Cellulose isolation from *Gracilaria* Genus and its Potential as Bioethanol Raw Material

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Abstract
The availability of crude oil is very limited and it is less environmentally friendly. Therefore, we need alternative energy sources which are also environmentally friendly. Indonesian seashores have diverse types of macroalgae, and one of them is Gracilaria. The advantage of this genus is that it has a high level of carbohydrate and therefore it can be used as bioethanol raw material. This research is a preliminary research that utilizes Gracilaria cellulose from the south seashore of Java Island as bioethanol raw material.

Cellulose isolation was obtained in base condition. Cellulose degradation into glucose was catalyzed by Cellulase from Trichoderma viride while glucose fermentation into ethanol was catalyzed by various glycolysis enzymes from Saccharomyces cerevisiae. The concentration of cellulose from 135 grams Gracilaria was 32.38%. This cellulose is further used as the substrate for T. viride celullase. The maximum concentration of reducing sugar was 0.66 mg/mL from 0.25 grams substrate, and the yield of reducing sugar was 0.26%. The optimum fermentation condition was at pH 4.5 for 24 hours, creating 1.75 mL ethanol. Ethanol detection was done by gas chromatography and reaction between ethanol and potassium dichromate in acid solution. From that reaction, ethanol was detected as a green color.

Keywords: *Gracilaria*, bioethanol, cellulose, glucose.

Introduction

Bioethanol is ethyl alcohol (C_2H_5OH), liquid, clear, colorless, biodegradable and does not cause corrosion. Bioethanol is generally produced through biochemical processes (fermentation) and thermochemical processes (gasification) using biological raw materials. Bioethanol is a type of alternative energy because it has high oxygen content, high octane number and easily decomposes. It is also a renewable energy source. High oxygen content will increase combustion efficiency and will reduce pollution caused by flue gases such as hydrocarbon emissions, carbon monoxide, and particulate emissions, or greenhouse gases[1]. Regular sources of bioethanol materials that have been used are cassava, sugar cane juice, sorghum, coconut palm, sweet potatoes among others.

Unfortunately, most of these raw materials have weaknesses. For instance, the planting requires a large area and the bioethanol obtained is not maximal There is, however, an alternative solution, which is the use of cellulosic material (lignocellulose) as sources of bioethanol. Macroalgae (seaweed) has high content of cellulose and it does not need a large area to be planted.

Seaweed is a multicellular algae that lives in marine waters belonging to the Thallophyta division. Seaweed has not been de-differentiated into roots, stems, and leaves as commonly happen with plants at high levels[2]. In addition, seaweed contains a very important organic compound in the microbial world. Seaweeds contain high biomass and oil content, are widely developed, are less competitive with land agriculture, absorb carbon dioxide well, are suitable for waste treatment, and they can be utilized as a renewable energy source[3]. Based on data from the Indonesian Ministry of Marine Affairs and Fisheries in 2014, Indonesia has a total area for seaweed cultivation activities reaching 1,110,900 Ha, but the development of new seaweed cultivation utilizes 222,180 hectares of land or 20% of the potential area.

Based on FAO 2014 statistical data, to date the types of seaweed developed in Indonesia include *Kappaphycus alvarezii* (cottonii), *Eucheuma denticulatum* (spinosum) and *Gracilaria* sp. *Gracilaria* comprises carbohydrates (41.68%) and fat (0.68%). The carbohydrate of *Gracilaria* can be used as the main ingredient of bioethanol production[4].

Research about bioethanol was conducted in the year 2009. The research was about producing bioethanol from the substrate of the macroalgae of *Eucheuma* and *Gracilaria*. Based on the study, it produced reducing sugar content of 0.090 mg / mL, and ethanol was only 0.698%[5]. Therefore, it needs another approach to increase the amount of reducing sugar and ethanol. From the explanation that has been given, it is necessary to conduct further research in order to increase the amount of reducing sugar and ethanol by using cellulose from *Gracilaria* as the raw material of bioethanol. Therefore, the aim of this research is to isolate the *Gracilaria* cellulose and ferment them into bioethanol.

Material and Methods

Materials: *Gracilaria* was obtained from Cidaun seashore, West Java, Indonesia. *Saccharomyces cerevisiae* to ferment glucose, *Trichoderma viride* is to hydrolyze cellulose, sodium hydroxide is used to isolate cellulose. PDA, PDB and potassium dichromate acid are used for ethanol qualitative detection.

Methods: The cellulose isolation method used is known as the Bertoniere and King method[6]. Cellulose was isolated in alkaline condition using sodium hydroxide concentration variation. The cellulose was hydrolyzed in two step producing intermediate compound namely cellobiose which it will hydrolyze into glucose. Determination of reducing sugar was using Somogyi-Nelson method[7]. Glucose fermentation method was referred to glycolysis using *S. cerevisiae*[8]. Gas chromatography analysis was performed using Gas Chromatography GC-9A Shimadzu. The temperature GC column was 120°C.

Results and Discussion

Cellulose Isolation: 135 g biomass of *Gracilaria* was dried up producing 12.19 g dry weight of *Gracilaria*. To isolate cellulose, 4.5 g *Gracilaria* was soaked in potassium hydroxide (3%, 6%, 9%). After the cellulose treatment process, the cellulose obtained can be seen in table 1. The maximum yield of cellulose was 32.38 %.

Table 1
Cellulose isolation

NaOH (%)	*Gracilaria* mass (g)	Cellulose mass (g)	Yield (%)
3	1.5006	0.4082	27.20
6	1.5010	0.4861	32.38
9	1.5015	0.4418	29.42

Cellulose Degradation: We use Cellulase from *T. viride* to hydrolyze cellulose. The hydrolysis process was optimized by variation of substrate concentration and the time taken for hydrolysis. The variation concentration substrates are 0.125, 0.25, 0.375 and 0.5 g per 5 mL hydrolysis medium. The variation of hydrolysis time is 24, 48, 72 and 96 h. The obtained glucose was calculated as reducing sugar level.

The reducing sugar determination was done using the Somogyi Nelson method. The optimum reducing sugar level was obtained at incubation time 48 h and substrate concentration 0.25 grams per 5 mL medium. The result of reducing sugar level can be seen in figure 1.

Substrate Hydrolyzate Fermentation by *Sacccharomyces cerevisae:* The optimum condition for fermentation of 0.25 g glucose into ethanol was maintained for 24 h at medium pH 4.5 producing 1.75 mL ethanol. The optimum time for catalyst reaction by various glycolysis enzymes produced by *S. cerevisae* is 24 h. At more than 24 h, *S. cerevisae* will be at stationary phase. Glycolysis enzymes produced by *S. cerevisae* have an optimum pH 4.5. The fermentation process went slower when the medium pH is 3.

The ethanol was detected through reaction between ethanol and potassium dichromate. The reaction was successful when there is a change in color solution, from orange to blue-green color. The oxidation reaction was described as reaction 1.

$$3C_2H_5OH + 2K_2Cr_2O_7 + 8H_2SO_4 \rightarrow 3CH_3COOH + 2Cr_2(SO_4)_3 + 11H_2O + 2K_2SO_4 \quad (1)$$

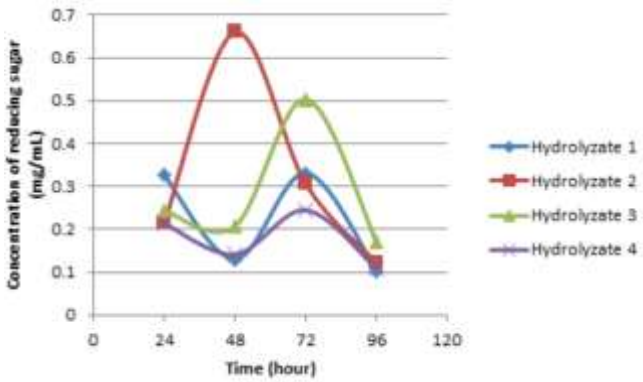

Figure 1: Reducing sugar concentration of the hydrolyzate. Hydrolyzate 1, hydrolyzate 2, hydrolyzate 3 and hydrolyzate 4 are results when substrate concentration of 0.125, 0.25, 0.375 and 0.5 g/5 mL respectively used in the hydrolysis process.

The oxidation reaction of ethanol showed that Cr(VI) ion was reduced into Cr(III) ion by ethanol. This reaction is an explanation for color solution changes. We also measured the absorbance of Chromium ion at maximum wavelength. There is absorbance reduction from 0.182 to 0.068 indicating that chromium ion was reduced.

Gas Chromatography Detection: The ethanol standards for gas chromatography were 0.1%; 0.5%; 1% and 2%. The standards were injected to GC as much as 0.2 µL. Retention time of standard ethanol was 0.433 and 0.436. Same procedure was applied to the ethanol sample. The chromatogram showed that retention time of bioethanol was 0.436. The chromatogram can be seen at figure 2.

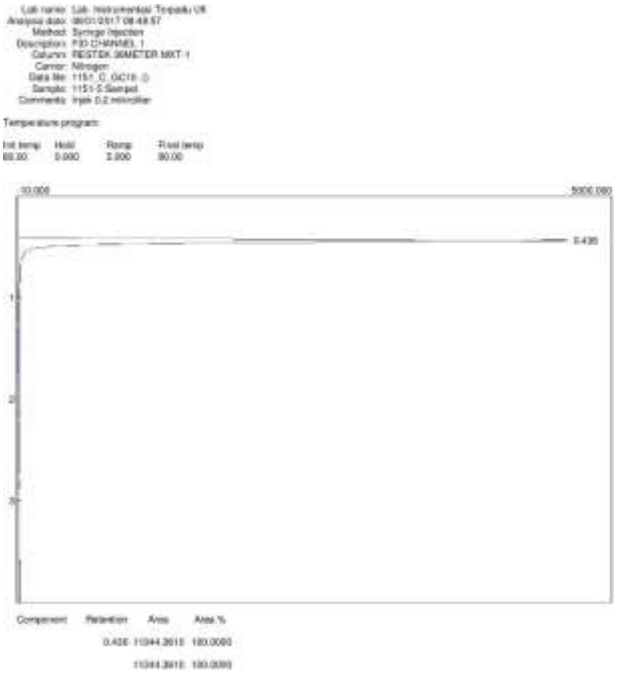

Figure 2: Bioethanol Chromatogram.

The concentration of ethanol was 1.47% and the yield of the ethanol was 0.02 mL/mg to 0.06 mL/grams macroalgae substrate.

Conclusion

Isolation of cellulose from *Gracilaria* macroalgae was successfully performed. The concentration of cellulose was 32.38%. The cellulose could be used as material for bioethanol. It was proven by degradation of cellulose into glucose, glucose fermentation, and bioethanol detection by oxidation reaction and GC analysis. The concentration of bioethanol was 1.47%.

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