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Bioethanol Production Using Green Algae (*Chaetomorpha*) As Renewable Energy

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Abstract

Biologically, green algae (Chaetomorpha) are a group of chlorophyll plants consisting of one or many cells and in the form of a colony. The main organic materials contain in algae for example polysaccharides, vitamins, and minerals. So far the use of algae as an industrial raw material is still relatively small. Though the chemical components containing in algae are very useful for raw materials for bioenergy. The green algae have a potential as raw material in bioethanol (C_2H_5OH) production as one of the alternatives and renewable energy. The purpose of these experiments was to determine the effect of a variety of concentrations of green algae on reducing sugar and bioethanol production through liquefaction, saccharification, and fermentation. Green algae flour was obtained by drying in an oven at 60°C for 24 h, then was ground. The reducing sugar was obtained by two steps, i.e., liquefaction and saccharification. In liquefaction, the algae flour was put in an Erlenmeyer 2000 ml and added by 40 mg/l $CaCl_2$, α-amylase, and water until the slurry volume was achieved 2000 ml, then heated at 90°C-100°C for 2 h. The next step was for saccharification, in this step HCl was added in the solution to achieve a pH of 4, and glucoamylase was added, then was heated at 60°C for 4 h. The final stage was fermentation, this solution was then added with urea, NPK, and yeast, then incubated for 72 h. After fermentation, the liquid was distilled to obtain bioethanol. The results showed that the addition of enzymes with a concentration of 2% yielded reducing sugar of 64.62, 34.38, 8.46, and 1.14 g/l, with green algal concentrations of 10, 30, and 40% (w/v), respectively. The bioethanol obtained was 0, 0.81, 2.92, and 5.29%, with reducing sugar of 64.62, 34.38, 8.46, and 1.14 g/l for 0, 24, 48, and 72 h, respectively. However, the addition of enzymes with a concentration of 5% produced reducing sugar of 122.22, 55.32, 10.23, and 1.3 g/l, respectively. The bioethanol obtained was 0, 0.92, 3.71, and 8.16% for 0, 24, 48, and 72 h, respectively. It concluded that the maximum bioethanol obtained was 8.16 % at algae concentration of 40% (w/v) for 72 h of fermentation.

***Keywords**: Amylase, bioethanol, glucoamylase, algae, reducing sugar, saccharification.*

Introduction

Bioethanol is a very flammable fuel. The results of this bioethanol combustion in the form of water, carbon dioxide (CO_2), and heat. Where the amount of CO_2 produced is less than fossil fuels from petroleum that are commonly used. The CO_2 produced from combustion itself is a greenhouse gas causing global warming, if CO_2 is reduced through the use of bioethanol as fuel, this can reduce the pollution of CO_2 gas in the environment which results in decreased global warming. NOx gas emissions produced during combustion are also lower. Bioethanol has properties similar to petroleum and can be used as a substitution or partial substitution for petroleum fuels of up to 5% without going through modification.

Indonesia is an archipelago that has a very high diversity of seaweed species, even by seaweed experts say that Indonesian waters are seaweed barns. The development towards seaweed industrialization, Indonesia is still far behind with other countries such as Japan, Korea, Taiwan, and China. In Indonesia, the production of seaweed is still limited to the food industry and export commodity raw materials. To use seaweed as an ingredient in the food, cosmetic, pharmaceutical, medical, and agricultural industries, it is still necessary to learn from countries that are experts in seaweed processing. Therefore, future actions still need research on the use of microalgae/seaweed in a sustainable manner. Green algae (*Chaetomorpha*) is one of the most abundant seaweed in the waters in Indonesia. *Chaetomorpha* belongs to the

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group of green algae. The carbohydrate content of algae is 42, 81% and for the starch content is 16.71%, so that green algae can be used as raw material for bioethanol. In Indonesia, algae are still very poorly utilized by the community. Usually, algal fishermen are only considered as useless seaweed. However, in marine life, algae are very beneficial for marine biota such as zooplankton which makes *Chaetomorpha* an ideal home for them. Some different conversion mechanisms to produce bioethanol, such as anaerobic digestion, fermentation, transesterification, liquefaction, and pyrolysis processes (Alfonsin *et al*., 2019; Chen *et al*., 2015; Soeprijanto *et al*., 2019). Shokrkar (2017) showed that cellulose and starch in the biomass were decomposed to release simple sugars by dilute acids using mixed microalgae culture, then fermented into bioethanol. Their experiments were carried out using microalgal biomass with concentrations from 25 to 50 g/l, and reducing sugar and glucose yielded after 40 min remained constant at about 86 and 81%, respectively. The use of a mixture of enzymes is better for seaweed/ microalgae biomass hydrolysis since seaweeds/microalgae are composed of various polysaccharides other than cellulose such as laminarin, mannitol, alginate, agar, carrageenan, and ulvan (Ge *et al*., 2011; Chirapart *et al*., 2014; Kostas *et al*. 2016). Microorganisms such as yeasts have an important role in bioethanol production by fermenting of sugars to ethanol. They have valuable properties in ethanol yield, ethanol tolerance, ethanol productivity, growth in simple, inexpensive media, and undiluted fermentation broth with resistance to inhibitors and retard contaminants from growth condition (Mohd Azhar *et al*., 2017). Yeasts are their population mainly depending on the type and content of organic matter available in the environment (Khambhaty *et al*., 2012). Khambhaty *et al*. (2012) showed that the utilization of marine yeast was conducted using acid hydrolysis by mixing 5 g of biomass with 100 ml of different acid concentrations. They observed that the yeast could ferment sugars such as melibiose, lactose, maltose, galactose, cellobiose, xylose, and raffinose. Kostas *et al*. (2016), however, showed the use of 24 types of yeast obtained from glycerol stocks stored. Experiments were carried out for reports on the selection of yeast strains that can metabolize a range of monosaccharides originating from the breakdown of seaweed polysaccharides.

These experiments aimed to evaluate the effect of a variety of concentrations of green algae on the production of reducing sugar through liquefaction and saccharification, and the production of bioethanol from fermentation.

Materials and Methods

Materials. Green algae were obtained from the Sumenep district, Madura. The chemicals used such as HCl, NaOH, NPK, Urea, Fehling A, and Fehling B, Methylene blue was purchased at Surabaya chemical stores. α-amylase and glucoamylase enzymes were obtained from PT. Sorini Agro Asia Corporindo, Pasuruan Regency.

Experimental Setup. Green algae were cleaned by washing them clean of impurities. This clean alga was heated on the oven at a temperature of 60°C for 24 h. Then the dried green algae were blended until it becomes flour.

Liquefaction process. The liquefaction was carried out by the use of the enzyme α-amylase through hydrolyzing the starch contained in the material into reducing sugars and other sugar compounds as shown in Figure 1. In this process, algae flour of 200-400 g (10-40%, w/v), 40 mg/l $CaCl_2$, 2-5% (v/w) α-amylase was put into a 2000 ml Erlenmeyer and then added with HCl solution to achieve a slurry pH 6.5-6.6. Then, the slurry was heated at 90-100°C for 2 h.

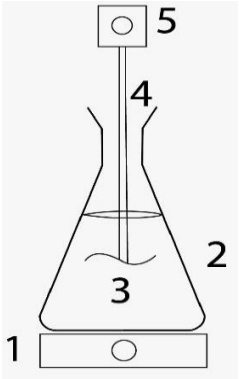

Figure 1. Liquefaction of green algae flour for sugar production using α-amylase. *Note:* 1 = hot plate; 2 = Erlenmeyer; 3 = Slurry of tapioca solid waste; 4 = propeller stirrer; 5 = stirrer motor.

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Saccharification process. After the liquefaction, the slurry was cooled to 60°C and passed on to the saccharification (Figure 2). The slurry was then added with a 2 N HCl solution to become a pH of 4. To complete the hydrolyze of starch to sugar, glucoamylase enzyme 2-5% (v/w) was added to the slurry. Then the slurry was heated temperature of 60°C for 4 h.

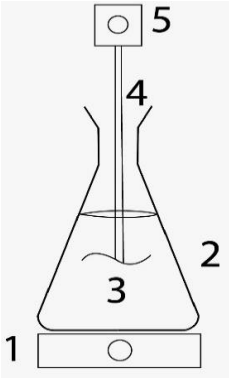

Figure 2. Saccharification of green algae flour for sugar production using glucoamylase. *Note:* 1 = hot plate; 2 = Erlenmeyer; 3 = Slurry of tapioca solid waste; 4 = propeller stirrer; 5 = stirrer motor.

Fermentation. Fermentation was carried out by the use of *Saccharomyces cerevisiae* to convert sugars to ethanol in the fermentation as seen in Figure 3. The sugar obtained from saccharification was put in a fermenter than was added with yeast 0.5%, urea 0.2%, and NPK 0.2% (w/w) of reducing sugar content in the slurry. This slurry was fermented for 72 h.

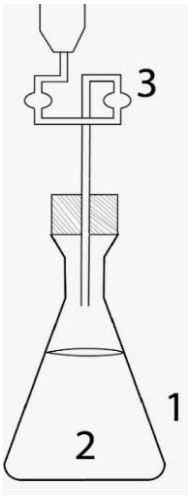

Figure 3. Batch fermentation of ethanol using *Saccharomyces cerevisiae* baker yeast. *Note:* 1 = fermenter; 2 = slurry of green algae flour; 3 = channel of CO2 gas produced.

Distillation. The content of ethanol produced from fermentation was then increased through the fractionation distillation process by heating the slurry at approximately 80°C. Then, the results of the distillate were analyzed for the ethanol content.

Analysis of reducing sugar. A solution of 10 ml of Fehling A and Fehling B was put into the Erlenmeyer, then 4 drops of methylene blue indicator solution were added. The mixed Fehling solutions were then titrated with a standard sugar solution of 10 g/l and the volume of solution needed was recorded. This work was repeated using a sample sugar solution. Reducing sugar was calculated as seen in Equation (1).

$$C_{spl} = \frac{C_{std} \times V_{std}}{V_{spl}} \left({}^{g}/_{l} \right) \quad (1)$$

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Where:

C_{spl} = sample concentration, g/l
V_{spl} = sample volume, ml
C_{std} = standard concentration, g/l
V_{std} = standard volume, ml

Analysis of starch content.

The green algae flour were weighed as much as 2-5 grams and were then added with 50 ml of distilled water and stirred for 1 h. Then the slurry was filtered to separate solids and liquids and washed with distilled water to obtain 250 ml of filtrate. The residue on filter paper was then washed 5 times with 10 ml of ether, then allowed to evaporate the ether from the residue. The residue has been washed again with 150 ml of 10% ethanol to further free up dissolved carbohydrates. In the next stage, the residue was transferred to the Erlenmeyer by washing 200 ml of distilled water and adding 20 ml of 25% HCl. The filtrate was then heated with a water bath for 2.5 h in a closed Erlenmeyer. After that, the filtrate was cooled and neutralized with 45% NaOH solution and diluted to a volume of 450 ml then filtered.

The determination of sugar content is expressed as glucose from the filtrate obtained and the determination of glucose such as reducing sugars. The weight of glucose multiplied by 0.9 is the weight of starch.

Weight of starch = weight of glucose x 0.9

Results and Discussion

Liquefaction

Figure 4 shows that in the process of liquefaction the reduced sugar concentration obtained at the addition of α-amylase of 2% (v/w) with algal concentrations of 10, 30, and 40% (w/v) producing sugar of 7.33, 13.75, and 15.28 g/l, respectively. Whereas in the addition of α-amylase 5% (v/w) with algal concentrations of 10, 30, and 40% (w/v) producing sugar of 8.46, 22, and 27.5 g/l, respectively. The maximum reducing sugar concentration obtained during the liquefaction was 27.5 g/l at 40% (w/v) algae concentration with the addition of 5% α-amylase. This is consistent with the literature which explains that the greater the concentration of the addition of enzymes, the greater the activity of enzymes in the media so that more starch is converted into reducing sugars (Alfonsin *et al*., 2019; Soeprijanto, 2013).

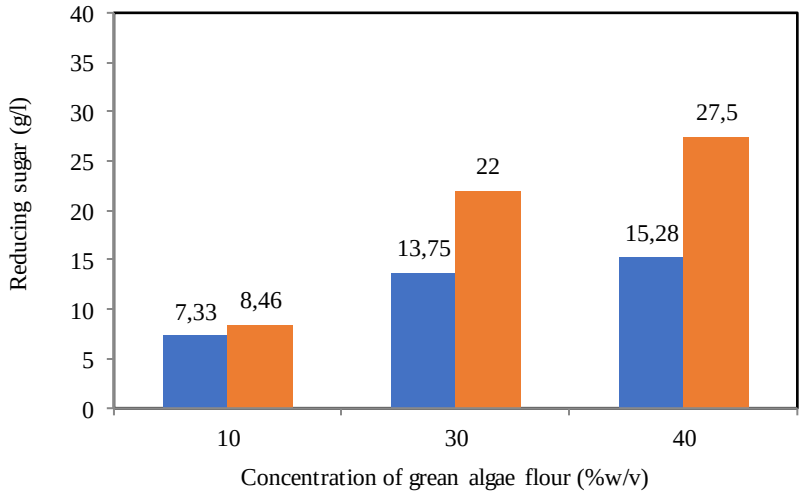

Figure 4. Comparison of reducing sugar content after the liquefaction for 2 hours. *Note:* ■: Enzyme = 2%; ■: Enzyme = 5%.

The yield of reducing sugar in liquefaction

Figure 5 shows that in liquefaction the yield of sugars obtained on the addition of the enzyme of α-amylase 2% (v/w) with algal starch concentrations of 10, 30, and 40% (w/v) was 0.0733, 0.04583, and 0.0382 g sugar/g flour, respectively. While the addition of the enzyme of α-amylase 5% (w/v) with algal concentrations of 10, 30, and 40% (w/v) produced

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0.0846, 0.0733, and 0.0687 g sugar/g flour, respectively. The maximum yield of sugar obtained during the liquefaction was 0.0846 sugar/g flour at 10% (w/v) algae concentration with the use of 5% (w/v) α-amylase enzyme. This is consistent with the literature which states that the yield of reducing sugars increases with the increase in the concentration of the enzyme α-amylase (Alfonsin *et al*., 2019; Soeprijanto, 2013).

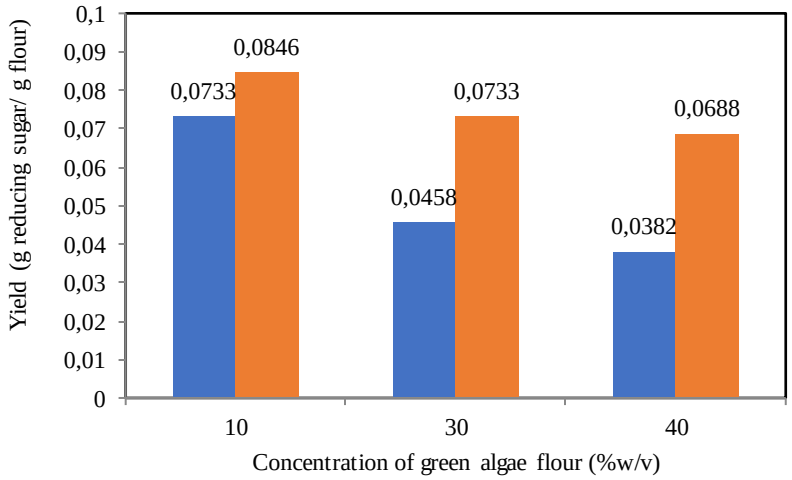

Figure 5. Comparison of reducing sugar yield after the liquefaction for 2 h. *Note*: ■ = Enzyme (2%); ■ = Enzyme (5%).

Saccharification

Figure 6 shows that in the saccharification the reduced sugar concentration obtained in the addition of the enzyme glucoamylase 2% (w/v) with algal concentrations of 10, 30, and 40% (w/v) producing sugar of 19.64, 57.89, and 64.62 g/l, respectively. Whereas in the addition of the glucoamylase 5% (w/v) with flour algal concentrations of 10, 30, and 40% (w/v) produced sugar of 29.72, 91.67, and 122.22 g/l, respectively. The maximum reducing sugar concentration obtained during the saccharification was 122.22 g/l at an algal flour concentration of 40% (w/v) with an additional concentration of the enzyme glucoamylase 5% (w/v). This is consistent with the literature which explains that the greater the concentration of the addition of enzymes, the greater the activity of enzymes in the media so that more starch is converted into reducing sugars (Alfonsin *et al*., 2019; Soeprijanto, 2013).

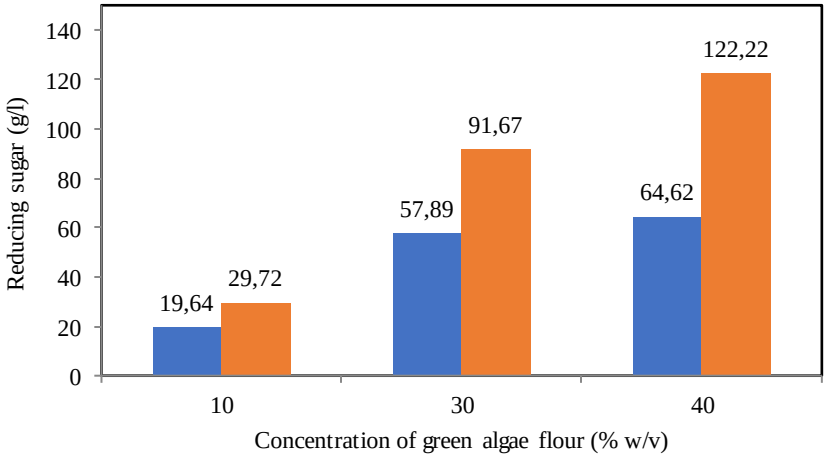

Figure 6. Comparison of reducing sugar content after the saccharification for 4 hours. *Note:* ■: Enzyme =2%; ■: Enzyme = 5%.

The yield of reducing sugar in saccharification

Figure 7 shows that in the saccharification the yield obtained on the addition of the enzyme of glucoamylase 2% (w/v) with algal concentrations of 10, 30, and 40% (w/v) produced 0.1964, 0.1930, 0.1615 g sugar/g flour, respectively. While

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in the addition of the enzyme glucoamylase 5% (w/v) with algal flour concentrations of 10, 30, and 40 (w/v) produced 0.2972, 0.3056, and 0.3055 g sugar/g flour, respectively. The maximum yield of sugar obtained during the saccharification was 0.3056 g sugar/ g flour at 30% (w/v) algae flour concentration with the addition of α-amylase 5% (w/v). This is consistent with the literature which states that the yield of reducing sugars increases with increasing concentrations of the α-amylase enzyme (Alfonsin *et al*., 2019; Onay *et al*., 2018; Soeprijanto, 2013).

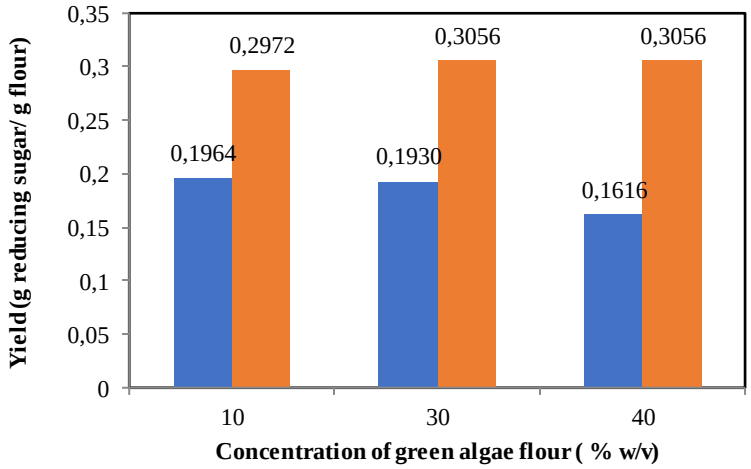

Figure 7. Comparison of reducing sugar yield after the saccharification for 4 hours. *Note:* ■ = Enzyme (2%); ■ = Enzyme (5%).

Fermentation

Figure 8 shows a correlation between sugar reduction and ethanol production. The results indicated that the use of enzymes at a concentration of 0.2%, the sugar reduced from 64.62 g/l to 1.14 g/l for 72 h, and the ethanol production was from 0 to 5.29%. However, the addition of enzymes with a concentration of 0.5%, the sugar decreased from 122.22 g/l to 1.3 g/l, and the ethanol production was from 0% to 8.16%.

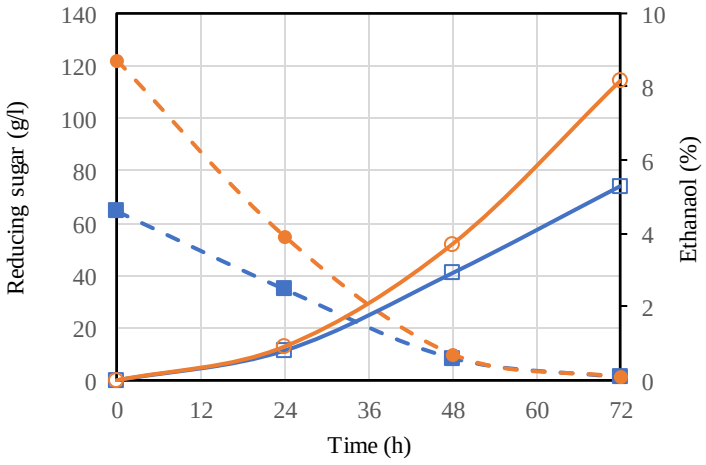

Figure 8. Effect of reducing concentration on ethanol production. *Note:* ● = Reducing sugar (0.5% enzyme); ■= Reducing sugar (0.2%); ○ = ethanol (0.5%); □ = ethanol (0.2%).

Conclusions

It concluded that the maximum reduced sugar was achieved by the concentration of green algae flour (*Chaetomorpha*) of 40% (w/w) with the addition of 5% (w/v) enzymes in the liquefaction by 27.5 g/l. The maximum sugar concentration

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was achieved by the concentration of green algae flour of 40% (w/w) with the addition of a 5% (w/v) enzyme in the saccharification of 84.615 g/l. The maximum ethanol concentration was achieved with the concentration of green algae flour of 40% (w/w) with the addition of an enzyme of 5% (w/v) of 8.16% on the 72 h.

Acknowledgment
The authors would like to thank the Department of Industrial Chemical Engineering, Faculty of Vocational Studies, Institut Teknologi Sepuluh Nopember Surabaya to support a Laboratory Facility.

List of Notation
C_{spl} = sample concentration [g/l]
V_{spl} = sample volume [ml]
C_{std} = standard concentration [g/l]
V_{std} = standard volume [ml]
P = pressure [atm]
T = temperature [^oC]
t = time [second, hour]
V = volume [ml, l]

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Lembar Tanya Jawab

Moderator : M. Maulana Azimatun Nur (UPN "Veteran" Yogyakarta)
Notulen : Aditya Kurniawan (UPN "Veteran" Yogyakarta)

1. Penanya : Aditya Kurniawan (UPN "Veteran" Yogyakarta)
   Pertanyaan : Dalam penelitian ini apakah suhu dijaga atau dibiarkan? Apakah pengaruh suhu terhadap proses yang dijalankan?
   Jawaban : Suhu diusahakan untuk dijaga pada suhu 60 C. Suhu akan mempengaruhi kinerja dari bakteri.

2. Penanya : M.M. Azimatun Nur (UPN "Veteran" Yogyakarta")
   Pertanyaan : Drying membutuhkan energi. Apakah pada penelitian ini, proses drying ini bisa dihilangkan? Selain karbohidrat, apakah ada zat lain yang adapat kita ekstrak sehingga dapat menghemat biaya proses?
   Jawaban : Lebih baik jika langsung difermentasi tanpa drying. Dalam percobaan ini, drying digunakan hanya untuk menghitung massa kering. Alga hijau memiliki banyak jenis. Sudah banyak pemanfaatannya, selain untuk agar-agar.