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Biosugar Production From Algae *Spirogyra peipingensis* by acid and enzymatic hydrolysis processes

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Abstract. *Spirogyra peipingensis*, green algae, is a suitable feedstock for biosugar production. This study analyzes biosugar production by the hydrolysis of *S. peipingensis* conducted by acid and enzymatic hydrolysis processes. Acid hydrolysis process was conducted by H_2SO_4 0,2 M acid with various concentrations (0,2,4,6,8, dan 10%). Enzymatic hydrolysis process was held by using amylase enzyme with vary concentrations (0,25,50,75,100 dan 125 KNU). The result showed that the hydrolysis process with H_2SO_4 acid generates higher sugar concentration than compared with the amylase enzyme. Enzymatic hydrolysis method produces the optimum sugar concentration which is made up 0,45 g/g in adding α-amylase enzyme 50 KNU. In contrast, hydrolysis acid process able to produce the optimum sugar concentration in 0,55 g/g in adding of H_2SO_4 10% 0,2 M acid.

1. Introduction

Algae is one of the organisms that can grow in a wide range of condition on earth [1]. Alga is an organism which has quick growth process [2]. Algae has gigantic benefits, such as natural polysaccharides, which are widely used in the fields of food technology, biotechnology, microbiology, and even medicine [3]. Algae are renewable biomass resources consisting of carbon-containing sugar polymers, which can be used to create biodegradable and high-quality biochemical products, especially biosugar [4].

Production of biosugar from algae can be a realistic solution to replace the function of canes as. It was caused by the ingredients of carbohydrate in algae is quite high and the growth also very quick [5]. Biosugar production from algae does not disrupt food security and has high carbohydrate concentration. One of the potential carbohydrates is in Spirogyra algae because the carbohydrate concentration of Spirogyra algae can reach 64% [5].

Spirogyra algae are alga which the population were spread out on earth [6]. Spirogyra algae is a group of green algae from Zygnematales ordo which can do photosynthesis and has a eukaryotic cell [7]. Spirogyra algae have chloroplasts in spiral ribbon shape with a nucleus [8]. Reproduction process in vegetative in fragmentation way and generative reproduction with conjugation [9]. The length of Spirogyra algae can attain one foot (30,48 cm) which arranged by transparent protoplasm and each cell has one or more chloroplast that extends from end to end in a spiral shape [10,11]. In ribbon chloroplast, has pyrenoid which was surrounded by flour granules and consisted of high carbohydrate concentration [12,13].

Carbohydrate degradation process from algae to monosugar can do in two methods generally, i.e acid hydrolysis method and enzymatic hydrolysis method [14]. One of effective hydrolysis method is enzymatic hydrolysis method. Biosugar production should produce sugar value more than 20%. The advantage of enzymatic hydrolysis is it has potential in revealing high result and low operational cost because it does not need any corrosive materials [15]. Another method in degrading carbohydrate is the acid hydrolysis process. Besides its effectiveness, the method also has cheaper cost but it is quite

not environmentally friendly [16,17]. This study aimed to determine biosugar production by the hydrolysis of *S. peipingensis* conducted by acid and enzymatic hydrolysis process.

2. Materials and Method

2.1. Algae Strain

S. peipingensis algae were obtained from the wetlands of Tamalanrea District, Makassar at LS coordinates 05 ° 12'65,229 "and BT: 119 ° 49'18,695". Algae that have been obtained are then cultured on sulfahri-01 medium with 3,000 lux illumination at 12 hour afternoon intervals and 12 night hours. The culture process for 2 weeks at 30 °C.

2.2. Pretreatment Alga S. peipingensis

Spirogyra algae that have been cultured are then harvested and then dried using an oven at 80 °C for 24 hours. *S. peipingensis* algae that have been dried crushed using a hammer mill (Fomac-Miller FCT-2100) at a speed of 12,000 rpm for 5 minutes until they were crushed and sieved with a 40 mesh sieve. *S. peipingensis* algae which passed the 40 mesh sieve were weighed with 100 g biomass and added distilled water until the volume reached 900 ml, then stirred. *S. peipingensis* was then hydrolyzed by heating, followed by the addition of the α-amylase enzyme.

2.3. Enzymatic hydrolysis Process

The pretreatment *S. peipingensis* was put into the Erlenmeyer and heated on a hot plate. Heating is done while stirring. The heating process lasts for 2 hours with a heating temperature of ± 100 ° C then cooled to temperatures of ± 45 ° C, and α-amylase (Liquozyme Supra, Novozymes, Denmark) as much as 25 KNU (Kilo Novo Unit), and incubated for 80 minutes [15]. After hydrolysis, the hydrolyzate is filtered using filter paper for the supernatant to take. The supernatant obtained was then centrifuged at 9,000 rpm for 15 minutes, the centrifugation supernatant then measured the sugar content.

2.4. Acid Hydrolisis Process

S. peipingensis which has undergone a pretreatment process is hydrolyzed using sulfuric acid (0.2 M H_2SO_4) with each different concentration 0%, 2%, 4%, 6%; 8%; 10% then heated for 2 hours at 1000C. After hydrolysis, the hydrolyzate is filtered using filter paper for the supernatant to take. The supernatant obtained was then centrifuged at 9,000 rpm for 15 minutes, the centrifugation supernatant then measured the sugar content.

2.5. Measurement of Reducing Sugar Levels

The reducing sugar was measured using the Luff Schoorl method. Culture samples were taken as much as 5 ml and put into Erlenmeyer, then added 50 ml of distilled water. After that, $Al(OH)_3$ drop by drop was added until the sample became clear. Then added distilled water until the sample volume reaches 100 ml, and filtered using filter paper. The filtrate is stored in 200 ml measuring flask and sufficient anhydrous Na_2CO_3 is added, then distilled water is added to 200 ml, then stirred and filtered again. 25 ml of the sample which was free of Pb were taken and 25 ml of Luff-Schoorl solution was added. The solution is then heated to boil for 10 minutes and then cooled. After cold, add 15 ml of crystal KI 20% and 25 ml of 26.5% H_2SO_4 solution and then tightly closed for 10 minutes. The sample was then added to the 3 ml starch indicator and titrated with 0.1 N sodium thiosulfate as a titrant until the black blue colour disappeared for the first time. The volume of sodium thiosulfate solution used as titrant for sam = an ml. In distilled water, the same procedure is carried out with the sample treatment. The volume of sodium thiosulfate solution used as titrant for blank = b ml. Use of sodium thiosulfate: (b - a) ml. The reducing sugar contained in the sample is identical to (b-a) ml. reducing sugar is then calculated by converting the value (b-a) ml to the Luff table (attachment 2) so that glucose is obtained.

2.6. Statistical Analysis

The design of this study used RAL (Completely Randomized Design) with replications 3 times. The parameters measured were sugar levels. Data were analyzed statistically using Analysis of Variance (Anova) with a 95% confidence interval ($\alpha = 0.05$). The Analysis was conducted to compare the process of acid hydrolysis and enzyme hydrolysis process to the level of reducing sugars produced. If there is an effect, then continued with Duncan test at the 95% confidence level ($\alpha = 0.05$) to know pair of a group the same and different data in each treatment.

3. Results and Discussion

3.1. Enzymatic Hydrolysis

The result of the research showed that the hydrolysis process with enzyme produces higher sugar value than without enzyme. Hydrolysis method with α-amylase enzyme addition (Liquozyme Supra, Novozymes, Denmark) 25 KNU generate sugar value 0,35 g/g, while hydrolysis method without enzyme produces 0,22 g/g sugar value in each repeating. This showed that the addition of enzyme produces higher sugar value than without enzyme. It was caused by an enzyme which has the biocatalyst characteristic which increases the reaction process [18]. In addition of α-amylase enzyme with 50,75, 100 and 125 KNU reveal do not significantly different on 0,45-0,46 g/g.

Based on ANOVA analysis on 95% confidence interval show that the amount of enzyme can effect in sugar value which was produced by enzymatic hydrolysis process *Spirogyra peipingensis* algae. Additionally, based on Turkey's test, reducing sugar production in addition enzyme 50 KNU, 75 KNU, 100 KNU and 125 KNU were not significantly different. Therefore, the enzymatic hydrolysis method that produced the most effective reducing sugar was 50 KNU because the resulting reducing sugar concentration was higher and not significantly different with 75 KNU, 100 KNU and 125 KNU (Figure 1).

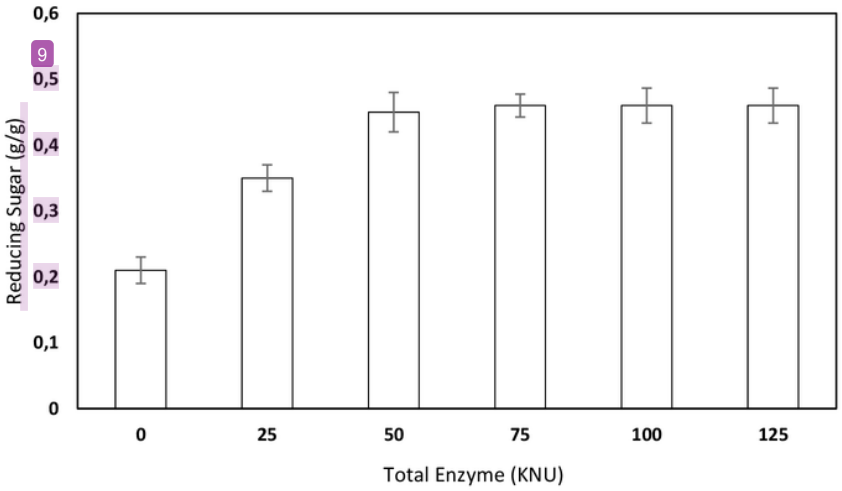

Figure 1. The Influence of The Amylase Concentration to Reducing Sugar Value Result

The substrate of *S. peipingensis* is carbohydrate types. Spirogyra algae have high carbohydrate concentration with 64% [19,20]. The biomass composition of algae are k Spirogyra algae are 45,1% sugar, 22,0% rough protein, and lipid 3,6%. The carbohydrate that consisted of Spirogyra is starch carbohydrate [14] so as it can be separated by the amylase enzyme.

Amylase enzyme is able to abbreviate amylum in *S. peipingensis* algae to be monosugar. Amylase enzyme is an enzyme which has the capability to break down glucoside bond of the starch polymer.

The excess addition of α-amylase enzyme may devastate specific bond, such as α-1 bond, and 4-glucoside to produce glucose [21]. The obtained sugar concentration in the present study by using algae *S. peipingensis* to be higher than other studies. Hydrolysis of *Saccarina japonica* use amylase enzyme which can generate biosugar whereas hydrolysis of the Ulva fasciata with cellulase enzyme can produce 0,21 g/g monosugar [22,23]. Thus, the algae *S. peipingensis* may be more suitable for successful biosugar production by enzyme hydrolysis.

3.2. Acid Hydrolysis

The result of the research showed that the hydrolysis process with H_2SO_4 acid generates higher sugar value than without enzyme. Enzymatic hydrolysis method produces optimum sugar value by adding α-amylase enzyme by 50,75,100 and 125 KNU. Meanwhile, acid hydrolysis can produce 0,55 g/g sugar value by adding H_2SO_4 10% 0,2 M.

Base on ANOVA analysis result, acid concentration affect significantly reducing sugar value. By ignoring acid addition, reducing sugar production is made up only 0,34 g/g. However, addition 6% acid may produce reducing sugar with 0,49 g/g. Additionally, based on Tukey's test, reducing sugar production pada H_2SO_4 2% dan H_2SO_4 4% were not significantly different. reducing sugar production pada H_2SO_4 6%, H_2SO_4 8%, dan H_2SO_4 10% also not significantly different. Therefore, the hydrolysis method that produced the most effective reducing sugar was H_2SO_4 6% 0,2 M because the resulting reducing sugar concentration was higher and not significantly different with H_2SO_4 8% and H_2SO_4 10% (Figure 1).

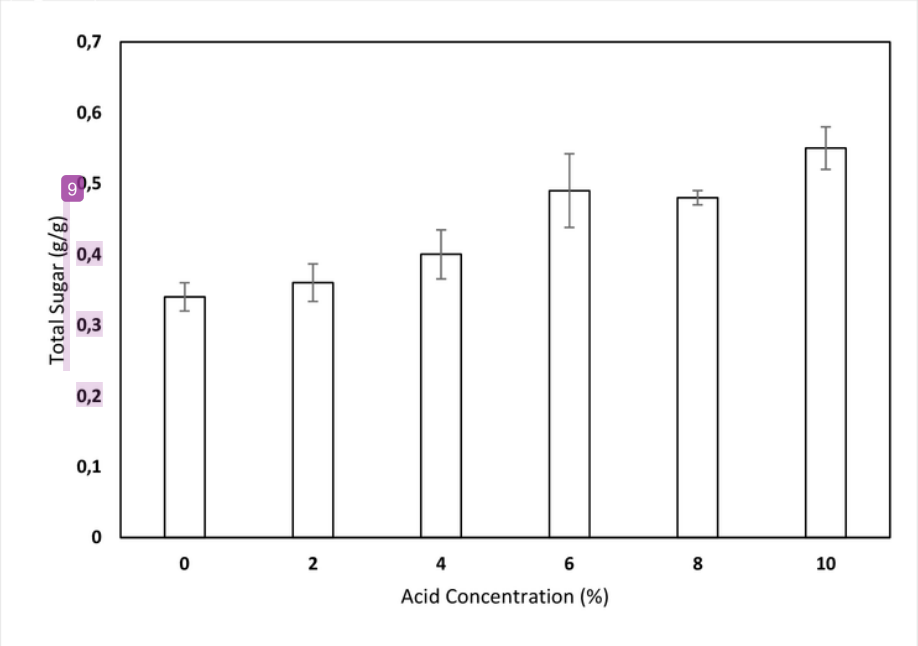

Figure 2. The Influence Of The H_2SO_4 Acid To Reducing Sugar Result

The result showed that based on ANOVA analysis, enzymatic hydrolysis produces different sugar value significantly with acid hydrolysis while reducing sugar value that was generated by acid hydrolysis is higher by comparing with enzymatic hydrolysis. This result bears a strong novelty that acid hydrolysis is more effective to use on breaking down carbohydrate of *S. peipingensis*. Result of this research which was found that in this study has been supported by other studies which also found that acid hydrolysis is beneficial because producing sugar value with low cost [3]. However, acid

hydrolysis produce hydroxymethylfurfural (HMF) and levulinic acid (LA) that as an inhibitor in biosugar development of hydrolysis product to be bioethanol, methanol, glutamate etc. Hence, acid hydrolysis result needs further action to erase hydroxymethylfurfural (HMF) and levulinic acid (LA). [24,25]. Nevertheless, even though enzyme hydrolysis produces low sugar value, but it is environmentally friendly and does not produce inhibitor product, such as hydroxymethylfurfural (HMF) and levulinic acid (LA) of acid hydrolysis.

4. Conclusion

The result of the research showed that the hydrolysis process H_2SO_4 acid produces higher sugar value than enzymatic hydrolysis with amylase enzyme. Enzymatic hydrolysis method produce optimum sugar value with 0,45-0,46 g/g by adding 50,75,100 and 125 KNU, while acid hydrolysis can produce optimum sugar value in adding H_2SO_4 10% 0,2 M.

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