



Design Process of Hydrolysis and Fermentation Bioethanol Production from Seaweed *Euचेuma cottonii* to Renewable Energy Sovereignty

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Abstract

The aim of this research was study of *E. cottonii* to produce bioethanol fermentation substrate with a high reduction sugar content and low Hidroxymethylfurfural (HMF). Fermentation done by instant yeast and *Saccharomyces cerevisiae* culture of FNCC 3012. The best treatment was obtained in the combination of 2% of H_2SO_4 by time reaction of 120 minutes in 80°C produced 15.61 g/l reducing sugar and 5.03 g/l HMF. In fermented process, the hydrolysate with instant yeast starter delivered much more efficiency in 3.63 ml CO_2 volume, 87.53% in fermentation efficiency, and 1.96 g/l reducing sugar on fifth day of fermentation.

Keywords: bioethanol, *Euचेuma cottonii*, fermentation, hydrolysis, process design

1. INTRODUCTION

Indonesia's seaweed production reached 4,305,027 tons (Marine and Fisheries in Figures, 2011) which potentially for the development and utilization of seaweed. In addition, the climate in Indonesia is also supportive for the growth and cultivation of seaweed so that Indonesia has a great diversity of seaweed. It has been used as a source of raw materials of food industry, pharmaceutical industry, and environmental remediation. Other utilization of seaweed is as a producer of biofuels such as bioethanol, biodiesel, and biogas. Conversion seaweed into ethanol will provide widespread impact on the national economic also increasing the income of farmers and fishermen, the increasing in revenues from exports, and strengthening the resilience of countries in the field of energy, especially renewable energy.

Seaweed is categorized into three groups: red seaweed (*Rhodophyta*), green seaweed (*Chlorophyta*), and brown seaweed (*Phaeohyceae*) (Percival, 1979 in Park *et al.*, 2011). Among the three types of seaweed, red seaweed known to have a high carbohydrate content (Park *et al.*, 2011). Carrageenan is a polysaccharide extracted from red seaweeds such as *Chondrus*,

Gigartina, *Euचेuma*, *Furcellaria*, and *Phyllophora* (Meinita *et al.* 2011). According Rachbini *et al.*, (2011) in Nurdin (2012), a type of seaweed that is widely cultivated in Indonesia consists of *Euचेuma cottonii* and *Glacilaria*, with the yield ratio of about 70:30. Due to the high production of these types of seaweed, so in this study used red seaweed of the type of *E. cottonii*.

According to the results of previous studies on the seaweed group, it can be seen that the seaweed in particular of the type of *E. cottonii* can be processed into ethanol by fermentation method and utilizing carbohydrate content. Puspawati *et al.* (2015) conducted a seaweed *E. cottonii* hydrolysis using cellulase enzymes. The best reduction sugar produce after hydrolysis for 12 hours at 19 AU cellulase enzymes at 50 °C resulted in reduction sugar 7.853 g / l.

In that study, the raw material used seaweed dried first. Before hydrolyzed, dried seaweed should be smoothed beforehand to expand the surface and facilitate contact with the catalyst material. The process required energy and high cost. Therefore, in this research made efforts to simplify the production process by doing seaweed washing and soaking before hydrolysis.

2. MATERIAL AND METHODS

2.1 Material and Equipment

2.1.1 Material

Dried seaweed *E. cottonii* was supplied from Buton, Southeast Sulawesi. The chemicals used include H₂SO₄, distilled water, glucose anhydrous, NaOH, alcohol, and various reagents for analytical purposes such as arsenomolibdat, nelson A and B, Carrez solutions I and II, and others. Instant yeast for fermentation “Fermipan” brands imported by PT Sangra Ratu Boga, yeast inoculum FNCC 3012 from the Central Laboratory of the Inter-University, Gadjah Mada University, and *Glucose Yeast Extract*.

2.3 Methods

2.3.1 Seaweed Characterization

E.cottonii rinsed with distilled water to remove salt, sand and other impurities. Characterization of seaweed carried by proximate test to determine water content,

fat content, protein content, ash content, and fiber content to determine the potential of *E. cottonii* as bioethanol feedstock.

2.3.2 Hydrolysis of polysaccharides

Seaweed must first be soaked in water for 24 hours then hydrolysed by using dilute acid (H₂SO₄). The experiment design was randomized block design complete with three factors as presented in Table 1. The hydrolysate then neutralized by NaOH to pH 7. Furthermore, the hydrolysate was analyzed of reduction sugar Nelson-Somogyi and HMF.

2.3.3 Fermentation

Fermentation was done by *S. cerevisiae* yeast (Fermipan) with starters as much as 10% at 30 °C for 72 days. It also conducted a pure culture of *S. cerevisiae* FNCC 3012. Criteria for selecting the best method based on the efficiency and performance of the fermentation process.

Table 1. Treatment variations on seaweed hydrolysis process using H₂SO₄

Notation	Parameter	Treatment 1	Treatment 2	Treatment 3
A	H ₂ SO ₄ con.	1%	2%	3%
B	Time	60 minutes	90 minutes	120 minutes
C	Temperature	80°C	90°C	100°C

3. RESULT AND DISCUSSION

3.1 Characterization of *E. cottonii*

Table 2 showed that the water content of *E. cottonii* from Buton was 40,73% (after solar drying). Whereas, the optimal water levels in seaweed 31-35% for *Eucheuma sp.* (Anonymous, 2008). In addition, spoilage can occur if the algae has a high water content. This means that the seaweed that has a high water content has a great opportunity to experience decay if not promptly drained back. Crude fiber was the residue of food or agriculture after being treated with an acid or alkali boiling (Fardiaz et al., 1986 in Sudieman, 1990). Crude fiber in seaweed *E. cottonii* amounted to 4.09%. Determination of crude fiber content, in principle, that separates the coarse fiber of

non-starch polysaccharides by hydrolyze. But, the acid hydrolyzed able to destruct all polysaccharides to glucose that can be converted into bioethanol.

Table 2. Characterization of *E. cottonii*

Compound	Concentration (%)	
Water	40,734	±0,077
Ash	33,775	±0,043
Protein	3,855	±0,007
Fat	0,078	±0,028
Fiber	4,087	±0,030
Carbohydrate	17,469	±0,0750

3.2 Acid Hydrolysis

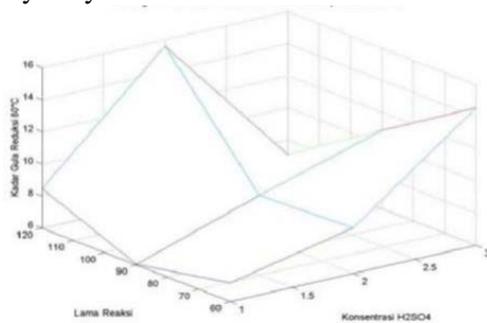
3.2.1 Hydrolysis Process

Pretreatment of *E. cottonii* was done by soaking the seaweed in water for 24 hours. The water content of seaweed is initially approximately 40.73%, after being soaked for 24 hours increased to 92.42% water content. Further size reduction was done using a blender for 1 minute. Soaking and size reduction were intended that seaweed can be easily hydrolyzed. Water contained in seaweed also served as a precursor reaction. This condition made the hydrolysis reaction optimally. Preparation of raw materials as applied required less energy compared to previous studies because it did not require drying and shrinkage to form a powder.

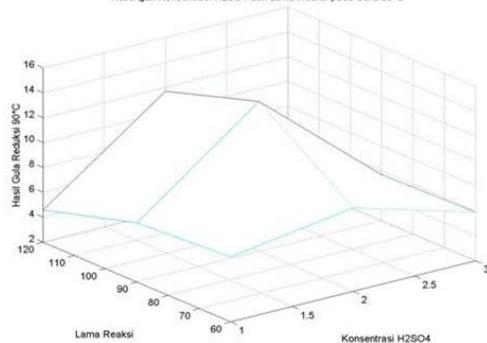
The acid hydrolysis mechanisms of carrageenan based on protonation of the glycosidic oxygen. High temperature and acid hydrolysis reported facilitate the glycosidic bond (Khambhaty *et al.*, 2012 in Tan *et al.*, 2013).

3.2.2 Reduction Sugar Content

The reducing sugar obtained in this research was 2.94% - 15.61%, the maximum achieved in combination treatment H₂SO₄ concentration of 2% with a reaction time of 120 minutes at a temperature of 80°C. Based on analysis of variance, known that H₂SO₄ concentration, reaction time, temperature, and the interaction of these three significant effect on hydrolysis of *E. cottonii*.



Hubungan Konsentrasi H₂SO₄ dan Lama Reaksi pada Suhu 80°C



Meanwhile, treatment groups were not significantly different. Test of orthogonal polynomials indicated that treatment with H₂SO₄ concentration and reaction time increases linearly reducing sugar levels at a concentration of 1% and 2%. but the 3% H₂SO₄ concentration, reducing sugar levels decreases with the time reaction

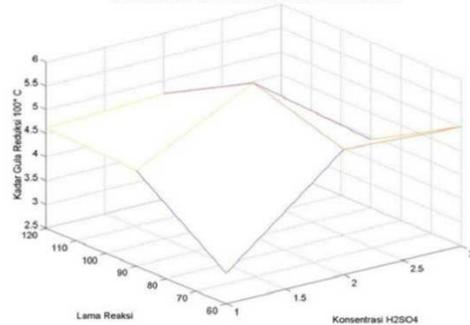


Figure 1. Reduction sugar on 80°C (a), 90°C (b), and 100°C (c).

Samsuri (2007) stated that the low concentrations of diluted acids may increase the quantity of sugar in the hydrolysis of lignocellulose as H⁺ ions in strong acid may decide glycosidic bond contained in cellulose.

At higher temperatures the reaction did not lead to the formation of monomeric sugars until the yield of sugar decrease at above 120 °C. The advanced reactions typically occurred as long as heat treatment of the polysaccharide. This reaction could not be avoided in the state of acidic hydrolysis. This causes sugars would be decomposed and resulted in low sugar yield (Irawan, 2012). Hydrolysis in the study conducted under conditions of treatment temperature of 80 °C without size reduction prior to powder form. So that this condition can be applied to make it easier for small and medium-sized industry.

3.2.3 Hydroxymethylfurfural (HMF)

In addition to acid hydrolysis produce glucose also produce furan compounds, phenolic, and acetic acid. These products were unexpected form because it would inhibit the fermentation process. Using acid hydrolysis process could also trigger the degradation of glucose so that the glucose and ethanol yield decreased (Howard *et al.*, 2003).

Interaction between the reaction time

and the H_2SO_4 concentration was significant effect on levels of HMF. As polynomial trials showed that increasing the parameter observation led to increase HMF levels from hydrolysate *E. cottonii*. Along with the increase in the reaction temperature, there was also increased levels of HMF in a linear trend. After hydrolysis, HMF levels fall between 2.81 g/l to 5.40 g/l. Also, the higher the concentration of H_2SO_4 , the higher the levels of HMF produced in line with the duration of fermentation.

The levels of HMF closely associated with glucose levels in *E. cottonii* that has been hydrolyzed in H_2SO_4 solution. The higher the acid concentration and temperature used, and the longer the reaction that lasts the greater the levels of HMF. At high temperature and pressure, glucose will be converted into HMF. If HMF further hydrolyzed to form levulinic acid and formic acid (Musatto and Roberto, 2004).

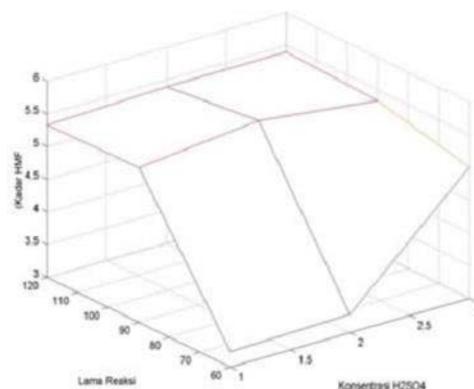
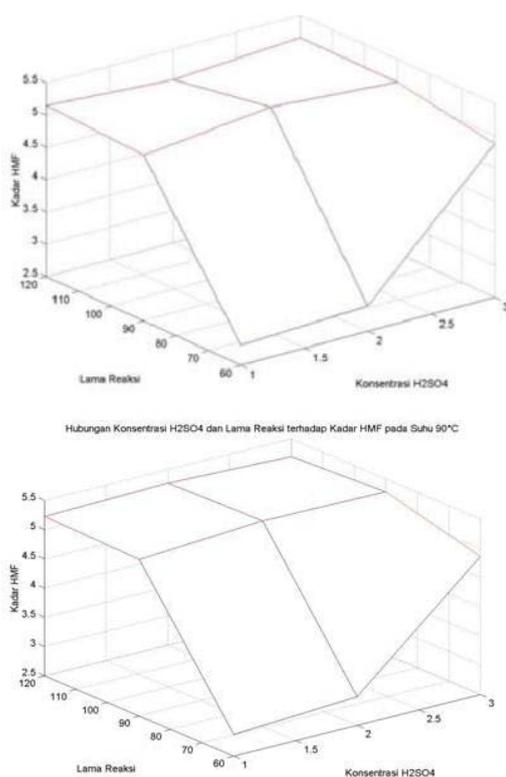


Figure 2. Concentration of HMF at 80°C (a), 90°C (b), and 100°C (c).

HMF is a reaction formation of side reactions are unavoidable in the circumstances acidic hydrolysis. The most important degradation products in terms of results and the possibility of its use is cyclic compound furfural (2-furaldehida) formed from pentose and uronic acid, and hydroxymethylfurfural (5-hydroxy methyl-2-furaldehida) from hexose sugars, primarily glucose. The results were higher than these compounds obtained only in concentrated acid and high temperatures. If the temperature is raised, the molecule HMF levulinic acid turns into acid and formic acid (Fengel and Wegener, 1995). The compounds could inhibit the growth of microorganisms used in fermentation into ethanol. This was because glucose and other sugars that are a major component in the formation of ethanol has been degraded into other compounds that are not expected.

Meinita *et al.* (2012) conducted research to hydrolyze 10 g powdered catalyst *E. cottonii* with H_2SO_4 0.2 M as much as 100 ml. The hydrolysis yield of HMF 4.85 g/l. The results tend to be not much different from that obtained in this research produced HMF 5.03 g/l for hydrolysis conditions that produce the highest reduction sugar.

3.2.4 Optimal Hydrolysis Treatment

The highest reduction sugar 15.61 g/l was obtained on condition of hydrolysis with H_2SO_4 2% for 120 minutes at a temperature of 80°C (A2B3C2). In this condition, the levels of HMF reached 5.03 g/l while the

lowest levels of HMF obtained at hydrolysis conditions using 1% H_2SO_4 for 60 minutes at a temperature of 80 ° C. While on the fermentation conditions, very low levels of reducing sugars and hemicellulose, cellulose, and lignin is not hydrolyzed properly.

Hemicellulose is hydrolyzed using low concentrations of acid (dilute) at moderate reaction conditions, but needed more extreme conditions to be able to hydrolyze cellulose. The main advantage with dilute acid hydrolysis is, no need for acid recovery, and the absence of loss of acid in the process (Iranmahboob *et al.*, 2002). A long time the hydrolysis reaction will lead to the formation of toxic inhibitors. Glucose was degraded to form HMF and reacted further to form formic acid. While the result of the degradation of lignin will be formed phenol compounds (Palmqvist and Hahn-Hagerdal, 2000). Those products could inhibit the growth of microorganisms in the fermentation process of ethanol formation.

3.2.5 Fermentation Hydrolisate *E. cottonii*

E. cottonii hydrolisate fermentation gel has been carried out by Chandra et al. (2011) with a reducing sugar of 15.8 mg/mL using baker's yeast capable of producing alcohol 4.6% during the 5-6 days of fermentation at room temperature. Meanwhile, in this research the fermentation was done by using the instant of *S. cerevisiae* starters as much as 10% at 30 ° C for 72 days. It also conducted a pure culture fermentation using *S. cerevisiae* FNCC 3012. Ethanol concentration of 1-2% (v/v) could interfere with the fermentation and the ethanol concentration of 10% (v/v) yeast growth rate will stop because yeast is a microorganism that is sensitive to ethanol (Clark and Mackie, 1984). According to Prescott and Dunn (1981), the maximum ethanol content that can be produced before the fermentation really stop is 13% (v/v). Mangunwidjaja and Suryani (1994) adds that the concentration of 40g/L (4%) of ethanol would be a good inhibitor for growth of biomass and ethanol production.

In Figure 6 shows that the CO_2 produced by fermentation using pure cultures of *S.cerevisiae* was smaller than the fermentation using yeast. This was

presumably due to differences in strains and the quality of their respective cultures. From hour-12 to 36th an increase in the rate of fermentation was demonstrated by the increasing rate of formation of CO_2 is very sharp. In this phase conditions were optimal fermentation process to produce CO_2 and high ethanol. After hours of 36th decrease the rate of formation of CO_2 which means a decline in the rate of fermentation. A decrease in the rate of fermentation was suspected because of the accumulation product is ethanol and the acid metabolite which then inhibit the rate of fermentation.

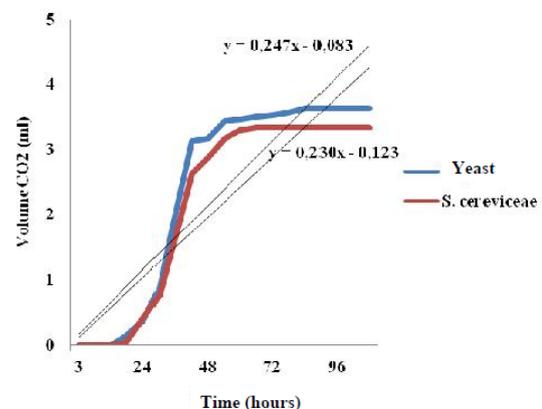


Figure 6. CO_2 volume while fermentation

Decreased levels of reducing sugars indicates the use of substrate by *S. cerevisiae* during fermentation. In Figure 7 below shows that there was an increase in the consumption of sugars by yeast are very significant. The consumed sugars stopped on the 2nd day of fermentation. After experiencing a phase of adaptation, the microbes start splitting at low speed because the new start adjusting (Fardiaz, 1988).

Based on Figure 7 below showed that until the 2nd day decreased in reducing sugar levels very quickly. This situation was due to *S. cerevisiae* yeast that still entering log phase, microbes are rapidly dividing and constant follow a logarithmic curve. The increasing of the sugar consumption indicated that substrate fermentation has not converted to ethanol completely. The substrate will continue to decrease with the length of the fermentation process.

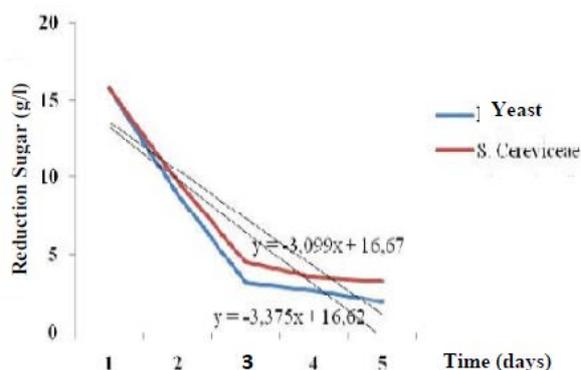
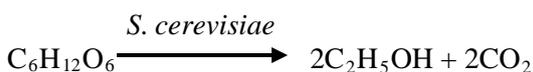


Figure 7. Decreasing of reduction sugar

In the fermentation process using the inoculum of *S. cerevisiae* yeast and pure, able to converted sugar into ethanol of 0,87 g/l and 0.79 g/l respectively, with substrate utilization efficiency average were 87.53 % and 78.90 %. Theoretical conversion of ethanol formation from glucose is 51% with the following reaction:



Based on the above equation, 1 mole of glucose can produce each 2 moles of ethanol and CO_2 . While from 1 g glucose theoretically be produced 0.51 g ethanol (Maiorella et al., 1981 in Ega et al., 2013). In fact, Isroi (2013) stated that ethanol derived only about 90% of the theoretical results because glucose was also used for the growth of *S.cereviceae*. Then the possibility of ethanol produced by 60-70% or 0.357g ethanol per 1g of glucose.

In this study, sugar was used in the fermentation process amounted to 15.61 g/l. If the whole substrate sugar was converted to ethanol yield 10.23 ml of ethanol. On the condition of fermentation using yeast inoculum obtained fermentation efficiency by 87.53%, the theoretical ethanol obtained was 8.9 ml and 8.1 ml inoculum for the fermentation process using a pure culture fermentation of *S. cerevisiae* with 78.9% efficiency.

Based on this research, it was known that the performance of yeast derived from yeast inoculum better than pure cultures of *S. cerevisiae*. Significant differences in the results indicated the possibility of the use of

yeast culture that was cheaper and easy to apply in small and medium industries.

CONCLUSIONS

1. *Eucheuma cottonii* hydrolysis optimal conditions, i.e. at a concentration of 2% H_2SO_4 for 120 minutes at a temperature of 80°C which results in reducing sugar 15.61 g/l; Hydroksymetilfurfural (HMF) 5.03 g/l.
2. Fermentation of *E. cottonii* hydrolyzate by yeast was able to achieve the substrate utilization efficiency of 87.53% and a consume in reduction sugar to the remaining 1.96 g/l on the fifth day of fermentation.

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