

Bioethanol: Indonesian Macro-Algae as a Renewable Feedstock for Liquid Fuel

T. Poespowati, E. Marsyahyo, R. Kartika-Dewi

Abstract—This experimental study aims at studying the conversion of macro-algae into bioethanol under several steps of procedure: preparation, pre-treatment, fermentation, and distillation. The main objective of this work was to investigate the role of buffer's type as a stabiliser of pH level and fermentation time on the yield of ethanol. For this purpose, experiments were carried out on biomass macro-algae to de-couple the pre-treatment and fermentation processes from those associated with distillation process. β -glucosidase was used as cellulose decomposer during hydrolysis step and yeast was used during fermentation process. The species of macro-algae utilised as energy feedstock was *Ulva lactuca* and it was harvested from southern coast of Central of Java Island – Indonesia. Experiments were conducted in a simple fermenter over a different buffer: citrate buffer and acetic buffer, and over a range of fermentation times between 5 to 20 days. The ethanol production was found to be significantly affected by both variables. The optimum time of fermentation was 10 days with citrate buffer; result in 0.88458% of ethanol, and the ethanol content after distillation process was shown 0.985015%.

Keywords—Fermentation, ulva-lactuca, buffer, β -glucosidase, bioethanol.

I. INTRODUCTION

RECENTLY, the world has a serious problem caused by the depletion of fossil fuel, meanwhile the demand of energy increases as the increase of population number. Billions of people around the world rely on fossil fuels for instance cars burn gasoline for energy and electricity as a form of energy for household appliances. Unfortunately, fossil fuels are non-renewable energy; they cannot be replaced easily once they are used. Burning fossil fuels releases CO_2 which leads acid rain, causes air pollution and increases global warming. Therefore dependence on fossil fuels should be reduced or might be stopped. There are many types of renewable energy sources able to replace fossil fuels; one of them is biomass, an organic material that can be converted into burnable fuel which becomes biofuel. Nowadays algae biomass farming is a growing business in coastal countries in line with the increasing demand of healthcare and food sectors based on algae. As they grow easily by using the sun, CO_2 and nutrients, and produce carbohydrates, they become one of the world most valuable and renewable fuel resources. Based on photosynthetic pigments, *Ulva lactuca* or sea lettuce is a green

algae which is classified as macro-algae with chlorophyll pigment [4]. There is very low concentration of lignin or no lignin in marine algae *Ulva lactuca*, they have higher growth rate rather than growth rate of other food crops and they do not compete with land used for food crop [5], [8]. The main biochemical compounds of algae are protein, carbohydrate and lipids. Carbohydrate content in *Ulva lactuca* is about 44.44% DW [1], 35% according to Kumar [2], and 42.31–51.37% DW as noted by Moustafa [3]. *Ulva lactuca* powder collected in Tunisia consist of 54% of fibers, these fibers comprises of hemicellulose (20.6%), cellulose (9%) and a small number lignin (1.7%) [7]. The two most biomass widely used in bioethanol production are biomass with sugar content and starch content. Meanwhile, biomass consists of lignocellulosic still needs to be developed in order to increase the efficiency of ethanol [8].

II. MATERIALS AND EXPERIMENTAL METHODS

A. Materials

Ulva lactuca was collected from Southern coast of central of Java. Firstly, washed the ulva due to released salt and other impurities, secondly dried the ulva under the sun for 4 days prior oven dried until the weight was stable, and stored the feedstock of ulva in a desiccator. Furthermore, the feedstock was grinded into 80 mesh of size in order to reach the efficiency of fermentation process. Analysis of cellulose, hemicellulose, lignin and ash content has been done on the feedstock; result in 34%, 18%, 0.7% and 1.3% DW respectively. *Saccharomyces cerevisiae* or yeast was used during fermentation process, this yeast commonly used for cassava fermentation and soybean fermentation. Beside yeast as a starter, 0.5 gram/liter of each Ureic and NPK (Nitrogen Phosphorus Potassium) fertilizers were utilized as nutrition during fermentation process. Citrate buffer and acetic buffer were used as stabilizers of pH of solution.

B. Experimental Methods

The feedstock was sent to a pretreatment reactor and to promoted hydrolysis process, the combination of steam and acid was utilized. The long cellulose chain was decomposed by using enzyme of β -glycosidase into individual and simple glucose consists of six carbons. In fermenter, glucose was fermented into cellular energy and thereby produced ethanol and CO_2 as metabolic waste product by using yeast as a microbe and nutrient. The microbe ate sugars and released ethanol as a waste product. In this step, one mole of six carbon sugar converted into two moles of ethanol and two moles of carbon dioxide in the presented of yeast. This conversion

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process was under anaerobe condition or in the absence of oxygen. After fermentation process, the solution was distilled in a distiller and the content of ethanol was analyzed by using a Gas Chromatography. The methods consisted of 6 steps as follow:

1. Pre-Treatment

There are many way to pretreat the feedstock: by using steam, hot water, or acid. This paper presents the combination of steam and acid pretreatment to promote hydrolysis process. *Ulva lactuca* was rinsed with tap water for 24 hours to release the salt; ulva was further washed three times with tap water as well due to remove the salt, sand and other impurities. Clean ulva then sun dried for 4 days, followed by oven dried until the weight of ulva become remain stable. The dry sample was grinded by using a blender and finally sieving process was carried out to produce ulva particle in 80 mesh of size. Prior experimental work, cellulose content and lignin content in the algae sample both before and after pre-treatment were analyzed by using Chesson method [10]. The result of both analyses shows in Table I:

TABLE I
CELLULOSE CONTENT & LIGNIN CONTENT BEFORE AND AFTER PRE-TREATMENT PROCESSES

	Cellulose content (%)	Lignin content (%)
Before Pre-treatment Process	12.5	6.5
After Pre-treatment Process	13	1.03

As can be seen from Table I, pre-treatment process or hydrolysis process reduced lignin content significantly, from 6.5% into 1.03%. On the other hand cellulose content remain stable, compared with the cellulose content before pre-treatment process, the cellulose content after pre-treatment process result in 0.5% increasing.

2. Acid Hydrolysis

Feedstock of ulva was hydrolysed into cellulose, lignin, and hemicellulose in the present of steam and acid in a hydrolysis reactor. 50 gram of dried ulva was added by 500 ml of 2% sulfuric acid solution, the solution was then hydrolyzed in an autoclave for 30 minutes at 121°C degree. Afterwards, glucose was analyzed on the solution.

3. Enzymatic Hydrolysis

Acid hydrolysis mentioned above was followed by enzymatic hydrolysis. First of all, NaOH was applied in order to control the pH level on 5, and then the pH level of filtrate was stabilized by using citrate buffer and acetic buffer. 0.001 ml of enzyme β -glucosidase was added into the solution and then incubated it for 42 hours at 40°C degree. Afterwards, glucose was analyzed on the solution. The long chain of cellulose was decomposed by the enzyme into short cellulose chain then the short chain was decomposed into cellobiose fragments, and finally the cellobiose fragment will be broken into individual glucose.

4. Starter Preparation

Due to increased the number of yeast, and adjustment situation during fermentation process can be maintained properly, starter was prepared prior to fermentation. For fermentation process, yeast should be able to grow quickly and produces enzyme for glucose conversion into ethanol. Ulva powder was mixed with water and took 5 ml of the mixture; pH of starter need to be adjusted and yeast was added in an amount of 5% by weight of sample.

5. Fermentation Process

Starter was added into hydrolyzed solution, followed by the addition of nutrients with the amount as mentioned above. Together with yeast, nutrients and antifoam, the glucose was sent into a fermenter tank. Fermentation was running under anaerobe conditions and under the certain period of time. Furthermore, ethanol content was analyzed on the sample after fermentation process. The fermentation reaction of glucose to ethanol is as follows:



6. Distillation Process:

The whole procedures were ended by distillation work under temperature of 80°C degree (slightly higher than the boiling point of ethanol), ethanol will be separated from the solution and removed and finally ethanol content was analyzed by using GC.

The experimental method is outlined in Fig. 1:

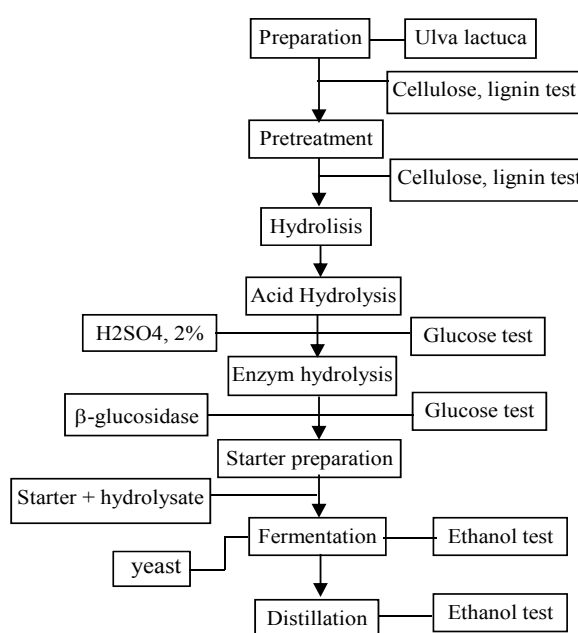


Fig. 1 Experimental method chart

III. RESULTS AND DISCUSSIONS

The growth curve of *Saccharomyces cerevisiae* at 10^{-6} of dilution was investigated by using a spectrophotometer. Adaptation phase occurred at the first hour. After the

adaptation phase, logarithmic phase or growth phase happened at the second hour, during this phase starter was mixed with fermentation media. The logarithmic phase did not follow by stationer phase, decay phase occurred instead. It means that there was no more cell division. The growth curve is figured out in Fig. 2:

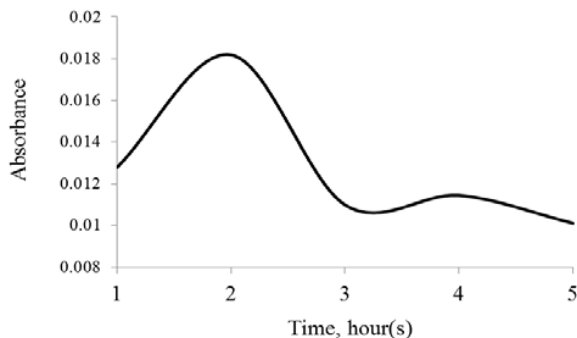


Fig. 2 *Saccharomyces cerevisiae* growth rate

Table II and Fig. 3 provide a summary of the glucose content after hydrolysis processing on samples. Two different types of hydrolysis process were conducted; they were acid hydrolysis and enzymatic hydrolysis. Two kinds of buffer were applied during enzymatic hydrolysis (acetic buffer and citrate buffer with pH levels of 5 and 4.8 respectively). pH levels of acid hydrolysis was 0.

TABLE II
GLUCOSE CONTENT AFTER HYDROLYSIS PROCESSES

Hydrolysis	Buffer	Volume ratio (acid:buffer)	pH	Glucose content (%)	Glucose content (g/kg ulva)	Yield (%)
Acid	-	-	0	34.1425	341.425	26.3234
Enzyme	Acetic	1:0.67	5	63.5305	635.305	40.9157
Enzyme	Acetic	1:1	5	67.4895	674.895	42.5222
Enzyme	Acetic	1:1.33	5	63.9360	639.360	41.0836
Enzyme	Acetic	1:1.67	5	68.9875	689.875	43.1120
Enzyme	Citrate	1:0.67	4.8	68.9875	689.875	43.1120
Enzyme	Citrate	1:1	4.8	61.6075	616.075	40.1094
Enzyme	Citrate	1:1.33	4.8	61.9875	619.875	40.2702
Enzyme	Citrate	1:1.67	4.8	18.0765	18.0765	1.7797

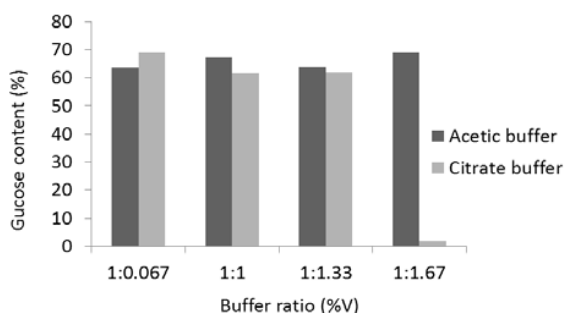


Fig. 3 Glucose content after hydrolysis processes

As can be seen from Table II and Fig. 3, the average of glucose content by using acetic buffer and citrate buffer were

65.9859% and 48.5975% respectively. These glucose contents were much higher than the glucose content founded by Yazdani [9] and Wang [6] which is only about 7.02% by weight. The high result of glucose content was primarily caused by the role of buffers as pH stabilizers of enzyme were very effective, therefore enzyme successfully broke down the linkage of glucose into individual glucose. The enzyme was activated optimally under the temperature of 42°C degree.

TABLE III
ETHANOL CONTENT AFTER FERMENTATION PROCESSES

Buffer	Ratio	Fermentation time, days	Ethanol content (%)	Ethanol content (g/kg alga)	Yield (%)
Acetic	1:1.67	5	2.18038e-1	2.18038	0.2176
Acetic	1:1.67	10	3.42291e-1	3.42291	0.3411
Acetic	1:1.67	15	6.66047e-1	6.66047	0.6617
Acetic	1:1.67	20	5.02292e-1	5.02292	0.4998
Citrate	1:0.67	5	3.01486e-1	3.01486	0.3006
Citrate	1:0.67	10	8.84580e-1	8.84580	0.8769
Citrate	1:0.67	15	6.35473e-1	6.35473	0.6315
Citrate	1:0.67	20	4.70724e-1	4.70724	0.4685

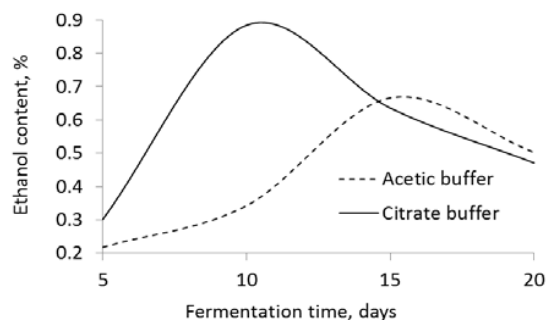


Fig. 4 Ethanol content after fermentation processes

Table III and Fig. 4 show the result of ethanol content after the variety of fermentation times. The highest percentage of ethanol was resulted by 10 days and 15 days of fermentations for citrate buffer and acetic buffer respectively. This simply because longer life time of yeast was under the pH level of 5, on the other hand yeast able to produced ethanol optimally under the pH level of 4.8.

The low result of alcohol content after fermentation process might be caused by the bad control of pH level during fermentation process; as the effect, the glucose could not be converted into ethanol by the microbe optimally. Moreover the sugar content after hydrolysis process was very high; therefore the yeast was not strong enough to eat the all sugar. The optimum condition of sugar content for fermentation process is about 14-18%.

TABLE IV
ETHANOL CONTENT AFTER DISTILLATION PROCESSES

Buffer	Fermentation time, days	Ethanol content (%)	Ethanol content (g/kg alga)
Acetic (1:1.67)	15	9.85015e-1	9.85015
Citrate (1:0.67)	10	4.72396e-1	4.72396

As can be seen in Table IV, the ethanol content after distillation process increases from 0.6604% becomes 0.985015% for acetic buffer; contrary, the ethanol content after distillation process decreases significantly from 0.88458% into 0.472396%. General point of view, the result of ethanol content after distillation process was only nearly 1%. Ethanol was analyzed by using GC. This is much lower than 4% to 5% as the standard of economically feasible in ethanol production [8]. It was indicated that there was not enough amount of yeast during fermentation process, the yeast died before all of sugar converted into ethanol successfully. Another problem might happen was the number of viable cells of the yeast decreased by the time start from the packaging procedure, or the yeast was not really fresh anymore caused by the lose yeast cells due to age.

IV. CONCLUSION

- When biomass grows at the different habitat, the photosynthesis process will produce different compounds of carbohydrate; therefore different source of biomass gives different ethanol content.
- In hydrolysis process, both citric buffer and citrate buffer give the same glucose content
- The best ratio of buffer is 1:1.67 for citrate buffer and 1:0.67 for citrate buffer
- The best fermentation time were 10 days for citrate buffer, and 15 days for acetic buffer
- In order to get a perfect fermentation, the amount of yeast should be enough.
- The condition of yeast must be really fresh; therefore the number of viable cells of the yeast will suitable with the number of sugar that will be converted into ethanol.

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