *Tetraselmis chuii* contains lignin absence which lead the microalga easy to convert for fermentable sugar and other building block compound. The highest glucose concentration was obtained 61 g/L at 100°C which produced when sulphuric acid concentration was 2.25% (v/v). Also, it was shown that temperature gave significant impact than acid concentration.

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