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Original Paper

STUDY ON BIOETHANOL PRODUCTION USING RED SEAWEED *Eucheuma cottonii* FROM BONTANG SEA WATER

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ABSTRACT

The increasing of energy demand for public transport and a rise of oil prices lead to intense of using green fuel for sustainable future. Red-seaweed polysaccharide consists of carrageenan can be used for production of bio-etahanol, as it supplies monosacharides. In this study, the possibility of bioethanol production using red-seaweed as raw material was examined. The purpose of this research was to determine the method of bioethanol production using red-seaweed. Two separate anaerobic fermentation following acid hydrolysis, each by different type of yeast, bread yeast (Saccharomyces cereviceae) and tapai yeast were conducted in this study. Acid hydrolysis for 2 h using H_2SO_4 of 5% at 100 ^oC of 100 g seaweed gel derived from 25 g of red-seaweed showed an optimal hydrolysis process yielded sugar content of 15.8 mg mL^{-1}. Tapai yeast was not suitable for fermentation of red-seaweed hydrolysate, while Saccharomyces cereviceae gave an alcohol content of fermentate of 4.6% after 5-6 days of fermentation at room temperature.

Keywords: Red seaweed, *Eucheuma cottonii*, acid hydrolysis, bioethanol, *Saccharomyces cereviceae*, bread yeast, tapai yeast.

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INTRODUCTION

The issue of renewable and eco-friendly energy resources combined with the high oil prices lead to the increasing of production of bioethanol every year (John *et al*., 2011). On the other hand, growth in the human population, pollution, overexploitation of land and lack of fresh water in the future will encourage use of seaweed (Jensen, 1993, Goh and Lee, 2010). Adam *et al.* (2009) compiled the huge potential of macroalgae compare to crops (wheat, maize, sugar beet, and sugar cane) by about 4-23 times for bioethanol production.

Indonesia with the longest seashore in the world has a huge potential in producing of seaweed. Using of seaweed as bioenergy source will be very important in the future including in Indonesia, because the use of bioethanol (E100) as energy source in transportation, industrial, and commercial is gradually planned to 15% to national energy demand in January 2025 (Ditjen Minyak dan Gas Bumi Kementerian Energi dan Sumber Daya Mineral, 2010).

Utilization of seaweed as bioenergy source could be combined paralel with food industry as nowadays many reports showed that floating residue or by-product of seaweed processing for food can be converted to bioethanol (Ge *et al*., 2010), however this efforts still need more innovation before it goes commercially (Waltz, 2009). Different types of seaweed will produce different yield of bioethanol (Lee *et al*., 2009), and different locations (seawater nutrition) and seasons will give different growth (Kotiya *et al*., 2011).

Eucheuma cottonii is now *Kappaphycus alvarezii* is still commercially called "cottonii", which is carrageenophytes belongs to red-seaweed (FAO, 2003). This red-

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seaweed is the main seaweed cultured in Bontang seawater. Its production continued to increase, from 1.82 million tones in 2003 to 181.5 million tones in 2007 (Dinas Kelautan dan Perikanan Kota Bontang, 2007). The usage of the seaweed is very broad, it is used in food, pharmaceuticals, cosmetics industries (Hurtado and Agbayani, 2000) and recently it promises an important role in bioenergy sources (John *et al*., 2011, Harun *et al*., 2009, Yoon *et al*., 2010a, Lee *et al*., 2009, Wi *et al*., 2009, Horn *et al*., 2000, Kim *et al*., 2011). Because of the huge potential production of this seaweed in Bontang seawater, it is interesting to explore the possibilities to provide benefit from seaweed. As seaweed is composed by carbohydrate of more than 62% (Anggadireja *et al.,* 2008), in this research we explored the possibility of using the red-seaweed from Bontang seawater as raw material for bioethanol. This bioethanol is proposed to be used for cosmetics, pharmaceutical, or as biofuel as reported by Yoon *et al*. (2010b). In this report, we determine the optimal condition for hydrolysis of *Eucheuma cottonii*, a red-seaweed from Bontang seawater, and the fermentation condition of the hydrolysate.

MATERIALS AND METHODS

Eucheuma cottonii red-seaweed was delivered from Bontang seawater at Bontang Kuala Sub-regency, Bontang city. H_2SO_4, NaOH, Na_2CO_3, rochelle salt, $NaHCO_3$, Na_2SO_4, $CuSO_4.5H_2O$, $Na_2HAsO_4.7H_2O$ NH_4Mo, were purchaced from Merck and Riedel-Haen. Bread yeast (*Saccharomyces cereviceae*) was purchased from local market ("Haan" brand), while "tapai" yeast were purchased from local market in Bogor, West Java.

Collection and preparation of seaweed

Seaweed was collected from seaweed collector at Bontang Kuala Subdistrict of Bontang City, East Kalimantan Province, Indonesia. The harvested seaweed was sun dried and packed in plastic. The dried seaweed transported to Laboratory in Samarinda, then was stored at room temperature before use.

Gel preparation

Dry seaweed of 100 g was placed in water of 300 mL for 2 h to develop, followed by boiling at 80 ^oC until gel was formed and then cooled at room temperature. From this treatment, the weight of gel produced was about 400 g. The gel produced was then cutted and weighed for 100 g to be applied for acid hydrolysis.

Acid hydrolysis

25 mL of 5% H_2SO_4 (sulfuric acid) were poured into a bottle glass containing 100 g seaweed gel, which was designed with reflux and boiled using stirrer hotplate for 30 to 120 minutes. The hydrolysis time was measured after the acid boiled. The hydrolysis temperature was maintained at 100 ^oC. The solution was then adjusted to pH 5 by adding dropwise of 0.1 M NaOH. Reducing sugar was measured from the hydrolysate using Nelson Somogyi method (Sudarmadji *et al*., 1984).

Fermentation

Hydrolisate was sieved and placed in erlenmeyer designed for anaerobic fermentation. Over night precultured bread yeast or "tapai" yeast of about 10% (v/v) was added as starter. The starters were precultured in sucrose solution of 10%. The fermentation was conducted at room temperature (28-30 ^oC) for 36 to 168 h. Alcohol was then analysed from the fermentate using alcoholmeter as follows: Alcoholmeter and test jar were washed with warm water and dry before use. The liquid tested was first shaked ten times and then poured into the test jar. The alcoholmeter was then dipped slowly into the liquid until it floats freely. The reading was taken at eye level at the level of the liquid surface. During the measurement, the hydrometer was kept out from bubble.

Experimental Design

This research was conducted into two steps experiment i.e. acid hydrolysis of seaweed gel and anaerobic fermentation of hydrolisate. The two steps experiments were single factor experiment arranged in complete randomized design. Acid hydrolysis of seaweed gel was performed with 4 levels of treatment

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(hydrolysis time) i.e. 30, 60, 90, and 120 min. Fermentation of hydrolysate was performed with 4 levels of treatment (fermentation time). Each experiment was repeated for 3 times. Data were analyzed using ANOVA (F test) and continued by least significant difference (α=5%). In fermentation step, two types yeast (bread yeast/*Saccharomyces cereviceae* and "tapai" yeast) were studied.

RESULTS AND DISCUSSION

Gel Preparation

Gel preparation for *Eucheuma cottonii* needs a portion of three times water of seaceds weight as reported by Syamsuar (2006) for general seaweed. In this experiment, we showed that the gel was not formed completely when less than 300 mL of water was used. On the other hand the gel was too soft when the water was added more than 300 mL. From 100 g of *Eucheuma cottonii*, appoxincetely 400 g of seaweed gel was performed by adding 300 mL of water and boiled at 80 ^{o}C for 2 h.

Hydrolysis of Seaweed Gel

Acid hydrolysis of 100 g gel corresponded to 25 g of *Eucheuma cottonii* using 25 mL of sulfuric acid of 5% at 100 ^{o}C for 2 h gave reducing sugar in hydrolysate of about 16 mg mL^{-1} or the yield of sugar was 16 mg g^{-1} of dry seaweed. Hydrolysis time gave significant effect on sugar content of hydrolysate (**Table 1.).** The trend of the data following regression analysis (**Fig. 1.**) showing that the hydrolysis condition can be developed i.e. by extending the hydrolysis time or increasing the concentration of sulfuric acid, as well as increasing the hydrolysis temperature, as reported by Yoon *et al*. (2010). Rachmania *et al*. (2009) reported that temperature used in acid hydrolysis using sulfuric acid is 125-214 ^{o}C, and Sudjatmiko (2008) using sulfuric acid by concentration of 7%. Sulfuric acid is the best catalyst for hydrolyzing compare to that of nitric acid and chloric acid as reported by Surraya *et al*. (2008) for starch from canna ("ganyong" in Indonesian).

Table 1. Influence of fermentation time on alcohol content of fermentate

Hydrolysis time (min.)	Reduction sugar content in hydrolysate (mg L^{-1})
30	(10.0 ± 2.0) a
60	(10.7 ± 1.7) a
90	(11.2 ± 3.9) a
120	(15.8 ± 3.8) b

Note: Data from three replications. The sugar reduction content followed by same character showed no significant difference by least significant difference test at α of 5%.

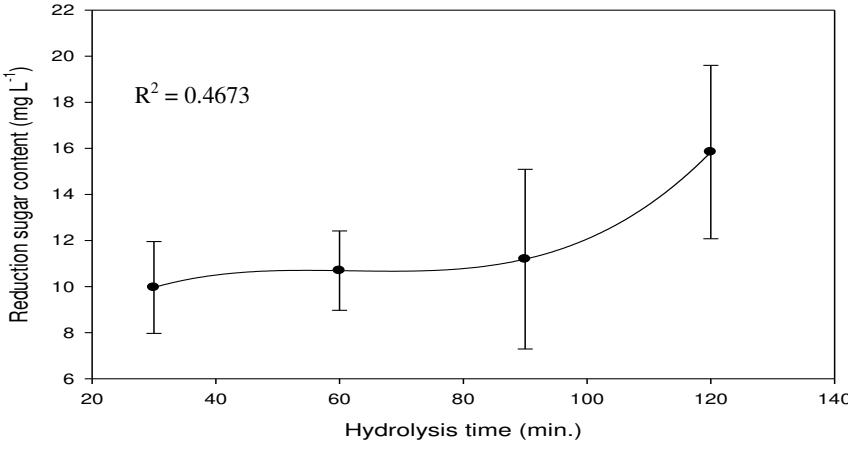

Fig. 1. Influence of hydrolysis time on reduction sugar content of seaweed hydrolysate by acid hydrolysis using 5% of sulfuric acid

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Combination of chemical and enzymatic hydrolysis method of seaweed has been reported by Ge *et al*. (2010). Using seaweed waste, which are rich in cellulose (30%) and bit of cellobiose (2.2%), they reported that about 277.5 mg g^{-1} of glucose could be reached using method of enzymatic hydrolysis following mild acid pre-treatment using sulfuric acid (0.1% w/v, 121 ^{o}C, 1.0 h). Wi *et al*. (2009) also reported that pre-treatment using chloride will transform lignin to soluble compound without any significant loss of carbohydrate, and it could increased glucose yield up to 70% with contrast of only 5% was obtained from non pre-treatment samples of seaweed Ceylon mass (*Gelidium amansii*).

Fermentation

Seaweed hydrolisate (sugar concentration of 15.8 mg mL^{-1}) produced by acid hydrolysis using 5% sulfuric acid at 100 ^{o}C for 2 h was used as raw material for fermentation process. The seaweed hydrolysate from 100 g of seaweed gel (25 g of seaweed) yielded alcohol maximal of about 4.6% in fermentate using bread yeast as fermentation agent (**Table 2.**). Regression analysis of the data of alcohol content in fermentate shows that for 100 g of seaweed, time fermentation to produce alcohol is around 130-140 h or about 5-6 days (**Fig. 2**). Time fermentation gave significant effect on alcohol content of fermentate using bread yeast. However, the yield was still low compare to work of Ge *et al*. (2010), which could get about 41.2% (corresponds to 80% of theoritical yield) of ethanol from seaweed waste hydrolysate fermentation using *Saccharomyces cereviceae* as bio-agent. In this experiment we showed that tapai yeast was not suitable for bio-ethanol fermentation of seaweed hydrolysate.

Table 2. Influence of fermentation time on alcohol content of fermentate

Fermentation time (h)	Alcohol content in fermentate (%)
36	(0.0 ± 0.0) a
72	(0.0 ± 0.0) a
120	(4.0 ± 0.0) b
168	(4.6 ± 0.0) b

Note: Data from three replications. The alcohol content followed by same character showed no significant difference by least significant difference test at α of 5%

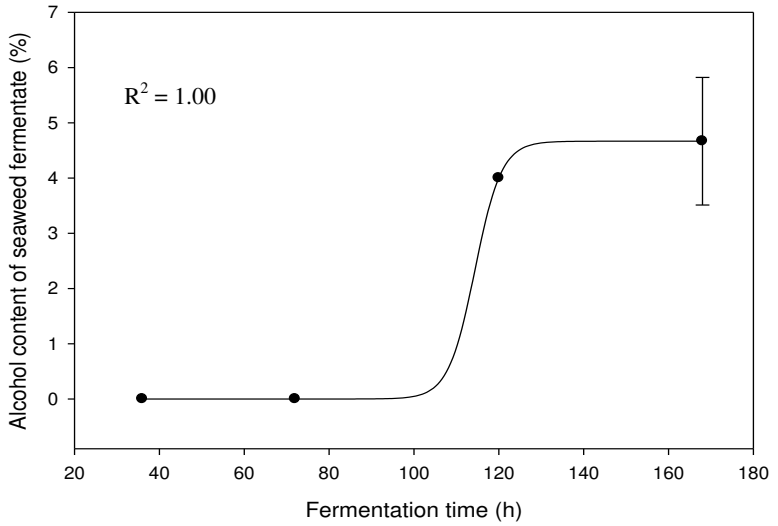

Fig. 2. Influence of fermentation time on alcohol content of fermentate of seaweed hydrolysate by acid hydrolysis using 5% sulfuric acid

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In preliminary experiment we have showed that "tapai" yeast we used is appropriate for "tapai" fermentation (Indonesian traditional fermented food from cassava). Azmi *et al.,* (2010) has reported that tapai yeast is the best biological agents among three yeast of *Saccharomyces cereviceae*, *Candida tropicalis*, and "*tapai" yeast*, which produced bioethanol of 20, 23, and 26 g L^{-1} in 72 h from 20% w/v of unhydrolyzed raw cassava starch, respectively.

Another method to increase the fermentation yield of alcohol are using recombinant bio-agent or simultaneous saccharification and fermentation (SSF). Bioethanol production from seaweed (*Laminaria japonica*) was demonstrated by Kim *et al.* (2011) using ethanogenic recombinant *Escherichia coli* KO11 with ethanol production of 0.4 g ethanol per g of carbohydrate, while SSF method was reported by Choi *et al.,* (2009) using cassava as carbohydrate source and *Saccharomyces cereviceae* as bio-agent could yield alcohol of 90.7%.

CONCLUSION

Acid hydrolysis for 2 h using H_2SO_4 of 5% at 100 ^oC of seaweed gel derived from 100 g of red seaweed (*Eucheuma cottonii*) yielded sugar content of about 15.8 mg mL^{-1} in hydrolysate. "Tapai" yeast was not suitable for bioethanol fermentation of seaweed hydrolysate, while bread yeast (*Saccharomyces cereviceae*) gave an alcohol content of fermentate of about 4.6% after 5-6 days (130-140 h) of fermentation at room temperature.

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