

UTILIZATION OF RESIDUAL CARRAGEENAN EXTRACT FROM *Euclima cottonii* SEAWEED INTO BIOETHANOL

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Abstract: In the process of processing seaweed will produce residual waste from carrageenan extraction, and the residue still contain cellulose, lignin, hemicellulose, pectin, and other organic materials that can be processed into bioethanol. This research aimed to utilize the residual carrageenan extracted from seaweed *Euclima cottonii* into bioethanol. The research method includes acid hydrolysis process using 3% sulfuric acid at a temperature of 70-80oC for 30 minutes, followed by a fermentation process using yeast *Saccharomyces cerevisiae* with a ratio of 1: 0.006 for hydrolyzate and yeast, fermentation time treatment 1, 3, 6, 9 and 12 days at temperature 25o-30oC. Fermentate at 78oC, measured in degrees of acidity (pH), volume, and levels of bioethanol. The results showed that the residual carrageenan extract containing carbohydrates as un-extracted carrageenan was 5.01%, hemicellulose was 7.12%, cellulose was 0.96%, and lignin was 8.26%. The level of bioethanol produced from the residual carrageenan extraction was 2.57% and, the yield was 32.64% with a fermentation time of 6 days as the optimal time.

Keywords: *Euclima cottonii*; residual extract; bioethanol; fermentation time

1. INTRODUCTION

Indonesia is an archipelagic country with a coastline length of around 81,000 km, is a coastal and oceanic area that has a variety of substantial and diverse biological resources. One of Indonesia's water resources that has the potential to be developed is seaweed. Seaweed is a sea plant that cannot come roots, stems and, leaves so that the whole body is called thallus, and based on the pigments it contains; it can classify as Rhodophyceae (red algae), Chlorophyceae (green algae) and Phaeophyceae (brown algae). The three types of seaweed contain chemical compounds that have high economic value. *Euclima cottonii* is a type of red seaweed that has cylindrical thallus, smooth surface and, cartilaginous. The contents of seaweed species of red algae including polysaccharides, carrageenan, agar, cellulose, lignin monosaccharides, glucose, galactose and, agarose.

E.cottonii seaweed is also one of the plants that have the potential to produce bioethanol. In the processing of seaweed will produce waste that can still use as bioethanol. Produce waste containing cellulose, lignin, hemicellulose, and other organic ingredients. The process of processing seaweed into carrageenan is carried out through extraction, which results in residual carrageenan extraction results. The filtrate further treated until carrageenan flour obtained, while the residue immediately

discarded / not utilized. The residual carrageenan extraction can use as bioethanol through a fermentation process that produces bioethanol with levels of 4.15% and a yield of 10.38% on the 6th-day fermentation.

Bioethanol is ethanol, which engineered from biomass (plants) through biological processes (enzymatic and fermentation). Bioethanol can be made by fermentation by khamir such as by *Saccharomyces cerevisiae* from polysaccharides found in waste residue from carrageenan extraction from seaweed *E.cottonii* including cellulose. Cellulose can be hydrolyzed into glucose using water media and assisted with an acid catalyst or enzyme. Cellulose hydrolysis can be carried out using acidic solutions such as sulfuric acid (H_2SO_4) or enzymatically using the cellulase enzyme from *Aspergillus niger*. The research carried out aimed at utilizing the residual extract of carrageenan seaweed *E. cottonii* into bioethanol

2. METHODS

2.1 Feedstock and chemicals

The residual carrageenan extraction results used in this study came from the research residue of Irawati (2015), sulfuric acid (H_2SO_4) 3%, sodium hydroxide (NaOH) 1 N, yeast (*Saccharomyces cerevisiae*), potassium dichromate ($K_2Cr_2O_7$) 0.2 N, Ferro ammonium sulfate (FAS)) 0.1 N standard, feroin indicator.

2.2 Hydrolysis Process

The hydrolysis process was carried out, according to Candra (2011). , the sample was weighed as much as 300 g and put into a boiling flask, then added 75 ml of H_2SO_4 3%. The mixture refluxed at 70-80oC for 30 minutes. The results of hydrolysis (hydrolyzate) stored in an Erlenmeyer for the determination of reducing and fermented sugar levels.

2.3 Measurement of Hydrolyzed Reduction Sugar Levels (Luff Schoorl Method)

The hydrolyzate is taken as much as 10 ml and diluted in a 100 ml volumetric flask. Dilute hydrolyzate is made as much as 10 ml into the Erlenmeyer flask, then add 25 ml of Luff Schoorl solution and 15 ml aquademin. The mixture refluxed for 10 minutes, then cooled, add with 30% KI of 10 ml, and 25% H_2SO_4 of 25 ml slowly. A standard solution of 0.1 N $Na_2S_2O_3$ is pulled up to turmeric yellow, then a 1 ml starch indicator is added, then pulled back until milky white.

2.4 Bioethanol Process

The degree of acidity (pH) of the hydrolyzate is set to 5.0 by adding 1N NaOH. The hydrolyzate stored in a fermenter bottle, according to Wiratmaja (2011), yeast is added in a ratio of 1: 0.006. The fermenter bottle tightly closed, and the condition is made to be anaerobic. Fermentation is carried out at 25-30oC with a treatment time of 1, 3, 6, 9, and 12 days. Each time the fermentation treatment uses 50 g of hydrolyzate and 0.3 g of fermented yeast. The results of the fermentation (fermentate) are filtered and accommodated to measure the degree of acidity (pH), volume, and determination of bioethanol levels. The fermentates are then used for the distillation process by measuring their volume, then distilled at 78oC for one hour. The results of distillation (distillate) are accommodated to measure the degree of acidity (pH), volume, and determination of bioethanol levels.

2.5 Measurement of Degree of Acidity (pH), Volume, and Bioethanol Levels

The measurement of the pH of the medium is carried out to determine whether there is a change in the pH of the medium. pH changes that occur indicate the occurrence of biological activity carried

out by microbes. Measurement of the degree of acidity used a calibrated digital pH meter. Fermentate and distillate volume measurements using a measuring cup.

Measurement of bioethanol fermentate and distillate levels carried out as follows where a sample of 1 ml put into an Erlenmeyer, then a 0.2 N 25 ml K₂Cr₂O₇ solution was added, then refluxed for 10 minutes and cooled rapidly. Then it is pulverized with a standard solution of Ferro Ammonium Sulfate 0.1 N until the greenish color is then added to the feroin indicator and pulled back until the endpoint color is brownish-red

3. RESULTS AND DISCUSSION

3.1 Content of the residual carrageenan extraction results

E.cottonii seaweed contains 61.51% carrageenan. Carrageenan is a polysaccharide composed of galactose. It means that carbohydrates contained in *E.cottonii* seaweed dominated by carrageenan and also means that most of the carbohydrates contained in the residual carrageenan extracted are carrageenan that not extracted.

In addition to carrageenan that not extracted, cellulose in the residual carrageenan extraction is also a material that has the potential to be a substrate for making bioethanol. The results of the analysis of the residual contents of the carrageenan extraction results shown in Table 1.

Table 1 Results of analysis of residual content of the extraction carrageenan

Component	Content (%)
Water	72.39
Carbohydrate	5.01
hemicellulose	7,12
Cellulose	0.96
Lignin	8.26

The moisture content is quite high in the sample of 72.39% due to being wet. Carbohydrates mostly carried into carrageenan extracts and, only a small portion, left in the residual extracted products. Carbohydrate content from the residual carrageenan the extraction results was 5.01%, hemicellulose 7.12%, cellulose 0.96%, and lignin 8.26%. Seaweed is biomass that contains lignocellulose with the main components of lignin, cellulose, and hemicellulose. This component is an important material to produce useful products such as sugar for fermentation, chemicals, and biofuels. Cellulose and hemicellulose are polysaccharides. Cellulose is a homopolysaccharide composed by α - (1,4) -D-glucose units and hemicellulose is heteropolysaccharide consisting of various simple sugars, namely pentose (xylose and arabinose); hexose (glucose, mannose, and galactose); and uronic acid (4-O-methyl glucuronic, D-glucuronic, and D-galactonic), while the molecular structure of lignin is very different when compared to polysaccharides because it consists of an aromatic system composed of phenylpropane units.

3.2 Hydrolysis Reduction Sugar

Reducing sugar is a simple sugar produced by hydrolysis of complex carbohydrates. The availability of reducing sugars in bioethanol production medium is one of the essential elements for the growth of *S. cerevisiae* because it functions as a carbon source for energy formation. The hydrolysis process uses 3% sulfuric acid at a temperature of 70o-80oC for 30 minutes to produce reducing sugars with

levels of 0.60%. The reducing sugar is calculated as galactose because most of the carbohydrates in the dried seaweed are carrageenan composed of galactose.

Table 2. Result of Reduction Sugar

Repeat	The weight of the sample (g)	Na ₂ S ₂ O ₃ (N)	Blank titration volume (ml)	Sample titration volume (ml)	Volume Na ₂ S ₂ O ₃ used	Number galactose (mg)	Galactose levels (%)
1	1,4986			23,0	2,4	6,86	4,12
2	1,3610	0,1036	25,4	22,5	2,9	8,31	5,50
3	1,2875			22,7	2,7	7,73	5,40
Rata-rata							5,01

The results of hydrolysis using acids show a small amount of reducing sugar; this is because the levels of carbohydrates contained in the sample are also low in number. The study of Adini, et al., 2015 also found that reducing sugar levels produced in the treatment using acid hydrolysis tended to be lower than in the treatment using enzymatic hydrolysis. The different types of polysaccharides found in a material can affect hydrolysis activity; more complex the nature of polysaccharide, the more difficult the catalyst to degrade.

3.3 Degrees of Acidity (pH), Volume, and Bioethanol Levels

3.3.1. Degree of Acidity (pH)

Measurement of the degree of acidity (pH) made on the results of fermentation (fermentate) and the distillation shows in Figure 2.

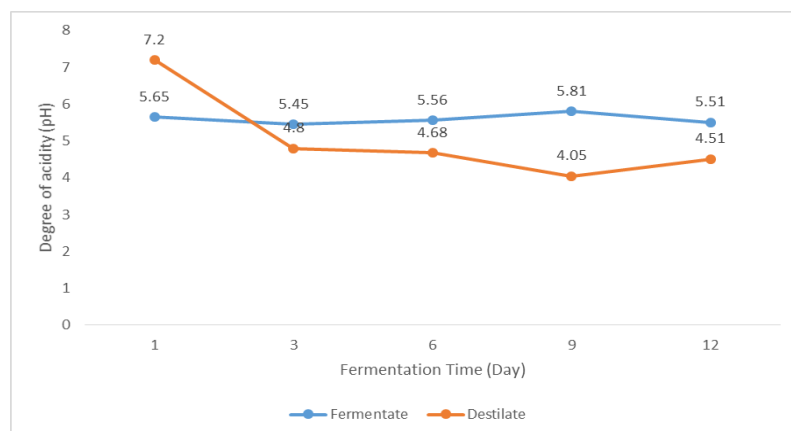


Figure 2 Results of Measurement The degree of acidity (pH) fermentate and distillate

Changes in pH in fermentation due to the activity of yeast cells (*S. cerevisiae*) produce ethanol as primary metabolites and also produce organic acids such as malic acid, tartaric acid, citric acid, lactic acid, acetic acid, butyric acid, and propionic acid; as a result side. These acids reduce the pH of the medium. Bioethanol production through fermentation can cause changes in the pH of the medium. The optimal pH range for *S. cerevisiae* growth is in the field of pH variations 4-6. Bioethanol

production through fermentation can cause changes in the pH of the medium. The results of pH measurements on fermentate and distillate show that almost all distillates have a lower pH than fermentate. The result indicates that the proportion of bioethanol in the distillate is more than fermentate. However, for a one-day distillate shows different things; this is because the bioethanol produced is still a little.

3.3.2 Volume

Making bioethanol from the residual carrageenan extraction results can not be fully converted into ethanol. This fermentation process, in addition to producing bioethanol fermentate, also provides the residual fermentation in the form of gel-shaped solids. The results of measurements of fermentate volume and the amount of residual fermentation shown in Figure 3 and Figure 4.

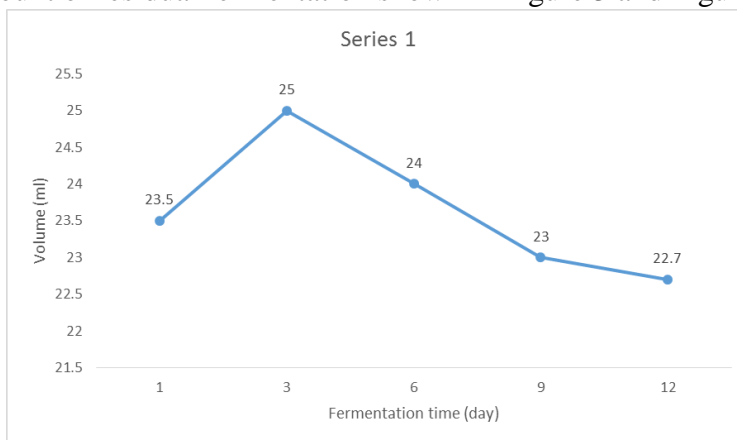


Figure 3 Results of Measurement Volume fermentate

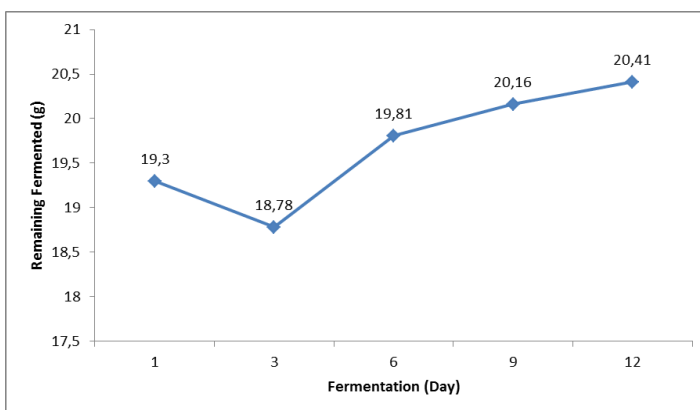


Figure 4. The Residual Amount of Fermentation

The fermentate volume measurement results show an increase in volume from 1 day to 3 days fermentation, then decrease in fermentation 6, 9, and 12 days. Conversely, the residual fermentation shows a decrease in fermentation 1 day to 3 days then increased in fermentation 6, 9, and 12 days. The amount of fermentate volume and the residual fermentation results are inversely proportional. Growth factors of *S. cerevisiae* influenced by production bioethanol.

The growth of *S. cerevisiae*, on fermentation 1 day to 3 days, shows an increase in the volume of fermentate; at this time, *S. cerevisiae* enters the log phase, which is the phase where *S. cerevisiae*

requires more energy than in other stage and is most sensitive to environmental conditions. At the end of the log phase, the speed of population growth decreases because the nutrients in the medium have decreased. The stationary phase occurs at 6 days; at this phase, the cell size becomes smaller because the cell continues to divide even though the nutrients are up, 9, and 12 days fermentation time the volume decreases. The reuse of ethanol causes use in volume after 3 days of fermentation is caused by the reuse of ethanol as the primary metabolite for the metabolism of acetic acid. Distillate volume measurements were carried out using 15 ml of fermentate as a distillation feed. Distillation is carried out at a temperature of 78oC for 1 hour. Distillates obtained presented in Table3.

Table 3 Results of measurement volume distillate

Long fermentation (days)	Distillate feed volume (ml)	Distillate volume (ml)
1	15	4.1
3	15	7.0
6	15	10.2
9	15	8.2
12	15	10.2

After calculating, the yield of each fermentation time is as follows: 1 day fermentation 12.85%; 3 days fermentation 23.34%; 6 days fermentation 32.64%; 9 days fermentation 25.15%; and 12 days fermentation 30.87%. The highest yield of bioethanol produced from fermentation for 6 days.

3.3.3 Bioethanol levels

The residual extract of carrageenan seaweed *E. cottonii* can be used as raw material for the production of bioethanol because it contains a lot of polysaccharide carbohydrates composed of many simple sugar monomers. These simple sugar monomers are the main precursors used by *S. cerevisiae* to convert into ethanol under suitable environmental conditions. The results of determining the levels of bioethanol fermentate and distillate shows in Figure

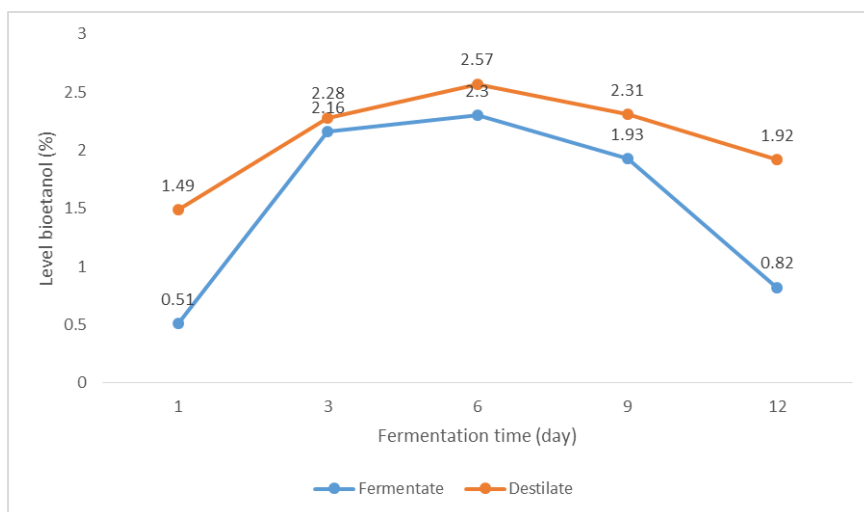


Figure 5 Results of Determination Levels of Bioethanol Fermentate and Distillate

The highest level of bioethanol on fermentate was obtained on the 6th day fermentation time of 2.3%, while the most top bioethanol distillate content on the 6th day fermentation time was 2.57%. These results are less than the results of a study conducted by Adini, et al., 2015 using the seaweed medium *Gracilaria* sp. produce the highest ethanol content of 5.50%; while the most top ethanol content for waste is 4.93%. One of the things that cause low levels of bioethanol is the acid hydrolysis process, which also produces inhibitor compounds that can interfere with the fermentation process.

4. CONCLUSIONS

The residual extract of carrageenan *E. cottonii* seaweed contains carbohydrates as un-extracted carrageenan at 5.01%, hemicellulose 7.12%, cellulose 0.96% and lignin 8.26%, which produces bioethanol fermentate levels of 2.57% with yield 32.64% and fermentation time for 6 days.

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