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Bioethanol from sargassum sp using acid hydrolysis and fermentation method using microbial association

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[a] Biology Education of Sanata Dharma University, Indonesia

Abstract

Sargassum sp is one of the abundant seaweed in Indonesian seas and it has the potential to be used as a renewable alternative energy source. Dry Sargassum sp contains 58.23% carbohydrate that can be used as bioethanol through a fermentation process. This research aims to determine the effect of fermentation time on the amount of ethanol content produced from Sargassum sp fermentation .The type of this study was laboratory experiment. The experiment was conducted by using microbial association namely Zymomonas mobilis, Tape Yeast and Bread Yeast with variation of fermentation duration of 4 days, 5 days, 6 days, and 7 days. Glucose test is done by using the DNS method . The measurement of ethanol content is done by using Gas Chromatography. In the data analysis the researcher uses correlation regression statistic method . The result of the research shows that the best treatment was 6 days fermentation duration with the amount of ethanol 24.67% with the reducing sugar content 54,570 ppm. The longer the fermentation time the higher the ethanol content produced except on the 7th day showing decline. The results of data analysis showed that the difference in fermentation time to ethanol content produced had a correlation coefficient that was positive and there was no significant relationship. © Published under licence by IOP Publishing Ltd.

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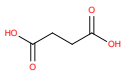

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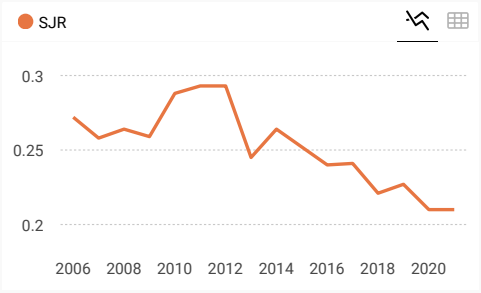

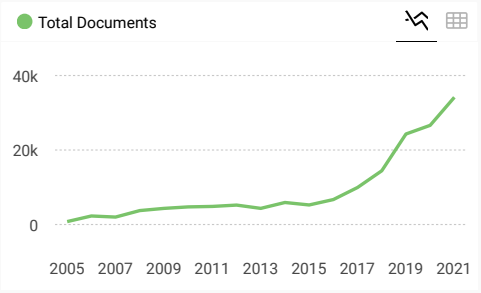

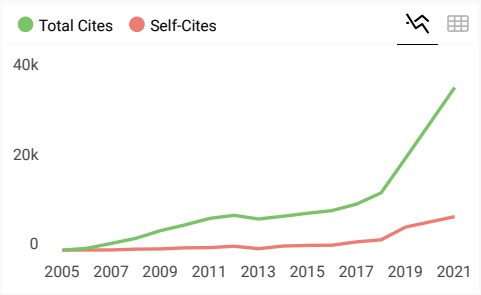

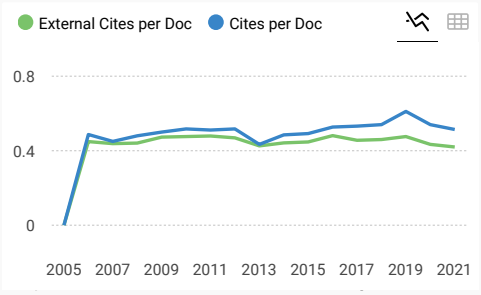

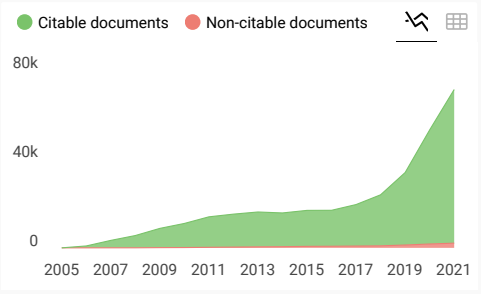

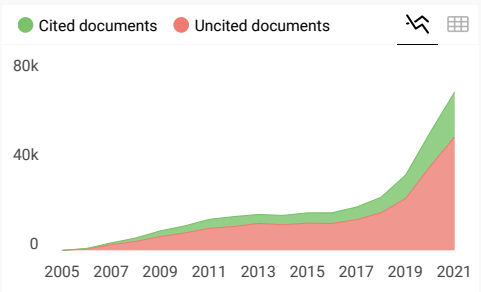

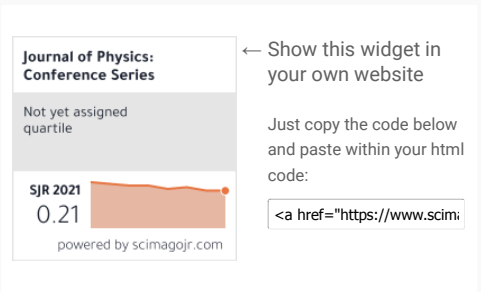

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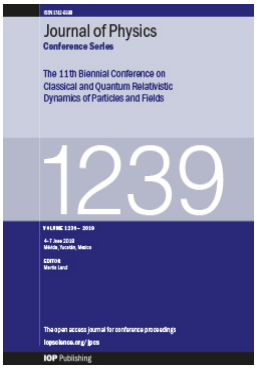

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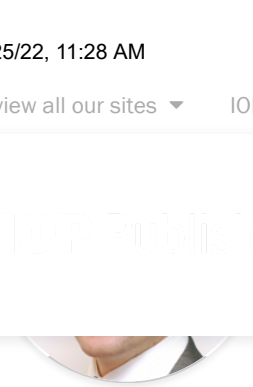

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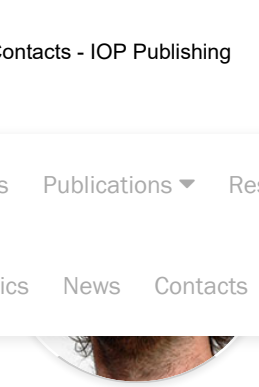

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Bioethanol from *Sargassum sp* Using Acid Hydrolysis and Fermentation Method Using Microbial Association

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Biology Education of Sanata Dharma University

Anggraenikusuma04@gmail.com

Abstract. *Sargassum* sp is one of the abundant seaweed in Indonesian seas and it has the potential to be used as a renewable alternative energy source. Dry *Sargassum* sp contains 58.23% carbohydrate that can be used as bioethanol through a fermentation process. This research aims to determine the effect of fermentation time on the amount of ethanol content produced from *Sargassum* sp fermentation.The type of this study was laboratory experiment. The experiment was conducted by using microbial association namely *Zymomonas mobilis*, Tape Yeast and Bread Yeast with variation of fermentation duration of 4 days, 5 days, 6 days, and 7 days. Glucose test is done by using the DNS method. The measurement of ethanol content is done by using Gas Chromatography. In the data analysis the researcher uses correlation regression statistic method. The result of the research shows that the best treatment was 6 days fermentation duration with the amount of ethanol 24.67% with the reducing sugar content 54,570 ppm. The longer the fermentation time the higher the ethanol content produced except on the 7th day showing decline. The results of data analysis showed that the difference in fermentation time to ethanol content produced had a correlation coefficient that was positive and there was no significant relationship.

1. Introduction

Fossil energy needs such as gasoline, diesel, coal are increasing every year. This is in line with economic growth, industry, population growth and scientific development. The increasing need for fossil energy is not proportional to the availability of fossil energy in nature. Fossil energy is non-renewable and causes pollution which results in global warming. Therefore, an alternative to environmentally friendly renewable energy is needed. One solution to overcome the crisis problem of energy sources is to use bioethanol. Bioethanol is ethanol produced from biomass which contains sugar, starch, or cellulose. Bioethanol is a renewable and environmentally friendly energy that can be used as a substitute for fossil fuels. Bioethanol is the main choice because it is easy to decompose, is safe for the environment, and burning of bioethanol only produces carbon dioxide and water [1]. Bioethanol has a clean energy predicate because it can reduce carbon dioxide production by up to 18% [2].

The source of biofuel (bio-fuel) has been derived from starch biomass which is also a source of food and feed so there is competition between the two [3]. Indonesia is a country with megabiodiversity so that it has great potential to develop and process its natural resources, supported by two-thirds of the total area of Indonesia which is water. One alternative is to use macroalgae as raw material to be used as bioethanol. Algae are one of the marine biological natural resources that have economic value and have a role and benefit to the surrounding environment. The main component in seaweed is carbohydrates (sugar

and gum). This component has the potential to be converted into bioethanol. One of the seaweed that can be used to make bioethanol is *Sargassum* sp.

Sargassum sp is one type of brown seaweed in Indonesia which has economic value, has a relatively short harvesting age, is widely distributed in Indonesian waters with a high production potential, but the production is still a lot derived from the harvest of natural supplies [4] . *Sargassum* sp has cellulose content which ranges from 23.97 - 35.22% [5]. High cellulose content in *Sargassum* sp is one of the potential to be used as raw material for bioethanol production as a renewable energy source. Raw materials for bioethanol production processes can be classified into three groups, namely starch, sugar and cellulose. The source of cellulose must be converted to sugar through the process of hydrolysis. Hydrolysis can be done either chemically using acid and enzymatically.

In this study using hydrolysis chemically with HCl acid. After the hydrolysis process, the fermentation process is continued. Ethanol fermentation is the activity of breaking down sugar into ethanol by releasing CO_2 gas, this fermentation is carried out using a microbial association namely *Zymomonas mobilis*, *Saccharomyces cerevisiae* found in yeast tape and bread yeast. Based on the problems and data that have been described, the researchers conducted a study entitled The Effect of Fermentation Time on Bioethanol Making from *Sargasum* sp Using Acid Hydrolysis Methods and Fermentation Using Microbial Associations (*Zymomonas Mobilis*, *Saccharomyces cerevisiae* in Tape Yeast and Bread Yeast).

2. Materials and Methods

Materials

The tools used in this study were incubators (Memmert), shakers (Langzaam type I SW09), waterbaths, fermentation bottles, UVmini 1240 (SHIMADZU) spectrophotometers, pH meters, hot plates, blenders, autoclave (GEA YX-2800 models) , digital scales (ACIS), destilators, gas chromatography, ovens, test tubes, erlenmeyer, ose needles, volume pipettes, spatulas, filters, glass cups 1 L, stoves, measuring cups, gloves, thermometers, stirrers, filter paper, aluminum foil.

The ingredients used in this study were dried seaweed *Sargassum* sp taken from Indrayanti Beach, Yogyakarta, pure culture of *Zymomonas mobilis* bacteria from PAU UGM, *Saccharomyces cerevisiae* found in bread yeast and instant tape yeast, hydrochloric acid (HCl), NA (Nutrient Agar), aquades, Dinitrosalicylic Acid (DNS), KOH, NB (Nutrient Broth), glucose, NPK, Urea, yeast extract, K_2SO_4, $MgSO_4$. 7H2O and$(NH_4)_2SO_4$.

Methods

This study was included in the research experiment, where changes were made (there were special treatments) to the treatments studied. In this treatment variation of fermentation time is 4 days, 5 days, 6 days and 7 days. *Saccharomyces cerevisiae* used is found in bread yeast and tape yeast. The volume of *Zymomonas mobilis* bacterial inoculum used was 10 ml, the volume of *Saccharomyces cerevisiae* in bread yeast and tape yeast were 5 ml each. Based on the place of research, this research is included in laboratory research. This research was conducted in March-May 2018 at the Biology Laboratory, Biology Education and the Laboratory of Chemical Biochemistry, Pharmacy, Sanata Dharma University, Yogyakarta. Testing of glucose levels is done using the DNS method. Measurement of ethanol levels was carried out using Gas Chromatography. Data analysis using correlation regression statistical method.

This research is divided into several stages, namely sample preparation (making seaweed pulp), chemical hydrolysis with HCl acid, measurement of glucose levels with spectrophotometer, *Zymomonas mobilis* bacterial cultivation, manufacturing of *Saccharomyces cerevisiae* nutrients in tape yeast and bread yeast, fermentation, distillation, measurement ethanol content with Brix-refractometer and measurement of ethanol levels using Gas Chromatography.

2.1 Sampel Preparation

Seaweed is taken from the coastal waters of Indrayanti, Gunung Kidul, Yogyakarta in February 2018. Seaweed is dried and mashed using a blender and sieved using a 100 mesh sieve, so that seaweed flour is obtained.

2.2 Acid Hydrolysis

Acid hydrolysis aims to convert the carbohydrates contained in the sample into glucose. Acid serves as a catalyst that is to accelerate the hydrolysis process. The catalyst used in this study is HCl. 1% HCl dilution was carried out in Erlenmeyer. A total of 20 grams of sample added to the erlenmeyer containing 200 ml of 1% HCl were put into an autoclave for 10 minutes at 121 ° C.

2.3 Measurement of Glucose Levels

Measurement of glucose levels was carried out by means of a sample of hydrolysis added 70% alcohol with a ratio of 1: 1. The sample is filtered using filter paper, and the remaining solids are washed with alcohol until neutral. The filtrate is heated on a 100 ° C water bath for 30 minutes then filtered. A total of 3 ml of the sample was put into a test tube and 3 ml of DNS reagent. Then the sample and reagent are placed in a boiling water bath for 5 minutes. Glucose levels were measured by spectrophotometer at a wavelength of 540 nm.

2.4 Zymomonas mobilis Cultivation

Zymomonas mobilis bacterial pure culture was recultured on the zig zag agar slope at 30 ° C for 24 hours on NA (Nutrient Agar) media. To enrich the number of cells in the fermentation process, NB (Nutrient Broth) liquid media is made. NB is made by 8 grams of NB dissolved in 1000 ml of distilled water. NB is then sterilized at 121 ° C for 15 minutes in the autoclave. Five cells of *Zymomonas mobilis* cells from reculture were put into 100 ml of NB and shaker for 24 hours at a speed of 120 rpm at 30 ° C.

2.5 Manufacturing Of Saccharomyces Cerevisiae Nutrients In Tape Yeast And Bread Yeast

Nutrition making aims to meet the needs of microbial nutrition so that it can carry out the fermentation process to produce ethanol. Making nutrients for tape yeast and bread yeast is different according to the type of microbes. A total of 1.4 g of yeast tape was grown on a medium consisting of glucose 10 g / l, yeast ekstract 1 g / l, $K_2SO_4$0.1 g / l, $MgSO_4$. 7H2O 0.1 g / l and $(NH_4)_2SO_4$ 0.1 g / l in an erlenmeyer 200 ml. Incubation is carried out on a shacker at a speed of 90 rpm at room temperature for 24 hours before being inoculated. 0.5 g of bread yeast was grown on a medium consisting of 10 g / l glucose, NPK 0.3 g and Urea 0.08 g in a shaker with a speed of 90 rpm at room temperature for 24 hours.

2.6 Fermentation

The fermentation process was carried out by adding 200 ml of hydrolyzate as a result of hydrolysis and then regulating the pH using 20% KOH solution at PH 5 and adding 10% (v / v) *Zymomonas mobilis* inoculum, 5 ml of tape yeast, 5 ml of bread yeast and incubated with old variations fermentation time 4 days, 5 days, 6 days and 7 days.

2.7 Distillation

The distillation process is carried out to obtain bioethanol with a higher level of purity. The distillation process is carried out using steam distillation at a temperature of 78 - 80 ° C for 1 hour. Results analysis using gas chromatography (GC).

2.8 Measurement of ethanol levels using Brix-refractometer

Ethanol measurement using Brix-refractometer aims to measure temporary ethanol levels to select samples with the highest ethanol content which is then tested using gas chromatography (GC).

2.9 Measurement of Ethanol Levels

The ethanol content of the distillation was tested using gas chromatography (GC) at

the Organic Chemistry Laboratory, Gadjah Mada University.

3. Results and Discussion

3.1 Acid hydrolysis and glucose level testing

Reduction sugar test was carried out using a UV-Vis spectrophotometer (SHIMADZU) with a wavelength of 540 nm. The standard curve of glucose solution was made to determine the reduction glucose level in the sample.

Table 1. Absorbance of standard solutions

Glucose (ppm)	Absorbance
0	0
5	0,25
6	0,299
7	0,353
8	0,398
9	0,441

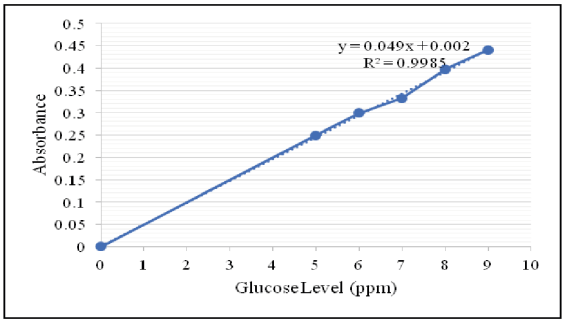


Figure 1. Glucose standard curve

Table 2. The results of glucose levels

Hydrochloric acid (%)	Time (minute)	Absorbance	Glucose level (ppm)
1	10	3,612	54.570

The use of hydrolysis of 1% HCl acid with a time of 10 minutes can produce a reduced sugar content of 54,570 ppm where the sugar will be converted to ethanol and CO2 during the fermentation process. Hydrolysis aims to break down carbohydrates into simple sugars so that they can be used in the fermentation process. Acid hydrolysis is used where acid hydrolysis has advantages compared to enzyme hydrolysis. In this study HCl acid was used as a catalyst in the hydrolysis process. HCl catalyst produces higher glucose than H2SO4. This is caused by H_2SO_4 is burning cellulose while HCl is not so that the use of HCl catalyst is more optimal in producing reducing sugar [6]. Acid hydrolysis was carried out with 1% HCl on autoclave 121 ° C 1 atm pressure with hydrolysis time for 10 minutes. Based on Prastika's research, in the study of *Sargassum* sp using hydrolysis of 1% HCl acid on auotoclave for 10 minutes to get the most optimal reduction sugar results [7].

Both *Zymomonas mobilis* and *Saccharomyces cerevisiae* can live in the pH range 4-7. So in the fermentation process it is necessary to add bases, in this study using 20% KOH. With the addition of 15 ml of KOH as much as 15 ml can change the pH of the sample from pH 1 to pH 4. The factors that influence the perfection of hydrolysis in addition to acid concentration are temperature and heating time [8]. Where in this study using dilute acid hydrolysis at high temperatures. If the hydrolysis temperature is too high or the hydrolysis time is too long then the formed monosaccharides can be further hydrolyzed into other materials. The main dissolved components in the final hydrolysis results are xylose, arabinose, glucose, galactose, mannose, hydroxymethyl furfural, and organic acids such as formic acid and acetic acid [9]. This can be proved by the pH of the results of hydrolysis samples having a pH of 1 (very acidic). The following stages of hydrolysis that occur chemically:

$$(C_6H_{10}O_5)_n + n\,H_2O \xrightarrow{HCl} n(C_6H_{12}O_6)$$

3.2 Fermentation and ethanol level testing

The fermentation results are distilled with a temperature of 78 - 80 ° C for approximately 1 hour. The results of the distillation were tested with Brix-refractometer to select the samples to be tested using Gas Chromatography (GC). Samples with the highest ethanol content were then tested using Gas Chromatography (GC) as in the following table:

Table 3. The results of ethanol content in the sample

No	Long Fermentation	Ethanol Level
1	Days 4	15,56 %
2	Days 5	16,70 %
3	Days 6	24,67 %
4	Days 7	19,81 %

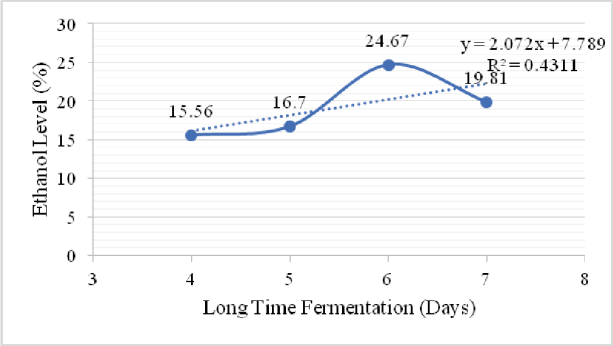


Figure 2. The Effect of fermentation time on ethanol levels

The graph above shows the effect of fermentation time on the ethanol content produced. The longer the fermentation time, the higher the ethanol content produced. On the 4th day of fermentation until the 6th day fermentation continues to increase ethanol levels. But on the 7th day fermentation experienced a decrease in ethanol levels.

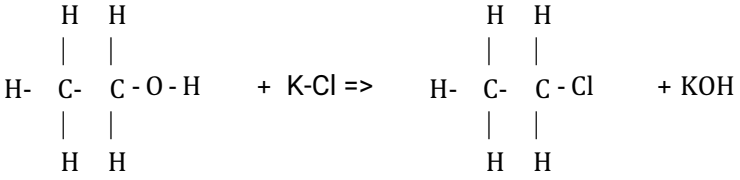
Fermentation is the process of breaking down glucose into ethanol and CO_2. Fermentation is carried out with a variation of time 4 days, 5 days, 6 days and 7 days. In this study using microbial associations (*Zymomonas mobilis*, tape yeast and bread yeast). *Zymomonas mobilis* is widely used in bioethanol companies because it has several advantages in various aspects. Able to grow facultatively anaerobically, can withstand high temperatures, the ability to achieve higher conversion, resistant to high ethanol levels and able to withstand low pH conditions. In tape yeast and bread yeast there is *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* is one of the most commonly used yeasts in ethanol fermentation which can produce high ethanol levels. *Zymomonas mobilis* and *Saccharomyces cerevisiae* have a period of fermentation for 4-7 days so the variation of fermentation time between the 4th day and the 7th day is used to determine the optimal time for ethanol formation from these microbial interactions.

Fermentation of *Saccharomyces cerevisiae* with Embden-Meyerhoff Parnas metabolic pathway (EMP). Whereas *Zymomonas mobilis* uses the Entner-Doudoroft (ED) line. Both microorganisms contain highly efficient homoethanol cycles, which convert pyruvate to acetaldehyde using pyruvate decarboxylase (PDC), then to ethanol using alcohol dehydrogenase (ADH). The existence of microbial interactions of these associations can produce more optimal ethanol. *Zymomonas mobilis* and *S. cerevisiae* can live in the same pH range of 4-7, temperature 27-30 ° C, tolerant to high ethanol so that it can work simultaneously to form ethanol. Based on the results of ethanol content testing using Gas Cromatography (GC), the highest results were obtained on the 6th day fermentation. Ethanol content test results can be seen on figure 2 that the longer the fermentation time, the higher the ethanol content produced. However, on the 7th day of fermentation, ethanol levels decreased due to depletion of available nutrients and both *Zymomonas mobilis* and *Saccharomyces cerevisiae* decreased productivity.

Figure 2 shows the effect of fermentation time on ethanol content produced. The longer the fermentation time, the higher the ethanol content produced. On the 4th day of fermentation until the 5th day of fermentation, ethanol levels continue to rise. However, on the 7th day fermentation experienced a decrease in ethanol levels because the productivity of microbes decreased and nutrients had begun to run out. In addition, ethanol fermentation produces ethanol as the main product and by-products such as carbon dioxide and organic acids such as pyruvic acid, succinic acid, lactic acid and other acids. These acids are produced as a by-product which makes the pH of the solution lower where the final pH after 7th day fermentation is 2. The line Y equation shows that the longer the fermentation time, the higher the ethanol content produced except on the 7th day fermentation which decreased. The increase in ethanol levels by 2.072-fold or an increase of 7.2% with the level of ethanol at the beginning of the 4th day to the 6th day respectively were 15.56% and 24.67% and decreased on the 7th day to 19.81 %.

Correlation value r = 0.657 indicates that the two variables have a strong and positive relationship. The value of R = 0.4311 means that the ethanol content is influenced by the fermentation time of 43.11% and the remaining 56.89% by other variables.

56.89% of the other variables that also influence the formation of ethanol are the excess of KOH in fermentation, the amount of nutrients decreasing and the formation of organic acids as a result of microbial metabolites at the end of fermentation. KOH when reacting with ethanol will produce alkyl halides as a consequence of the presence of halogen elements (Cl) used, OH in the ethanol chain will change with Cl element so that the ethanol chain is no longer pure and affects the ethanol content at the end of fermentation. With a microbial growth curve that decreases at the end of fermentation followed by decreasing available nutrients so that ethanol production decreases. In ethanol fermentation produces ethanol as the main product and by-products such as carbon dioxide and organic acids such as pyruvic acid, succinic acid, lactic acid and other acids. These acids are produced as a by-product which makes the pH of the solution lower.



4. Conclusion

Sargassum sp can produce ethanol levels on 4-7 days, respectively 15.56%, 16.70%, 24.67% and 19.81%. The longer the fermentation time, the higher the ethanol content produced with the highest ethanol content of 24.67% in the fermentation time of 6 days. While on the seventh day the amount of ethanol was decreased by 19.81%.

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Certificate

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This is to certify that

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has participated in

International Seminar on Bioscience and Biological Education (ISBBE)

organized by Department of Biology Education, Faculty of Mathematics and Natural Science, Universitas Negeri Yogyakarta, Indonesia on 29th–30th October 2018

as a

SPEAKER PARTICIPANT

with the paper entitled:

Bioethanol from Sargassum sp Using Acid Hydrolysis and Fermentation Method Using Microbial Association

Yogyakarta, October 30, 2018

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