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# Hydrothermal Acid and Enzyme of Indonesian Macro-algae (Ulva lactuca) for Ethanol Production

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**Abstract:** The world's global concerns about the availability of energy sourced from non-renewable materials will create another predicament if it does not immediately find a way out. As a solution, a renewable energy source derived from green algae Ulva lactuca can put into consideration, as its carbohydrate content allows it to be converted into bioethanol. This algae is widely available along the South Coast of Java Sea that has not been extensively used. The method used is to perform a pre-treatment processes (acid hydrolysis and enzyme hydrolysis), microbial fermentation using yeast or Saccharomyces and distillation process at a temperature slightly above the boiling temperature of ethanol. The study was conducted with variations of Sulphuric Acid concentration, the amount of enzymes and the types of enzymes that are used during the hydrolysis process. The results indicate that the highest levels of ethanol in the amount of 7.706273% V/V is by using sulphuric acid of 2% with 10 drops of beta-amylase enzyme. Although the level of Ethanol produced is not high, but there is a positive possibility to expand the bioethanol conversion technologies using the macro-algae Ulva lactuca.
**Keywords** : bioethanol, acid and enzyme hydrolysis, Ulva lactuca.

## Introduction

In the last ten years, marine natural resources have an important role in biotechnology [1]. As an archipelago country, Indonesia has an abundant natural wealth. Besides the beauty and richness of marine varieties, Indonesian beaches with a coastline around 108,920 km long [2] also provide other resources that can be utilized as raw material for alternative energy, i.e: bioethanol. One of the natural resources that can be converted into energy source is macro-green algae species Ulva lactuca. Its habitat is widely available along the coast of the South Island of Java Indonesia. Marine biomass has a higher growth rate compared to food crops, contains lipids [3], which is very high in carbohydrates [3,4] and also lignin [4]. Carbohydrate content in Ulva sp. is 60-70% dry weight [5]. Even other type of macro-algae, which is Cladophora sp contains 70% -72% carbohydrates [4].

Bioethanol can be produced from various types of feedstock derived from biomass. There are 3 types of bioethanol: first generation bioethanol originated from biomass such as corn, sugarcane, cassava; second generation bioethanol produced from feedstock of agricultural waste such as rice straw, wheat straw and sugar cane bagasse; and third generation bioethanol derived from a type of algae both micro-algae and macro-algae.

From all of the above, the first bioethanol had to be disused because of competition with food crop. As with the second generation bioethanol, its hydrolysis stage is difficult to do because agricultural waste feedstock is categorized as liqnocellulosic biomass, it contains quite high lignin, averaging above 20% by weight [6]. Biomass feedstocks for third generation bioethanol sourced algae both micro-algae and macro-algae with high carbohydrate content and low lignin content is now vastly considered [1,3,7] as the low lignin will facilitate hydrolysis process. Macro-algae has a good potential as a feedstock for the manufacture of bioethanol [4]. Before the alternative energy of macro-algae is developed, there were many researches done on alternative energy sourced of micro-algae.

The world's need on petroleum will increase by 38% in 2010-2040 and in Asian developing countries, including China, India and the Middle East rise by 85% [8]. Petroleum energy sources will be supplied by four energy sources; they are coal, oil, gas, and low carbon source [9]. With this fact, the challenge to find alternative energy sources need to be answered, one of which is to conduct a research on macro-algae Ulva lactuca conversion into bioethanol through the stages of the pretreatment process, hydrolysis process, fermentation process and distillation process. Ulva lactuca is the biomass from photosynthesis process that can be used as a raw material for the manufacture of bio-energy, can be cultivated and advantageous because it can be used as bioremediation [3,10]. The number of research on third generation bioenergy from macro-algae biomass is still relatively small. Not many researchers were conducting research on bioethanol from macro-algae, especially macro-algae species Ulva lactuca. Ulva lactuca studied in Denmark as a source of bioethanol containing a total of 58.4 grams of sugar per 100 grams of dry sample. Different places to grow Ulva lactuca will make a difference in carbohydrate content [3].

Based on the above phenomenon, the purpose of the research that has been done is to develop bioethanol energy from the third generation of renewable energy sources, namely macro-green algae species Ulva lactuca. Macro-algae species grow well in the coastal waters of the South Island of Java, such as beach Kukup Yogyakarta and beaches in South Malang, Indonesia. The advantage of macro-algae as feedstock energy is that it does not contain or contains very little amount of lignin (non-lignocellulosic biomass), so that the process of sugar content releasing is easier to do when compared to the second generation energy source that contains high lignin wherein deliqnification pre-treatment process is quite difficult. Besides, by utilizing Ulva lactuca as an energy source, it will not compete with the first generation energy source derived from food crops such as corn and sugarcane, which the first generation of this energy source is not allowed because of competition with its benefits as a source of human food.

Horn has conducted a research on making ethanol from brown macro-algae feedstock with pre-treatment process using a mill and sieve and its hydrolysate stored at low temperature. Microbe that was applied during the fermentation process is Zymomonas saccharobacter with pre-treatment temperature of 121 °C for 20 minutes, and the resulting ethanol content was 0.39 gr/gr [7]. Pre-treatment process of macro-algae Undaria pinnatifida was by blending followed by hydrolysis process using sulphuric acid buffer and citrate acid buffer at a temperature of 100 °C was able to produce the sugar content of 0.034 gr/gr. This sugar content indicated that the fermentation process can be done to transform sugar into ethanol [11]. Another biomass that can be converted into ethanol was sweet sorghum through a pretreatment process by means of acid hydrolysis in an autoclave. The microbe used was Saccharomyces cerevisiae in the fermentation temperature of 32 °C for 72 hours which will produce ethanol with a concentration of 1.6 - 1.8 g/liter.hour [12].

Hydrothermally pre-treatment and enzyme hydrolysis to Ulva lactuca done by Bruhn, the result of the conversion into ethanol of 0.141 gr/gr dry Ulva. Bruhn applied 4 types of enzymes: cellulant, novozym, spirizyme and α-amylase during the hydrolysis process. With Saccharomyces cerevisiae as a microbe during the fermentation process and hydrolysis process conducted at a temperature of 121 °C with a time of 45 hours. According to Bruhn, this result is considered minor, so he stated that the process of conversion on Ulva lactuca into bioethanol is a challenge or still need to be debated. To answer that further Bruhn together her team did further research by performing the cultivation of Ulva lactuca and convert Ulva lactuca into methane bioenergy. Bruhn concludes that Ulva lactuca is a promising biomass as feedstock methane gas that can be obtained through the anaerobic digestion process, and economically as well as continuity Ulva lactuca for energy production is profitable [3].

Other research conducted by Borines on the sargassum biomass pre-treatment process using sulphuric acid and cellulase enzyme during hydrolysis process. Microbes applied in the process of fermentation was Saccharomyces cerevisiae, in temperature of 40 °C and 48 hours to produce glucose by 89 % concentration [13].

This experimental study did assess whether the process of hydrolysis using a combination of acid and enzyme would give better results than previous researchers who used singly hydrolysis method. And whether the use of strong acids may increase the levels of ethanol concentration.

## Experimental Method

### Materials

Macro green algae species Ulva lactuca is obtained from Kondang Merak Beach, South of Malang, Indonesia. The harvesting process is done during the dry season when the tide is low at around 2-4 o'clock in the afternoon. During the study, chemicals used are mostly purchased from two local distributors, they are PT. Panadia Malang - Indonesia and PT Sari Kimia Raya Malang - Indonesia. Those chemicals are: Ethanol 98 % - Merck, sulphuric acid 98 % - Merck, Fehling A, Fehling B, sodium hydroxide, acetic acid, anti - moss (for distillation purposes), sodium acetate, anti - foam, beta-glucoamylase (supplied by Biotechnology Laboratory, Sidoarjo - Indonesia), yeast Saccharomyces cerevisiae (supplied by NKL, Surakarta Indonesia).

### Sample Preparation

Freshly harvested Ulfa lactuca are thoroughly cleaned from sand and other dirts, then washed repeatedly with water until it was completely clean, followed by sun drying then oven drying at a temperature of 50 °C to reach a stable weight. For a good mass transfer process, Ulva lactuca is pulverized by using a blender into about 100 mesh in size. To keep it dry, Ulfa lactuca is stored in a desiccator. HHCLA analysis (Hot Water soluble, hemicellulose, lignin, cellulose and ash) on the raw material has been done by using Chesson Method [14], and obtained 42 % of Hot Water soluble, 20 % of hemicellulose, 6.8 % of lignin, 30 % of cellulose, and 1.1 % of ash.

### Pre-treatment

Pre-treatment stage has been done by hydrolysis process using sulphuric acid (2% and 30%) in the autoclave at a temperature of 121 $^{o}$C [1,3,4,7] with a time of 90 minutes, followed by hydrolysis process using enzyme beta-glucoamylase and trichoderma enzyme (each 5 drops, 10 drops, 20 drops, and 40 drops). Different operation conditions were actuated on both enzymes: for beta-glucoamylase enzyme by heating in an autoclave for 42 hours at a temperature of 40 $^{o}$C, while for trichoderma enzyme performed for 42 hours at a temperature of 25-30 °C. There are 5 types of sugars contained in the biomass: glucose, xylose, mannose, galactose and arabinose (Yazdani). During the hydrolysis process, sugar chain will be broken into single sugars. Sugar content analysis has been conducted both qualitatively and quantitatively. Qualitative sugar analysis performed using solution of fefling A and fehling B until appeared a brick red precipitate, which precipitate indicates that it contains sugar. Meanwhile, quantitatively sugar analysis (reduction sugar, sucrose, and inverted sugar) has been done by using Luff Schoorl method.

### Fermentation

The next step was fermentation process by applying microbe of Saccharomyces cerevisiae carried out aerobically for 2 hours at room temperature followed by anaerobic at room temperature and keep the level of 4-4.5 pH to 7 days. During fermentation process, single sugar will be converted into alcohol by microbe. Nutrients such as urea of 0.5 grams and 5 drops of anti-foam was added during the fermentation process. Prior the fermentation process, it was necessary to curve the growth of Saccharomyces cerevisiae so it can be known when the exact application of microbe into substrate or hydrolysate. For 25 ml hydrolysate, Saccharomyces cerevisiae added was weighing 0.125 grams. The growth curve of yeast was performed by observing the number of colonies using opti-lab microscope (Euromex Holland), observed in 24 hours by recording in each time interval 2 hours. Starter preparation has been done with the addition of 2M NaOH solution into the hydrolysate and its pH is set at 5 and then pasteurized in an autoclave (YXQ.SG41, power 2000W, cubage 1 liter) for 30 minutes at a temperature of 90 $^{o}$C .

### Distillation

The final stage was distillation process at a temperature of 80 ℃ for 2 hours, where previously necessary pasteurization process was employed at a temperature of 60 ℃ for 15 minutes in a water bath. Ethanol content was analyzed by using Gas Chromatography (HP 6890 series).

## Results and Discussion

Microbial growth curves performed to make sure the right application time of microbe into microbial fermentation processes. The growth curve of saccharomyces cerevisiae is given in Figure 1. As can be seen from Figure 1, logarithmic phase started at the third hour, and the stationary phase apparently happened during 8th to 12th hour, then the decay phase is starting.

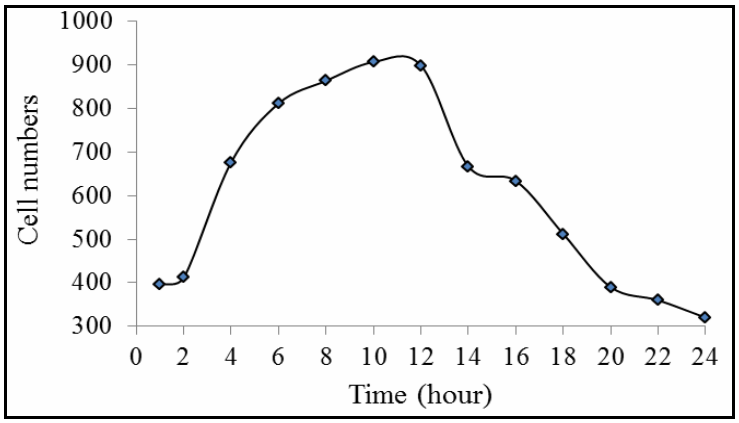


**Figure 1. Saccharomyces serevisiae growth rate**

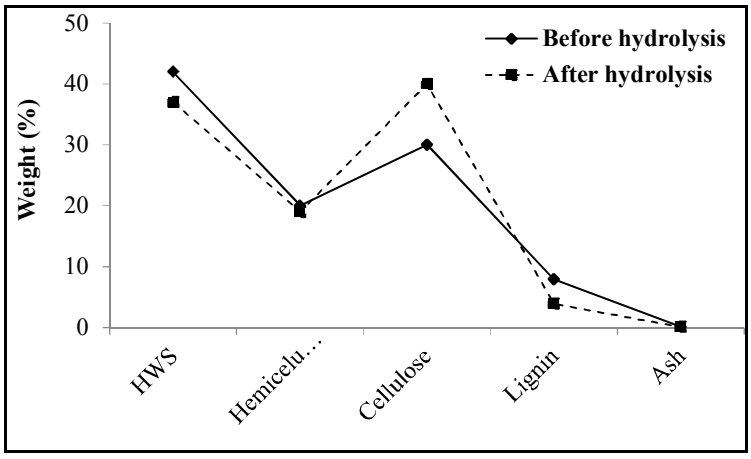


**Figure 2. HHCLA analysis before and after hydrolysis**

For the HHCLA analysis of the raw material Ulva lactuca on conditions before hydrolysis and after hydrolysis processes is given in Figure 2. The results of analysis performed 3 times and the average value is taken. As shown in Figure 2, the HWS level before hydrolysis is higher than after hydrolysis process, it was because a lot of dissolved content during the pre-treatment process. Meanwhile the content of the original cellulose is approximately 30%, rising to about 40%, while other components can be considered stable.

Sugar content test results conducted qualitatively is in the form of red sediment shown in Figure 3. The sugar content testing after acid hydrolysis 30% quantitatively performed with Luff Schoorl method with the highest result in the amount: 3.7255% sugar reduction, 16.6675 % sucrose, and 20.2176% total sugars.

**Figure 3. Result of sugar qualitative analysis**

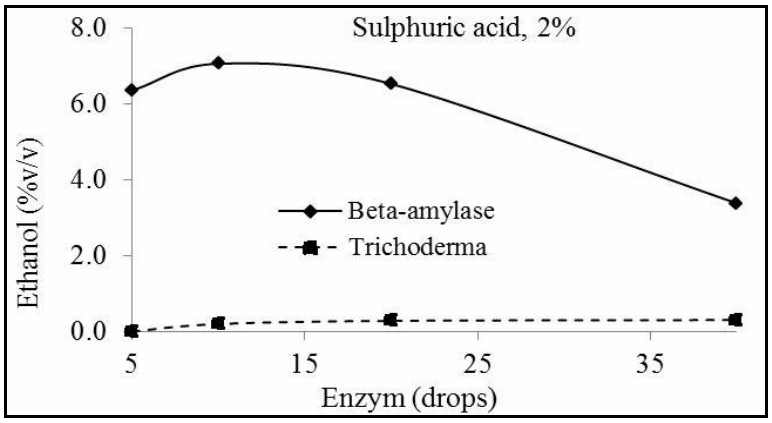


**Figure 4. Ethanol content in 2% sulphuric acid application as functions of enzyme type and drops of enzyme**

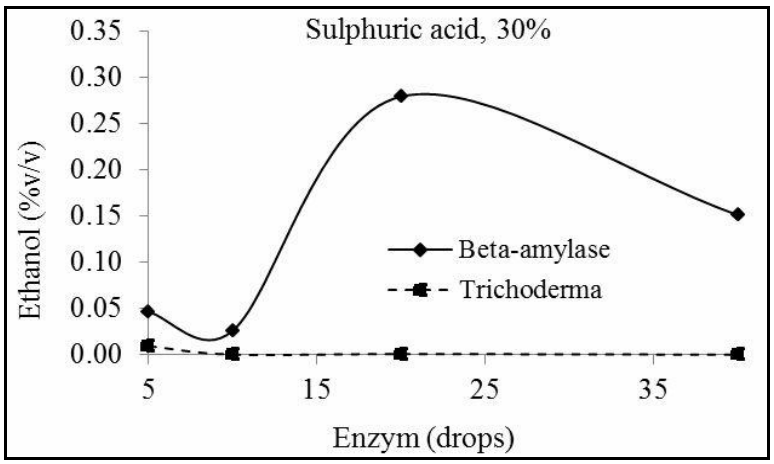


**Figure 5. Ethanol content in 30% of sulphuric acid application as functions of enzyme type and drops of enzyme**

The content of ethanol produced from the research is shown in Figure 4 and Figure 5. Hydrolysis process with the addition of the enzyme is an important phase because of there is a change from complex carbohydrates into simple monomers. As can be seen from Figure 4, the addition of sulphuric acid at a concentration of 2 %, the highest ethanol content of 7.06273 %v/v is given by the beta-amylase enzyme with doses of 10 drops, and for trichoderma enzyme highest ethanol content in the delivery of as many as 40 drops.

This indicates that the application of sulphuric acid 2 %, will make the role of trichoderma enzyme much weaker than the role of beta-amylase enzyme. This result does not consistent with the statement stated by Li that trichoderma is a producer of cellulase that most often used to extract the carbohydrate chains with less energy at a pH of about 4.5-5 and a temperature of 40-50 °C [15].

Another factor affecting the breakup process of carbohydrate chains is pH level, so that the pH level must be maintained at the level mentioned above by using an acid or base controller. Or it can be carried out with the use of buffer solution in the form of citrate buffer or acetate buffer to maintain the pH level of 5 with acetic acid buffer and level 4.8 using citric acid buffer [11,16].

Figure 5 shows ethanol content as functions of enzyme drops and type of enzyme with the addition of 30% sulphuric acid. Similar with the result gives in Figure 4, due to ethanol content, trichoderma enzyme does not have any power compared with beta-amylase enzyme. This is simply because in the high concentration of sulphuric acid, the role of trichoderma enzym much weaker than the role of beta-amylase enzyme. The other distinct feature in Figure 5 is that the highest ethanol content is given by 20 drops of beta-amylase enzyme. Unfortunately, trichoderma enzyme does not have any effect on the ethanol content.

As illustrated in both Figure 4 and Figure 5, this can be assigned to the fact that compared with beta-amylase enzyme, trichoderma enzyme does not have positive effect as long sugar breaker when applied in order with sulphuric acid. It can be indicated that during pre-treatment process, trichoderma enzyme inhibited the process and did not improve acid hydrolysis process.

## Conclusion

An experimental study on bioethanol production from green macro-algae Ulva lactuca has been conducted. Generally speaking, the yield of ethanol content of this study was not quite high, even lower than the result provided by the earlier researcher. However, this result still makes Ulva lactuca as a candidate for the future algal bioethanol. This is mainly due to ulva lactuca consist of glucose, available abundantly in nature, easy to cultivate, and it does not compete with food crop. Therefore bioethanol from Ulva lactuca is a challenge and further researches need to be conducted continuously on it.

Compared to high concentration of sulphuric acid, hydrolysis process with low concentration sulphuric acid gives more ethanol content. And trichoderma microbe does not support the hydrolysis process when its combine with sulphuric acid, especially with high concentration sulphuric acid.

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**INDEX Page-2.**

Next → 1 , 2 , 3

(Back to Journal section)

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