Bioethanol production from mixed culture microalgae biomass with temperature hydrolysis variation

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Abstract. This preliminary study aims to exploit the biomass of microalgae *Chlorella vulgaris; Scenedesmus obliquus*; and *Chlorococcum* sp. in the form of a mixed culture as raw material of alternative fuels. Microalgae were cultivated in the artificial growth medium of PHM (Provasoli Haematococcus Media) for 9 days to reach the exponential phase. Hydrolysis was carried out at a temperature variation of (ºC) 25; 80; 100; 120; 140; 160 within 30 minutes by adding hydrochloric acid. Biomass fermentation by adding 50% (v/v) *Saccharomyces cerevisiae* for 5 day to produce alcohol compounds. The last stage is separation of the alcohol compounds from another compounds by distillation. The result showed that carbohydrate levels with color change indicator in luff schoorl solution at hydrolysis and fermentation stages severely were 12.20 mg/L. Carbohydrate levels in fermentation stage produced 17% (v/v) alcohol compounds while in the distillation stage, 98% (v/v) alcohol will be separated into intermediate compounds such as linoleic acid and methyl alcohol as a characteristic of bioethanol. At 80ºC of hydrolysis temperature, 58% bioethanol was produced. Further research is needed, since the preliminary study proves the mixed culture of microalgae is potentially to be utilized in producing bioethanol.

1 Introduction

The demand of energy increasing and trigger us to seek alternative energy. Alternative energy demand has increased by 25% by 2015 [1-2]. Alternative energy such as bioethanol is easy to find and renewable from organic matter. Bioethanol is a fermentation product of organic material that can be made from carbohydrate-containing substrates (sugar, starch, and cellulose) with chemical formula C2H5OH in the form of colorless, volatile liquid, with a specific odor [3]. One source of renewable carbohydrates and an abundance in environments is the microalgae.

Microalgae are microcospic-sized unicellular living creatures that live in fresh or marine water, which have chlorophyll, so they can fix carbon dioxide, through the photosynthesis process [2]. Microalgae produce primary metabolites in the form of proteins, carbohydrates, fats, and nucleic acids. Research on microalgae as an alternative energy source has been widely conducted, but more focused on biodiesel and biogas production than bioethanol. Whereas theoretically microalgae can produce bioethanol naturally by optimizing its intracellular potential [4].

Environmental conditions optimization is indispensable in growing microalgae in batch culture to obtain high carbohydrate production. Suitable culture temperature, acidity degree between 6-7, and lighting between 2000 lux to 4000 lux, are such environmental conditions must be maintained to obtain high metabolic yields of microalgae [5-6].

In bioethanol production, there are 4 stages to be done, namely cultivation of microalgae, hydrolysis, fermentation and distillation. The hydrolysis process is highly dependent on temperature. The right temperature allows to breakdown of carbohydrates contained in the cell wall perfectly [7-8]. Therefore, this study aims to obtain the appropriate temperature for the ongoing hydrolysis process, as an effort to increase the production of bioethanol.

2 Research methodology

2.1 Cultivation

This study used 3 types of green microalgae namely *Chlorella vulgaris, Scenedesmus obliquus*, and *Chlorococcum* Sp. in the form of contructed consortium cultivated in PHM (Provasoli Haematococcus Media). The cultivation operational conditions as bacth cultures in the 3-liter photobioreactor are arranged as follows: acidity level at 6, temperature of 27ºC, light intensity of 4000 lux for 24 hours, and aerated in 0.3 liters/sec. Biomass is harvested from sediment through centrifugation 3000 rpm for 15 minutes.

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2.2 Hydrolysis

The resulting biomass is hydrolysed using a strong acid of HCl (hydrochloric acid). Hydrolysis was carried out by adding 25 ml of hydrochloric acid into 1 gram of dry biomass under conditions of temperature variation (°C) 80, 100, 120, 140, and 160 for 30 minute and room temperature as control.

2.3 Fermentation

The hydrolyzed biomass is fermented with *Saccharomyces cerevisiae* yeast at 25°C for 5 days. Further fluid formed will be moistened by using the distillation method.

2.4 Distillation

Distillation is carried out to separate between water and alcohol which is a product derived from the fermentation stage. The resulting destylate will then be tested by GC-MS to determine the characteristics of the ethanol compound.

3 Result and discussion

3.1 Cultivation of microalgae cosortium

The cultivated microalgae consortium in 3-liter photobioreactor is harvested by means of precipitation and then the sediment is centrifuged to separate the biomass with the remaining water. The harvesting time is carried out in the exponential phase that occurs on the 9th day.

In day 9th, the formation of biomass reach its highest rate due to the rapid nutrient absorption activity, which can increase the production of metabolite. If the harvesting of the microalgae is carried out at the stationary phase or more than 9 days, then the cell growth rate is balanced with the cell death rate. This can reduce the formation of metabolites [9].

3.2 The effect of hydrolysis temperature

The resulting dry biomass is then processed at the hydrolysis stage. Carbohydrate levels at each temperature variation are shown in Table 1.

Table 1. The effect of hydrolysis temperature on carbohydrate level.

Temperature (°C)	Biomass (mg)	Carbohydrate Levels (mg/L)			
		C_0		Cx	
		R1	R2	R1	R2
25 (room temp.)	305 ± 310	3.70	4.80	4.80	6.10
80		**10.48**	**10.22**	**12.20**	**12.20**
100		9.70	9.96	11.78	11.00
120		9.54	9.96	11.78	12.04
140		10.22	10.22	12.20	10.48
160		9.96	9.70	11.78	12.20

*Co = Concentration on t = 0 minute, Cx = Concentration on t = 30 minute

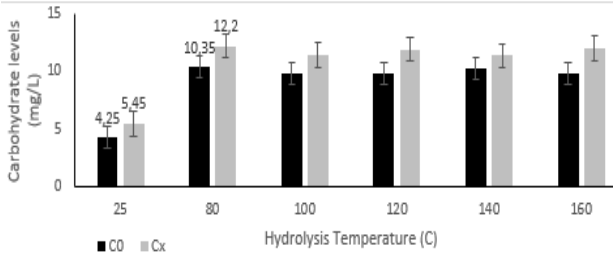

Fig. 1. The Effect of Hydrolysis Temperature on Carbohydrate Level.

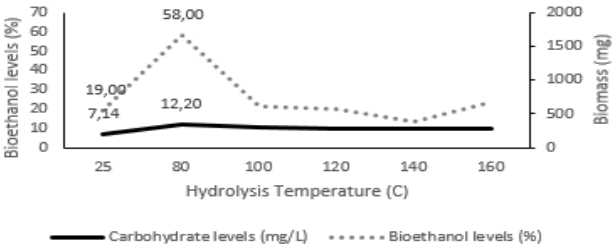

Fig. 2. Effect of hydrolysis temperature by production of bioethanol content.

Hydrolysis of dry biomass is done to break down complex carbohydrates produced from the metabolism of microalgae into simpler carbohydrates [10]. Based on these data, the highest carbohydrate levels were found at 80°C hydrolysis temperature. At these temperatures the content in the cell walls of cellulose, pectin, glycoprotein, and mannan which are complex carbohydrate compounds have been degraded [11-12]. This is in accordance with the statement of [12] that the content in the microalgae cell wall which is a complex carbohydrate compound that is easily degraded. In the green microalgae, the main constituent component of cellulose is galactomannan. Galactomannan consists of galactose, glucose, and mannose which is a hexose group having six carbon atoms in each monomer [11].

Galactomannan compounds are then to be fermented to be converted into bioethanol. As seen in Table 1, the determination of hydrolysis temperature greatly affect the carbohydrate levels obtained. Theoretically, the higher the hydrolysis temperature, the greater the carbohydrate levels obtained. The hydrolysis process is also influenced by the quantity of biomass particles to be

degraded [10]. As the research result show in Table 1, there is no significant increase in carbohydrate levels due to differences in the particle size of the biomass that affects carbohydrate levels so that the hydrolysis process does not occur completely. However, the hydrolysis process can also take place at room temperature but decomposition of carbohydrate biomass does not occur perfectly.

3.3 Bioethanol production

High carbohydrate levels will affect the production of ethanol in the fermentation process. Biomass that has been hydrolyzed in fermentation for 5 days are shown at Fig 2.

In Fig. 2, the higher the carbohydrate content, the greater the amount of bioethanol produced. This is confirmed by previous research that the higher levels of carbohydrates present in microalgae biomass the faster the process of changing from carbohydrates to bioethanol, which then increased bioethanol production by 25% (v/v) [13]. The process of hydrolysis using room temperature can also produce bioethanol content, but the formation of bioethanol does not occur perfectly. As seen in Fig. 2, there are several factors that need to be considered such as pH and temperature to support the fermentation process.

The process of purifying bioethanol by using the distillation method also greatly determines the level of bioethanol to be produced. If the distillation temperature is too high then the water mixed in the fermentation product will also vaporize and then reduce the concentration of the bioethanol. Hydrolysis at 80°C yields the highest bioethanol level of 58.00% (v/v). The bioethanol product is then analyzed using GC-MS to determine the ethanol characteristics contained in the bioethanol.

In Fig. 3, GC-MS results show that there are highest peak and some other peaks. This result indicate that the bioethanol produced is not 100% pure, that in the 58.00% (v/v) there are 37.25% (v/v) linoleic acid which is an acid compound contained in biofuels. The longer fermentation time of *Saccharomyces cerevisiae* could decompose carbohydrates into other compounds that could interfere the purity of bioethanol production [13-14].

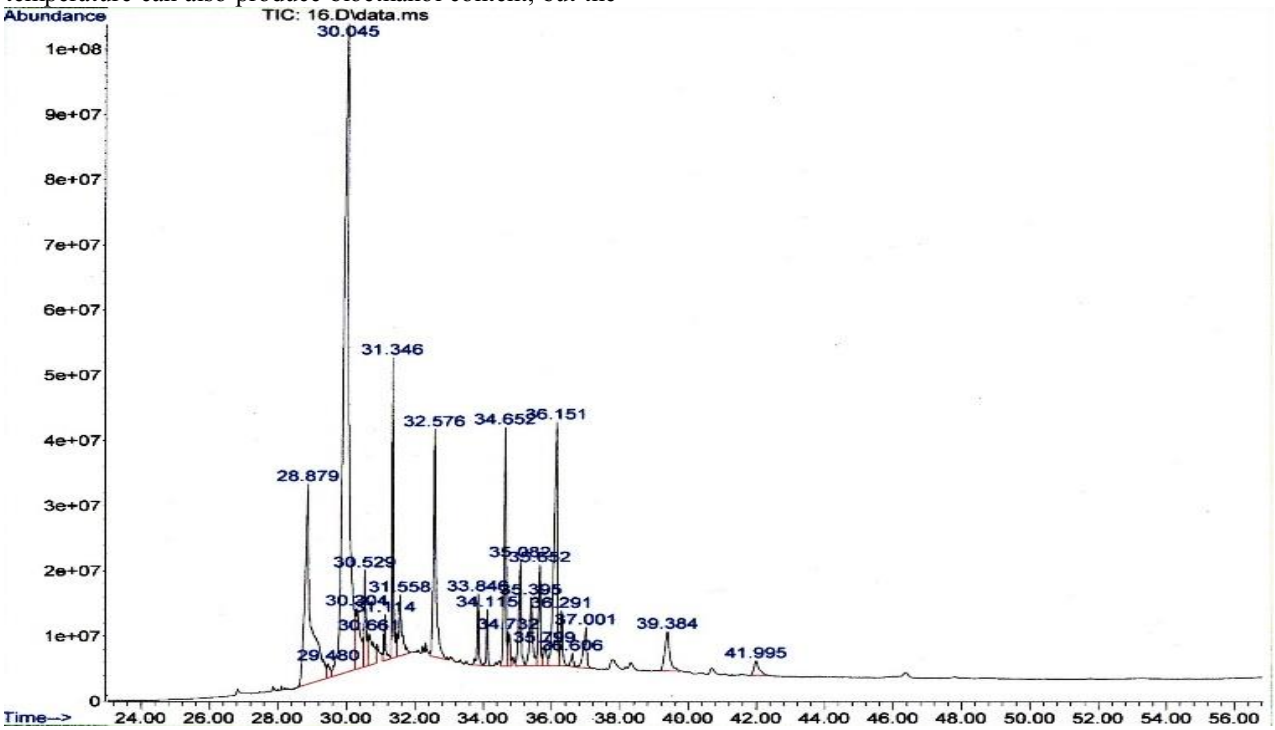

Fig. 3. GC-MS analysis results.

4 Conclusion

Carbohydrate levels obtained from the results of microalgae metabolism greatly determine the amount of bioethanol that can be produced. Hydrolysis temperature suitable for decomposing carbohydrate compounds occurs at 80°C. At that temperature produced the highest carbohydrate level of 12.20 mg/L and bioethanol production of 58.00% (v/v). There is influence of particle sized that can affect carbohydrate levels.

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