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Third Generation Bioethanol Production from *Chaetomorpha* sp. isolated from Pulau Seribu Seawater using Acid Pretreatment

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Abstract

Currently, the primary source of energy based on fossil fuel, thus promoting both the excessive use of fossil fuel and global warming. Bioethanol provides sustainable energy and serves to reduce the dependence on using fossil fuel. Third-generation bioethanol production from macroalgae provides alternative green energy. To observe the potency of biofuel resources of *Chaetomorpha* sp. isolated from Pulau Seribu Seawater, the effect of the acid pretreatment was evaluated by using two different acids (H_2SO_4) concentrations. Powdered *Chaetomorpha* sp. was prepared, followed by acid pretreatment using H_2SO_4 1% and 2% (v/v). After 72 hours of fermentation, 1% H_2SO_4 pretreatment produced 0.026 % of the ethanol from 3 grams of *Chaetomorpha* sp., whereas pretreatment with H_2SO_4 2% did not produce bioethanol.

Keywords: acid pretreatment, bioethanol, *Chaetomorpha*, macroalgae

Introduction

The demand for sustainable energy has been increasing with the increase of the human population. Currently, the primary source of energy is based on fossil fuel, thus promoting the excessive use of fossil fuel. This practice eventually leads to the scarcity of fossil fuel and trigger global warming (Behera *et al.,* 2015). Bioethanol is considered as a promising solution to reduce the dependence on using fossil fuel. It is mainly used to replace gasoline. Bioethanol provides a noticeable benefit by reducing the emission of CO^{-2}, toxic gases, for instance, carbon monoxide, nitric oxide, and other volatile compounds (Alia *et al.,* 2019).

Bioethanol production varies in the type of feedstock. The first-generation bioethanol uses starch (from barley, corn, and potato) and sugar as a carbon source to produce bioethanol. The second-generation bioethanol production uses lignocellulosic materials such as straw, wood, and grass (Alia *et al.*, 2019); meanwhile, third-generation bioethanol production utilizes microalgae and seaweed (Dragone *et al.,* 2010). Bioethanol production from first-generation is well implemented but also received negative views and certain restrictions such as energy consumption, utilization of arable land, and increase in food debate. The second generation, meanwhile, has its advantage, in which this practice requires less expensive biomass and non-edible feedstock (Lee *et al.,* 2013). However, the reliance on expensive and sophisticated technologies makes second-generation bioethanol less profitable for commercial purposes. Among these three types of bioethanol production, third-generation bioethanol production from macroalgae is considered as an ideal option. It has been focused on research since it can overcome the disadvantages of the first and second-generation bioethanol production (Alam *et al.,* 2015).

Macroalga is a multicellular organism which has similar characteristic with plant and usually concentrated in coastal areas (Roesijadi *et al.,* 2010). Macroalga could be used as a source of sustainable feedstock for the production of renewable energy (Schultz-Jensen *et al.,* 2013). Macroalga has been studied for its potential sources for biofuel production, such as *Ankistrodesmus* TR-87, *Botryococcus braunii*, *Isochrysis* sp., and *Chlorella protothecoides* (Carlsson *et al.,* 2007).

Macroalgae *Chaetomorpha linum* has rigid cell walls consist of crystalline cellulose and an inner amorphous part consisting of a complex polymer such as arabinose, xylose, and galactose. Schultz-Jensen investigated the feasibility of bioethanol production from C. linum. It is considered as an ideal feedstock compared to the other macroalga because it contains higher cellulose (Schultz-Jensen *et al.,* 2013). Moreover, in recent years, the amount of *C*. *linum* has been increasing.

This study aimed to observe the potential of *Chaetomorpha* sp. as a feedstock to produce bioethanol. The study showed the feasibility of using *Chaetomorpha* sp. isolated from Pulau Seribu Indonesia as a carbon source for bioethanol production. Moreover, as a maritime and tropical country, Indonesia possesses great potential in growing macroalgae, with a high intensity of sunlight and a suitable ecosystem.

Materials and Methods

Raw Material

Macroalga *Chaetomorpha* sp. was collected from a coastal area in Pulau Seribu, Jakarta Utara Indonesia.

Chemical and R eagent

Chemicals that have been used in this experiment were Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), H_2SO_4, Distilled Water, chloramphenicol, Yeast Extract, Glucose, Dextrose, Malt Extract, peptone, sucrose, and agar.

The methodology of this study consists of the collection and preparation of macroalga *Chaetomorpha* sp., Acid Hydrolysis, preparation of starter, fermentation and ethanol production, pH, and Growth measurement.

Preparation of Macroalga *Chaetomorpha sp.*

Macroalga *Chaetomorpha* sp. was collected from the coastal area of Pulau Seribu, Jakarta Utara, Indonesia. The macroalga sample was washed thoroughly with fresh water to remove the salt dan was sun-dried for three days. After drying, the macroalga sample was ground using mortar and pestle.

Sulphuric Acid (H_2SO_4) Pretreatment

According to Bensah & Mensah (2013), sulphuric acid (H_2SO_4) is very efficient in hydrolysing lignocellulose such that in this study, we used the variation of sulphuric acid (H_2SO_4) concentration which was 1% and 2% (v/v). Three grams of powdered macroalga *Chaetomorpha* sp. were prepared and transferred into an Erlenmeyer flask containing 25 ml of 1% and 2% H_2SO_4, respectively. The two samples then were autoclaved at 121 atm for 15 minutes. The solution was then filtered to separate the residue and filtrate. The pH of the filtrate was adjusted to pH 4.5-5 by adding dropwise of 0.1M NaOH (Hossai *et al.,* 2015). The filtrate was used as a fermentation medium.

Preparation of fermenter *Saccharomyces cerevisiae*

One gram of fermipan (brand name of baker's yeast) was inoculated into 9 ml distilled water in a test tube (10^{-1} dilution) and homogenized. The solution was then diluted until 10^{-8} dilution. 100 ul of solution from 10^{-8} dilution was transferred into malt yeast agar (MYA) supplemented with chloramphenicol and incubated at 30oC for 48 hours (Febriyanti *et al.,* 2016). The grew colony of S. cerevisiae was then used as a fermenter.

Fermentation and Bioethanol Production

Bioethanol fermentations were carried out in a 15 ml centrifuge tube. The centrifuge tubes were filled with 10 ml of fermentation medium. The mediums were inoculated with 10% (v/v) of S. cerevisiae and incubated for 72 hours at 30o (Muhibuddin *et al.,* 2017) without shaking. The fermentation products were centrifuged to get the supernatant, and the ethanol concentration was analysed with HPLC-system (High-Performance Liquid Chromatography; Hi plex Ca (Duo) using water with a flow rate of 0.4 mL/s. Bioethanol chromatogram can be seen in figure 4.

Yeast Growth Measurement

S. cerevisiae cell growth was done by measuring the optical density at 600nm (Olivares-Marin *et al*., 2018) using an Optizen POP UV/VIS spectrophotometer. Cell growth was measured at the end of the fermentation period.

Measurement of pH

pH measurement was done to observe the change of pH in the fermentation medium after 72 hours of fermentation. The change in pH shows the biological activities of the bacteria. pH condition at the end of the fermentation period was measured by using pH meter (Atmodjo, 2006).

Result and Discussion

The Effect of Sulphuric acid (H_2SO_4) Pretreatment on Bioethanol Production of *Chaetomorpha* sp. by *S. cerevisiae*

Chaetomorpha sp. contains cellulose and glucan, which further can be converted into bioethanol. In order to facilitate the conversion of *Chaetomorpha* sp. into bioethanol, acid hydrolysis is highly required (Schultz-Jensen *et al.,* 2013). To evaluate the effect of Sulphuric acid (H_2SO_4) pretreatment on bioethanol production, in this study, we applied two different concentrations of H_2SO_4, which were 1% and 2%.

The-treated *Chaetomorpha* sp. with 1% H_2SO_4 was fermented to ethanol by S. *cerevisiae* and resulted in 0.026% of ethanol, whereas the pretreatment with 2% H_2SO_4 did not produce ethanol at 72 hours of fermentation (figure 1).

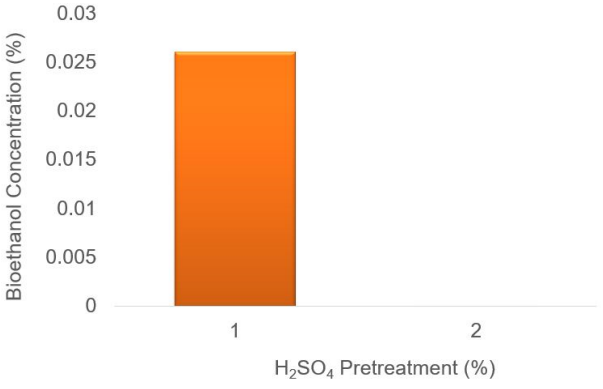

Figure 1. Bioethanol production at 72 hours of fermentation from H_2SO_4 1% and 2% pre-treated *Chaetomorpha* sp. fermentation medium.

In this study, we observed that in terms of bioethanol production from macroalga *Chaetomorpha* sp. by S. *cerevisiae*, H_2SO_4 1% pretreatment was more efficient that H_2SO_4 2 %. A similar result was reported by Adini *et al.,* (2015),

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the ethanol production from seaweed *Gracilaria* sp. was obtained higher in H_2SO_4 1% pretreatment compared to pretreatment using fungus *Aspergillus niger*. Sritrakul *et al*., (2017)., selected the treatment condition of 1% v/v H_2SO_4 for ethanol production from sugarcane bagasse pith because it produced the highest glucose yield thus provided more carbon source for ethanol production.

However, this result was lower compared to the previous study in which Neifar *et al.,* (2016) were done with a study of bioethanol production from *C*. *linum*. They were able to produce 8.6 grams of ethanol from 100 grams of dry matter by using enzymatic saccharification. The difference result obtained because of the availability of reducing sugar, which is provided by different hydrolysis treatments. Reducing sugar is a pure sugar produced by hydrolysis of complex carbohydrates and required by the microbial agent for ethanol production. The presence of reducing sugar was affected by the type of hydrolysis treatment given. The concentration of reducing sugar on *Chaetomorpha* sp. obtained in this study was low. This is due to the complex polymer of the cell wall of *Chaetomorpha* sp. (Adini *et al.,* 2015) such as arabinose and galactose. According to Schultz-Jensen *et al.,* (2013), *C*. *linum* consists of 9 to 10 grams of arabinan, 34 to 38 grams of glucan, 6 grams of xylan, 14 grams of pectin, and 7 grams of non-hydrolysable organic compounds. The more complex the polysaccharide, the more difficult to degrade, which resulted in less reducing sugar.

Yeast Growth

The growth of S. *cerevisiae* in mediums that have been pre-treated with H_2SO_4 1% and 2% is shown in figure 2. The OD of yeast after 72 hours of fermentation in pre-treated H_2SO_4 1% *Chaetomorpha* sp. medium was 0.94 and 0.84 on H_2SO_4 2% pre-treated medium.

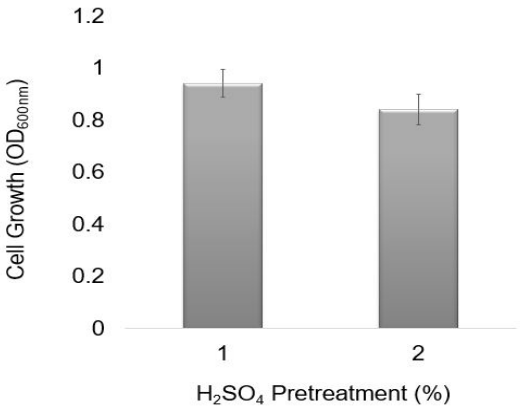

Figure 2. The Growth of *S. cerevisiae* at 72 hours of fermentation in H_2SO_4 1% and 2% pre-treated *Chaetomorpha* sp. medium. The error bars represent the standard deviations of three biological repeats.

Based on the result, the treatment of H_2SO_4 1% and H_2SO_4 2% on *Chaetomorpha* sp. showed there was no significant impact on S. Cerevisiae Growth. Both cell culture growths were slightly lower compared to the previous study in which S. Cerevisiae's growth reached OD of around one on medium supplemented with 15% glucose by the end of fermentation of 72 hours (Wardani *et al.,* 2013). The growth of the yeast after 72 hours of fermentation was low due to the depletion of nutrients. According to (Adini *et al.,* 2015), the growth of S. *Cerevisiae* started to decrease at 72 hours until 120 hours of the incubation period. The decrease in growth triggered by the loss of nutrients and the production of the toxic compound.

The pH of Medium at the end Fermentation

The pH of pre-treated with H_2SO_4 1% and 2% *Chaetomorpha* sp. fermentation mediums were evaluated. The pH of pre-treated with 1% H_2SO_4 after 72 hours of fermentation was 3.66. This result showed that there was small a decrease in the pH of fermentation culture in which the initial pH of the fermentation culture was 4.7 (Figure 3 A). The change in pH occurred because of the activity of yeast cells during fermentation.

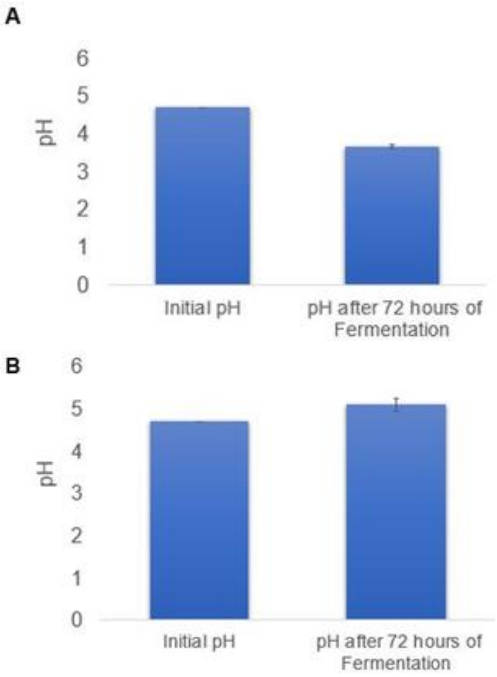

Figure 3. (**A**) pH of H_2SO_4 1% pre-treated *Chaetomorpha* sp. fermentation medium (**B**) pH of 2% H_2SO_4 pre-treated *Chaetomorpha* sp. fermentation medium. The error bars represent the standard deviations of three biological repeats.

In contrast with 1% H_2SO_4pretreatment, the pH in fermentation culture with was pre-treated with 2% H_2SO_4 showed a slight increase in pH medium after 72 hours of fermentation (Figure 3

B). The changed was observed from pH 4.8 became pH 5.1 at the end of fermentation.

The pH of medium affected the bioethanol production after 24 hours till 120 hours of incubation (Adini *et al.,* 2015). Initially, at 0 hours of fermentation, the pH of the acid pre-treated medium was adjusted to 4.5-5, as this pH range is the best condition for S. cerevisiae growth. As the fermentation proceeded, the pH changed. The highest amount of ethanol concentration was found at pH 3.66 on H_2SO_4 1% pre-treated *Chaetomorpha* sp. fermentation medium. Ogbonda *et al.,* (2011) reported that higher amounts of ethanol were produced at lower pH conditions. Ethanol production during fermentation pH 5-5.5 produced a lower concentration of ethanol (Wachid, 2011). As the acidity increased, the amount of ethanol decreased.

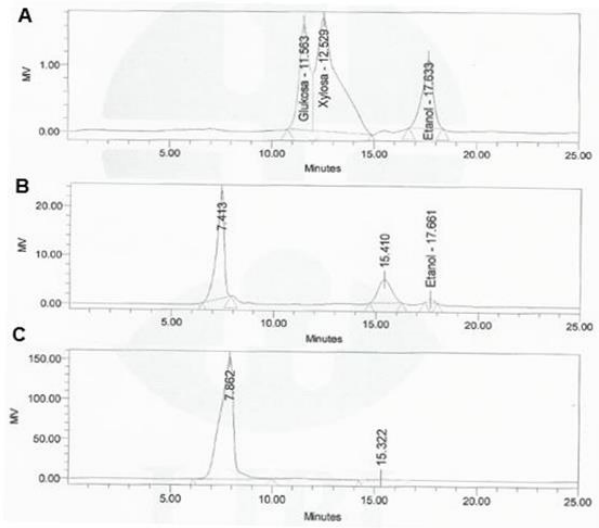

Figure 4. The chromatogram of (a) ethanol standard, (b) bioethanol at H_2SO_4 1% pre-treated *Chaetomorpha* sp. fermentation medium, (c) bioethanol at H_2SO_4 2% pre-treated *Chaetomorpha* sp. fermentation medium.

Conclusion

Chaetomorpha sp. as a feedstock enabled bioethanol production using S. cerevisiae as a fermenter. The highest bioethanol production was 0.026% using 1% H2SO4 pretreatment, thus implies the 1% H2SO4 pretreatment more suitable than H2SO4 2% pretreatment in bioethanol production from *Chaetomorpha* sp.

Further study on the bioconversion of *Chaetomorpha* sp. into bioethanol is required since it possesses a high potential for the production bioethanol from an abundant macroalga. Also, carrying out a study of bioethanol optimization in this study is strongly required for the third-generation study.

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