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Pretreatment and Hydrolysis Processes on Synechococcus Pcc 7002 Cells for Glucose Production

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ABSTRACT

Microalgae are potential alternative source for renewable energy such as bioethanol. To produce bioethanol pretreatment methods such as sonication and acid hydrolysis for carbohydrate production are required. Pretreatment is important to release the carbohydrate from the microalgae cell wall as well as to increase the production. These processes were applied to *Synechococcus* PCC 7002, a type of cyanobacteria species. The aim of this project was to release the glucose from cyanobacteria cells prior to bioethanol production. Three parameters were studied which were sonication time (15, 30 and 45 minutes), sulphuric acid concentration (1%, 3%, 5% and 7% v/v) and hydrolysis time (15 and 30 minutes). The response studied was carbohydrate yield. The result showed that, most of the samples with 15 minutes of sonication gave higher carbohydrate content compared to 30 and 45 minutes. In contrast to that, highest concentration of sulphuric acid at 7% and longer hydrolysis duration at 30 minutes hydrolysis gave higher yield of carbohydrate. It was concluded that neither glucose nor sucrose yields from sonicated and hydrolyzed biomass. This could be due to insufficient of heat to break the glycosidic linkage between the oligosaccharide.

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INTRODUCTION

Biofuel is renewable and environmental friendly. It is one of promising alternative energy to the nonrenewable fuel. It is derived from organic matter and can be categorize into two categories; primary and secondary biofuels [1]. Primary biofuels normally from crude/unprocessed substances like firewood, animal waste, forest and crop residue, meanwhile secondary biofuels either bioethanol or biodiesel which are produce from biomass processing for industrial and transportation. Technological process, raw material and development will further classified the secondary biofuels into three generations [1].

Biofuel production is facing a problem in it mass production due to shortage of resource. Alternative to the common sources such as sugar cane or starches, microalgae could be the potential substrate to biofuel production. Recently, microalgae have become the most desired source in broadening the production of biofuel. They have been considered by many in producing bioethanol and by-products [2] due to widely production with low capital and energy requirement [3], easily replenish and cultivation period over years [4]. Unlike first generation biofuels, some microalgae do not compete for freshwater or affect the natural landscapes because algae do not compete for agricultural land and can be grown in an enclosed bioreactor or open pond system. Therefore it has less conflict with food security which also a criteria that should be met to have energy security [5].

Microalgae, is a photoautotrophically which can be found in freshwater and marine system. It exist either individually, in chains or in groups. The colors can be varies from brown, red and green. Microalgae are mostly eukaryotes and conduct photosynthesis in the chloroplast, a membrane bound structure. Cyanobacteria is a prokaryotes and have characteristics of bacteria and similar to algae in term of size. Besides, due to it photoautotrophs characteristics cyanobacteria also known as blue-green algae and green pigments which result from photosynthesis.

Microalgae are known to consist of multiple layers of cell wall which is rich in sugar within it cellulose. Cellulose which composes of glucose monosaccharide units promotes microalgae as a source for conversion to bioethanol [6]. Therefore it is important to gain the glucose from the microalgae cellulose by rupturing it cell

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wall, either by physical or chemical process before hydrolysis with mineral acid to release the sugars. An optimum hydrolysis process is essential prior to ethanol production by fermentation of yeast. Thus, the earlier step will determine that ethanol is produce efficiently. This pretreatment process is important to attain larger surface area for further process like bioethanol fermentation and also to release the component like glucose, fructose and maltose within the cell wall [7].

On top of that, the concentration of sulphuric acid (H_2SO_4) with high temperature plays an important role in the production of glucose. H_2SO_4 will solubilize the starch and the rate of hydrolysis into monosaccharide will increase under high temperature condition [8].

The objective of this study is to compare carbohydrate yield of wet biomass of *Synechococcus* PCC 7002 pretreated using sonicator at different sonication time and hydrolyzed using different process conditions.

MATERIALS AND METHODS

Microalgae strain:

Synechococcus PCC 7002 strain was obtained from American Type Culture Collection (ATCC)® 27264. The strain was cultured in ATCC medium 957 with 20 µg/L of vitamin B_{12} at 26°C under 2000-3000 LUX of aerobic light.

Cell preparation:

Once the wet cells have been harvested, total cell number (TCN) was determined using hemocytometer chamber. Then, the wet cell biomass was transferred into 50 mL falcon tubes for the cell disruption method.

Cell disruption by sonication:

Wet cells biomass was processed with Labsonic®P homogenizer at 25 kHz in 100% power level for 15, 30 and 45 minutes as shown in Table 1. The ruptured cells were then centrifuge at 9000 rpm for 15 minutes. The collected supernatant and the pellet were then separated in different falcon tubes and stored in -20^0C.

Hydrolysis of microalgae:

Different concentrations of 3 ml H_2SO_4 (1%, 3%, 5% and 7%) were added into test tubes contained 2 ml of wet cell biomass as shown in Table 1. The test tubes where then underwent two different heating times (15 and 30 minutes) at 120°C and cooled at room temperature afterward. The biomass was centrifuged at 9000 rpm for 15 minutes and supernatant was collected for carbohydrate analysis by using high performance liquid chromatography (HPLC).

Table 1: Sample number with respect to its process condition.

Sample no.	Sonication time (minutes)			Sulphuric acid concentration (%)	Heating at 120°C (minutes)
1	15	30	45	1	15
2				3	
3				5	
4				7	
5				1	30
6				3	
7				5	
8				7	

HPLC analysis:

The standards of glucose and sucrose with molar concentration of 0.2, 0.4, 0.6, 0.8 and 1.0 M were prepared. HPLC was set up by using IC-Pak Ion Exclusion column with SH-1011P pre-column at temperature of 75°C. The flow rate was 1.0 mL/min and 0.5 mM of sulphuric acid as a mobile phase.

Results:

Total cell number (TCN) was determined before the experiment conducted in order to standardize the total cell in every tube. The TCN for all samples before sonication were estimated between ranges of $2.00\text{-}3.00\times10^7$ cells/mL.

Figure 1 showed chromatograms of samples compared to glucose and sucrose standards. The figure showed that sucrose and glucose standards appeared at minute 4.9 and 5.6 of retention time, respectively. Meanwhile, samples peaks appeared at retention time at minute 3.9 before the standards peaks.

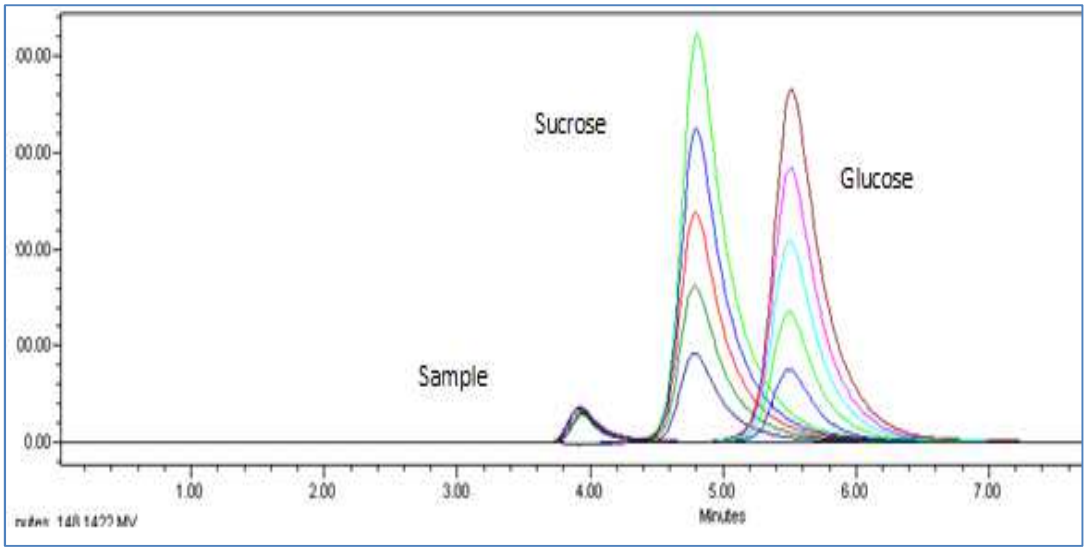

Fig. 1: Standard peaks of sucrose and glucose appeared at retention time of 4.80 and 5.50 minutes, respectively and sample in the range of 3.90 minutes.

Figures 2 and 3 showed different of peak area of oligosaccharides determined from chromatograms at different sonication time for respective samples as shown in Table 1. Peak area was measured to make quantitatively comparison of process conditions for oligosaccharides production. From both Figures 2 and 3, samples with lowest sonication time (i.e. 15 minutes) in general gave higher oligosaccharide content compared to higher sonication time (i.e. 30 and 45 minutes). In contrast to that, the higher sulphuric acid concentration and the longer hydrolysis process gave higher yield of oligosaccharides when compared with lowest concentration of acid sulphuric (i.e. 1%) and 15 minutes hydrolysis. Comparing both sulphuric acid concentrations and sonication time's correlation in both Figures 2 and 3, it showed that there was strong relationship between high concentration of sulphuric acid and low sonication time. The highest amount of oligosaccharides was obtained from samples which was least sonicated (i.e. 15 minutes) and hydrolyze at highest sulphuric concentration (i.e. 7%) at 120°C. Meanwhile, between period of hydrolysis and concentration of sulphuric acid respectively at 30 minutes and 7% concentration gave better yield when compared to 15 minutes and 1% concentration. The relationship between contact time and sonication time, influence the result where yield increase when both contact time and sonication time increase.

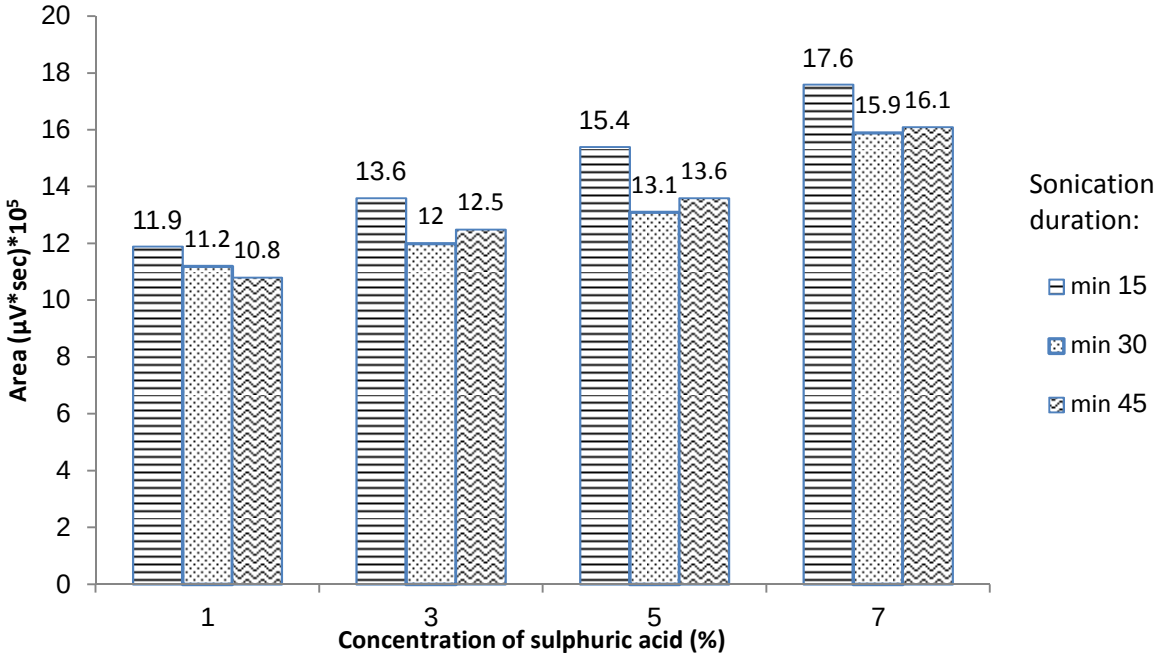

Fig. 2: Chromatogram area of samples hydrolyzed at 15 minutes retention time.

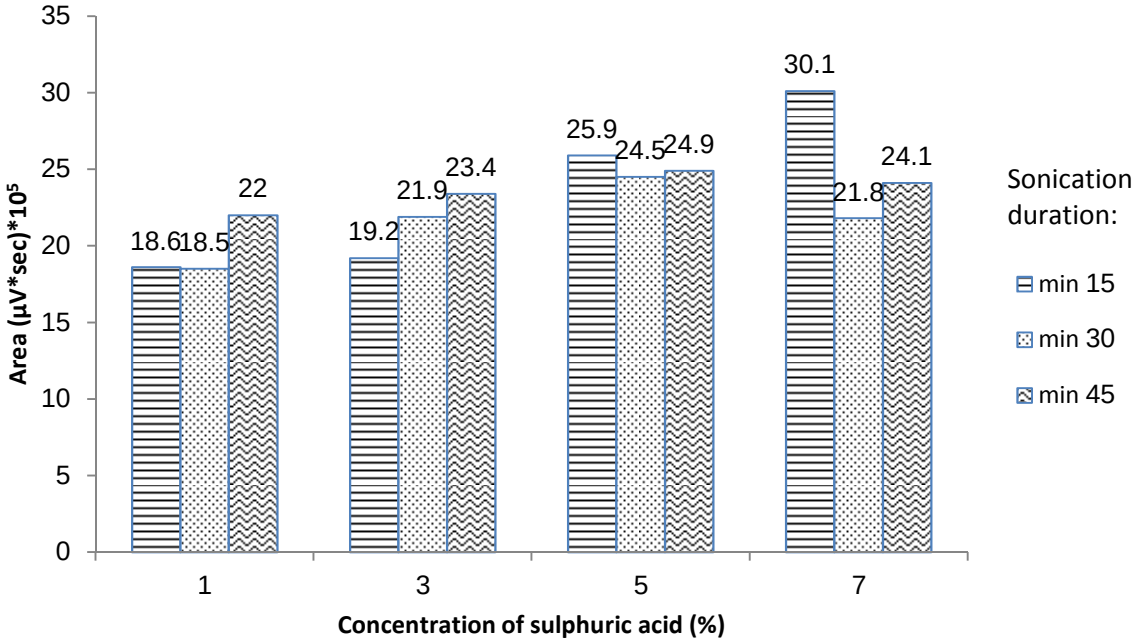

Fig. 3: Chromatogram area of samples hydrolyzed at 15 minutes retention time.

Discussion:

From the chromatogram showed in Figure 1, this confirmed that the samples neither glucose nor sucrose. The samples are suspected having higher chain of carbohydrate which belongs to oligosaccharide based on its retention time. The existence of oligosaccharide which was not completely hydrolyzed into monosaccharide or disaccharide such as glucose or sucrose, respectively, resulted of the sample to appear at earlier retention time than the standards peaks. Hydrolysis was needed to break the glycosidic linkage in order to get the constituent of monosaccharide units. These results may be due to the low temperature during hydrolysis process where the temperature used was 120°C. According to [2] Harun et al. (2011), 4-6% of glucose was obtained after *Chlorococum humicola* was hydrolyzed with 1% sulphuric acid at 140°C for 30 minutes. The research was conducted by using low concentration of sulphuric acid which is contrasted to [9] Yazdani et al. (2011) found higher concentration of sulphuric acid (i.e. 7%) gave better yield of carbohydrate content using different temperature and hydrolysis duration. Hence, to break further the oligosaccharides to monosaccharide (i.e. glucose), different pretreatment and/or hydrolysis conditions may be required.

Sonication was one of the methods to rupture the cell wall of cyanobacteria. Cyanobacteria cellulose is present in matrix form which inaccessible for chemical and enzyme activity. Therefore pretreatment was require to attain larger surface area and increase the susceptibility of microalgae cellulose to chemically or enzymatically hydrolysis. This method has been proved by [10] Bao (2011), where the researcher successfully isolated the cyanobacteria content by using sonicator. On the other hand, finding from [11] Mioni et al. (2011) showed that cell preservation was lacking when the sonication was applied. In this current study, the higher yield was obtained from 15 minutes of sonication. This showed that, least sonication time gave higher yield than longer sonication time. [12] Zechmann et al. (2010) reported that the cell can be ruptured at 4 minutes (in 1-min intervals) at 40 W on ice in order to release the content in cyanobacteria. Figures 2 and 3 on sonication time did not showed significant differences on the carbohydrate yields. This might be due to lack of uniformity which caused from of ultrasounds propagation. However, these results confirm that higher yield of carbohydrate content can be obtained when the minimum time for sonication process was applied.

Figures 2 and 3 showed that higher concentration of the sulphuric acid (i.e. 7%) can release more carbohydrate content. Similar finding was also obtained by [9] Yazdani et al. (2011). They reported that increase of concentration from 0.5% to 7% of H_2SO_4 increased the fermentable sugars. Meanwhile, many finding showed that the concentration of sulphuric acid is inversely proportional to time, where, low concentration of sulphuric acid was performed in longer time and vice versa [1,6]. This study utilizing dilute acid hydrolysis required longer hydrolysis or retention time to produce or release higher carbohydrates. Higher yields were obtained from longer hydrolysis process where 30 min of hydrolysis time produce more compared to 15 min (Figures 2 and 3).

Conclusion:

The results showed that samples were neither glucose nor sucrose. This could be affected by the different concentration of sulphuric acid and hydrolysis temperature to break the glucosidic linkage between the oligosaccharide. The carbohydrates were oligosaccharides types. The releasing of carbohydrate content from the cell wall was proportionally influenced by duration of hydrolysis and concentration of sulphuric acid. However, the carbohydrates are inversely proportional to duration of sonication. Further experiment and analysis may be required for glucose production.

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