The Effect of Differentiation of Hydrolysis Time towards Ethanol Levels Produced Through *Ulva Lactuca* Fermentation

IOP Conf. Series: Journal of Physics: Conf. Series **1241** (2019) 012010 doi:10.1088/1742-6596/1241/1/012010

The Effect of Differentiation of Hydrolysis Time towards Ethanol Levels Produced Through *Ulva Lactuca* Fermentation

Hendrika Micelyn Amelia Ngamput, Retno Herrani

micelynamelia07@gmail.com
Sanata Dharma University, Indonesia

Abstract. Indonesia as one of the largest maritime territories in the world has potential of marine products which can be used as the alternative source of bioethanol substrates, known as macroalgae. *Ulva lactuca* can be used as the substrates because it contains 16.42% hemicellulose and 19.58% cellulose. The acid hydrolysis stage is required to damage the cellulose structure and break down the hemicellulose to simple materials that can be converted by microbes. The purpose of this study was to determine the effect of acid hydrolysis time on ethanol levels produced from *Ulva lactuca* and to determine which of the treatments can produce the highest level of ethanol. This research was an experimental research with four kinds of treatment time of acid hydrolysis: 15, 30, 45, and 60 minutes. The fermentation was done with the help of microbes isolated from bread yeast and *tape* yeast. The sample products were distilled and tested for the ethanol content. The data obtained were then analysed using one-way ANOVA test. The results showed that the acid hydrolysis time have a significant effect on the ethanol levels produced from *Ulva lactuca*. The highest ethanol level was resulted from 60 minutes hydrolysis time, which was 13.17%.

Keywords: *Ulva lactuca*, *acid hydrolysis, bread yeast,* tape *yeast, ethanol.*

1. Introduction

The world's needs of energy is increasing as the population grow every year. Almost all sectors of human life need energy to function. Until now, the energy is still supplied from fossil fuels, which source is unrenewable and not environmentaly friendly. The bursting process of fossil fuels produce greenhouse gases such as carbon dioxide (CO_2), sulphur dioxide (SO_2), nitrogen oxide (NO_x)[1].

The global warning issue has been affecting many countries to start using alternative energy sources such as water, wind, and another ecofriendly sources to replace the use of fossil fuels. As a maritime country with 5.4 million km^2 total of sea area, the marine products are definitely potential to replace the use of fossil fuels as the source of energy. One of the potential products is macroalgae. Macroalgae is a multicellular algae group whose body is in the form of a thalus and does not have true roots, stems and leaves. This group of plants lives in marine waters that still get sunlight by sticking to a hard substrate[2]. Chlorophyta or green algae is one of the largest groups of algae with high species diversity. One of the species is *Ulva lactuca*.

The potential of this macroalgae has not been maximally utilized by the people who live around the area. Generally, *Ulva* sp. has only been processed as food product for sale, considering that Gunung Kidul is a tourism area in Special Region of Yogyakarta, Indonesia. Based on research on the chemical composition of green seaweed conducted by Santi *et al.* (2012)[3], it is known that *Ulva lactuca* contains

hemicellulose 16.42%, cellulose 19.58%, and lignin 2.9%. The hemicellulose and cellulose content makes *Ulva lactuca* potential to be used as bioethanol raw material through a fermentation process.

Bioethanol is ethanol derived from biological sources. According to John (2004)[4] bioethanol can be used as an alternative fuel because it is environmentally friendly, contains lower CO gas (19-25%)[5] and has the advantage of being able to reduce CO_2 emissions by up to 18% compared to emissions of fossil fuels like kerosene. The process of producing bioethanol requires several stages, such as pretreatment, hydrolysis, fermentation and distillation. In most cases, bioethanol production in Indonesia only uses raw materials such as cassava, sugar cane, sweet potatoes, and corn. These raw materials are included in food crops which cultivation is still limited, slow, requires extensive land and can lead to competition with human food needs. This has an impact on bioethanol production that has not been optimal[6]. This condition is a challenge as well as an opportunity to make new innovations by looking for alternatives of other raw materials which availability is abundant. Macroalgae is one type of raw material that has the potential to be used as raw material in ethanol production because of its abundant availability.

The fermentation of bioethanol generally uses *Saccharomyces cerevisiae*. The main reason of using this yeast is because it has several advantages, which are more adaptable to the environment, more resistant to high alcohol, and more easily obtained[7]. The use of S. *cerevisiae* is more profitable than *Zymomonas mobilis* because it is more easily maintained physiologically with high conversion power[8]. S. *cerevisiae* can be obtained from commercial bread yeast. Bread yeast contains S. *cerevisiae* which has been selected, mutated or hybridized to increase its ability to ferment sugar well and be able to grow quickly[9]. To optimize the resulting ethanol content, other microorganisms found in *tape* yeast are added. In *tape* yeast there are microorganisms that produce amylolytic enzymes such as *Rhizopus* sp., *Aspergillus* sp., *Mucor* sp., and *Bacillus* sp. The enzyme is able to break the bonds of amylose and amylopectin to glucose[10]. Glucose is then converted to ethanol during the fermentation process.

In this study, the acid hydrolysis stage was first carried out using strong HCl to break down the polysaccharide contained in *Ulva lactuca*, which known as cellulose. The perfect hydrolysis of cellulose will produce cellulose monomers, glucose. Glucose will be converted by S. *cerevisiae* to ethanol. Referring to previous research conducted by Kurniawan *et al.* (2014)[11] regarding the Effect of Yeast Tape and Bread Yeast Interactions on Bioethanol Levels of Tree Cassava (*Manihot utilissima* Pohl) Mukibat varieties prove that the second combination of yeast is effective in producing bioethanol levels up to 49.8%. In addition, the research of Utami, *et al.* (2014)[12] concerning the Effect of Hydrolysis Time and Acid Concentration on Hydrolysis of Potato Starch with Acid Catalyst showed that the time of hydrolysis and acid concentration had an effect on increasing glucose levels. The longer the hydrolysis time, the higher the glucose level produced. Based on those researches, the researcher varied the length of acid hydrolysis time on bioethanol production from *Ulva lactuca* to determine its effect on the ethanol content produced by the help of microorganisms from bread yeast and *tape* yeast during the fermentation process.

2. Materials and Methods

2.1. Materials

The equipment used in this study were autoclaves, blenders, filters, measuring cups, beaker cups, funnels, magnetic stirrers, analytic scales, erlenmeyer 500 ml, erlenmeyer 100 ml, test tubes, drop pipettes, volume pipettes, thermometers, measuring flasks, bottles fermenters, distillation flasks, heating mantles, test tube shelves, universal indicators, bunsen burners, spatulas, stirring rods, stoves, aluminum foils, buckets, pans, UV-vis spectrophotometers, refrigerators, rubber hoses and digital cameras.

The materials used in this study were *Ulva lactuca*, fermipan, *tape* yeast, aquades, 96% alcohol, 1% HCl, 10% NaOH, urea, NPK, $K_2Cr_2O_7$, sugar, cover plugs, plastic wrap, umbrella paper, label paper, gauze, tissue, plasticine, gloves.

2.2. Methods

Processing

Dried macroalgae was taken from Krakal Gunung Kidul Beach, Special Region of Yogyakarta, Indonesia. Before being given the initial treatment, the macroalgae sample was washed with fresh water to remove dirt that is still attached to the surface of the body. The sample was then dried under the sun to remove the water content. Drying time depends on sun intensity but generally between 2-3 days. Pretreatment was done physically by blending the ingredients. Then sieved with a filter to obtain macroalgae flour.

Starter Preparation

The starter used was commercial bread yeast, Fermipan, and tape *yeast*, each of them was grown in growth media. The growth medium consisted of 500 ml of sterile distilled water with 50 grams of granulated sugar (10% concentration) prepared in a 500 ml erlenmeyer size (modified from Elevri and Putra, 2006)[13]. In addition, as much as 0.4 g/l of urea and 0.5 g/l of NPK were added to each medium as a nutrient for microorganisms[14]. After all the ingredients were added, then homogenized using a magnetic stirrer for 10 minutes then sterilized using an autoclave at 121°C for 15 minutes. After it reaches a temperature of 30-33°C, as much as 5% (v/v) of bread yeast and 5% of tape yeast are put into each media. Then incubated at 30°C for 24 hours in the incubator (modified from Tipteerasri *et al*., 2009 in Azizah, *et al.,* 2012)[7].

Acid Hydrolisis

A total of 30 grams of macroalgae flour was put into a 500 ml fermenter bottle and then added 300 ml of 1% HCl then hydrolyzed in an autoclave at 121 °C for 15, 30, 45, and 60 minutes according to each treatment. This stage was carried out in the Pasteur Laboratory, Sanata Dharma University. After hydrolysis finished, the pH of each medium was set to 5 by adding 10% NaOH. The addition of NaOH is also intended to detoxify the toxic content produced during the hydrolysis process. pH was measured using a universal indicator.

Reducing Sugar

In this study the presence of reducing sugar was measured qualitatively using the Fehling test. As much as 1 ml of the solution resulting from hydrolysis of each treatment was put in a test tube and 1 ml of Fehling A and Fehling B solution was added to each test tube. Then the test sample was heated in boiling water for 1 minute. The color changes that occur were observed and recorded.

Fermentation

In each treatment, the hydrolyzed slurry was added aseptically with 10% starter (v/v) consisted of starter from bread yeast and *tape* yeast and then stirred using a shaker for 10 minutes. After that, each erlenmeyer was connected with a rubber hose. The end of the hose were inserted into the water to avoid direct contact with air. Then fermented for 3 days at room temperature. Fermentation was carried out in the Pasteur Laboratory, Sanata Dharma University.

Distillation

This stage was carried out in Wallace Laboratory, Sanata Dharma University. The distillator was assembled by connecting a distillation flask with a condenser, which is also attached to the top and bottom of the hose. A bent pipe was installed at the end of the condenser and the end of the bent pipe that has been installed, was inserted in the erlenmeyer to accommodate the results of the distillation. The fermented liquid sample is put into a distillation flask and closed using a cork. The distillation flask that has been closed was installed under the heating mantle to be heated. Heating temperature was maintained at a temperature of 70-80°C. This process is carried out until it produces 5 ml of distillate liquid. The liquid from the distillation was then tested for ethanol content in the CV Laboratory. Chem-Mix Pratama, Bantul, Special Region of Yogyakarta.

3. Result and Discussions

3.1. pH measuring

Table 1. The Results of pH Measuring

Treatment	Final pH			The Average pH
	Replication 1	Replication 2	Replication 3	
A	3	3	1	2.3
B	3	3	3	3
C	4	3	4	3.67
D	3	3	3	3

A: Hydrolysis time 15 minutes
B: Hydrolysis time 30 minutes
C: Hydrolysis time 45 minutes
D: Hydrolysis time 60 minutes

The degree of acidity (pH) is one of the crucial factors that need to be considered during the fermentation process. pH affects the growth of microorganisms used in fermentation. Therefore, before the fermentation stage took place, the pH of the subsrates was set to 5.

Based on the results of pH measurements using universal indicators, it is known that the final pH of each sample is lower than the initial pH which was set to 5. The average pH data for each treatment is presented in table 3. Changes in pH tend to be the same in all samples. The pH value ranges from 2.3 to 3.67. The pH value is affected by the product produced during the fermentation process. In this study, the product produced from fermentation is alcohol. As for the nature of alcohol, which is acid, it affects the pH value. This is similar with the opinion of Azizah *et al.* (2012)[7] that alcohol formed during fermentation causes the pH of the substrate to decrease. The longer the fermentation time, the lower the pH value. This is because the fermentation process will undergo pyruvate biosynthesis which produces acidic products, such as butyric acid, acetic acid, acetone, acetaldehyde and alcohol. Therefore the pH value decreases with increasing fermentation time. This is consistent with the opinion of Yuniarsih (2009)[15] that the results of the fermentation process will be converted into acetic acid, ethanol, and CO2.

The by-products produced during the fermentation period were CO_2 gas. In the opinion of Azizah *et al.* (2012)[7] increasing gas production was also followed by a decrease in pH value. If seen in table 3, the pH value of each sample decreases after 3 days of fermentation. The mean pH for each treatment ranged from 2.3 to 3.67. This proves that CO_2 gas was produced during the fermentation period. The presence of CO_2 gas is proven by the presence of bubbles as the main indicator. In addition, this is also in accordance with Kartohardjono *et al.* (2007)[16], that CO_2 gas is often called acid whey because it has acidic properties. CO_2 dissolved in water will form carbonic acid, as shown in the following reactions:

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

The amount of H_2CO_3 in water does not depend on pH, but depends on the amount of CO_2 (and several other factors such as temperature and salinity). H_2CO_3 formed will dissociate into H^+, HCO^{3-}, and CO_3^{2-}. These H^+ ions are produced which will reduce the pH value.

3.2. Reducing sugar

In this study, the Fehling method was used for qualitative analysis of reducing sugars. Fehling reagent used was a mixture of Fehling A and Fehling B. Fehling A is a solution of $CuSO_4$, while Fehling B is a solution consisting of NaOH and Na-K-tartrate. The working principle of Fehling reagent is the reaction between reducing sugar and Fehling B to form enediol, then enediol reacts with Fehling A to form Cu^{2+}

ions and a mixture of acids. Furthermore, Cu^{2+} ion in the form of $Cu(OH)_2$ will be reduced by reducing sugar to Cu^+ as CuOH, then insoluble Cu_2O is yellow or brick red[17].

The tested solution was first mixed with Fehling A and Fehling B reagents so that the color formed was blue. After being heated for 1 minute, each sample changes the color of the solution which varies depending on the level of reducing sugar contained in it. The color of the sample solution after heating ranges from dark green to brick red. In the sample with treatment A (hydrolysis time for 15 minutes) the color of the sample solution after being heated tends to be dark green, but for the A1 sample, the color of the solution tends to be light brown. Meanwhile, in the sample with treatment B (hydrolysis time for 30 minutes), the sample solution was dark green mixed with reddish brown. Whereas in the sample with treatment C (hydrolysis time for 45 minutes) the sample solution was light brown to brick red. In the sample with treatment D (hydrolysis time for 60 minutes) the sample solution was red with concentrated brick. The color of the solution is affected by the presence of Cu_2O deposits. This result is in accordance with Mathews *et al.* (2000)[17] that the presence of reducing sugars can be detected by forming dark green, yellow-orange Cu_2O deposits, or brick red.

The Fehling test will give brick red deposits to reducing sugars, this is because the copper monoxide ions from the fehling reagent will react with hydrogen atoms on the carbon atoms of the aldehyde group which produce brick red copper (II) monoxide deposits. The more aldehyde groups, the more red brick deposits produced will be. Therefore the sample changes color in the solution, this is because the copper monoxide ion which gives a blue color decreases and even reacts with the aldehyde group, and forms a brick red precipitate.

3.3. Ethanol Measuring

The data below is the result of ethanol content test in a sample of simple distillation. Ethanol levels were tested using Uv-vis Spectrophotometer at a wavelength of 470 nm at the Chem-Mix Pratama Laboratory, Bantul, Special Region of Yogyakarta, Indonesia. The result of ethanol content test can be seen in the following table.

Table 2. The Results of Ethanol Levels Measurement

No	Treatment	The Average of Replication 1 (%)	The Average of Replication 2 (%)	Total Average
1	A	4,489	4,611	4,55
2	B	8,133	8,156	8,15
3	C	11,644	11,599	11,62
4	D	13,156	13,178	13,17

The production of bioethanol from macroalgae *Ulva lactuca* includes 3 stages: hydrolysis, fermentation and distillation. Based on research by Santi *et al.* (2012)[3], it is known that *Ulva lactuca* contained 16.42% hemicellulose and 19.58% cellulose. Cellulose and hemicellulose are polysaccharide compounds that must be broken down into monosaccharide compounds to be fermented by microorganisms. Therefore, the process of hydrolysis using acid was first carried out.

Basically, the principle of hydrolysis is to break the polymer chain of materials into simpler monomer units with the help of catalysts. In this study the chain breaking process was carried out using HCl solution. In this case, HCl acts as a catalyst. According to Balat, *et al.* (2008)[18], the hydrolysis process of H_2SO_4 will react to form H^+ and SO^{4-} groups. If compared with the use of HCl in this study, it can be concluded that when the hydrolysis process occurs HCl will also react to form H^+ and Cl^- groups. Furthermore, the H^+ group breaks down the glycosidic bonds in both cellulose and hemicellulose, so that simple sugar monomers will be formed. The resulting monomer was still in the free radical group, but in the presence of OH- from the water it binds to the radical group to form a glucose group. In this process water functions as a free radical group stabilizer. The more water contained in the acid solution, the more it also stabilizes the radical group, so that the glucose formed will be more and more. Likewise

on the contrary, the higher the concentration of acid, the less water content that causes glucose to form, the less it will be.

This research was conducted by varying the hydrolysis time to determine the best time to produce ethanol levels. Hydrolysis time is one of the factors that influence the hydrolysis process. Hydrolysis and other fermentation variables are equalized for each treatment of hydrolysis time. The variation of hydrolysis time in each treatment is 15, 30, 45, and 60 minutes with 3 replications in each treatment so that there were a total of 12 samples to be fermented. The length of the hydrolysis time is related to the conversion heights reached to the optimum point. The reaction rate constant will increase with increasing reaction time. From table 4 it shows that the conversion of cellulose and hemicellulose to glucose is strongly influenced by the length of time of hydrolysis. The longer the hydrolysis time, the chance for cellulose and hemicellulose to decompose longer, so that ethanol levels also increase. The highest ethanol content was achieved at 60 minutes of hydrolysis time, which was 13.17%.

Yeast, mold, and bacteria can generally grow well at pH 3-6. Therefore, after the hydrolysis stage, the pH value was set to 5 by adding NaOH. After setting the pH, nutrients in the form of urea and NPK were also added. As much as 10% of yeast tape inoculum and bread yeast were added to the media and fermented for 3 days at room temperature. The duration of fermentation was determined according to Sari *et al.* (2008)[19] which states that the most optimal fermentation time for bioethanol production is 3 days. If fermentation is done more than 3 days, the alcohol content will tend to decrease because alcohol has been converted into other compounds, such as esters.

In anaerob condition, pyruvic acid produced from the glycolysis process will be converted to acetic acid and CO_2. Then, acetic acid is converted to ethanol. The process of changing acetic acid to ethanol was followed by changes in NADH to NAD^+. With the formation of NAD^+, glycolysis events can occur again. In this ethanol fermentation, only one molecule of ATP can be produced from one mole of glucose [8]. Conversely, under aerobic conditions, S. *cerevisiae* hydrolyzes sugar into water and CO_2.

After 24 hours, microorganisms found in bread yeast (S. *cerevisiae*) and *tape* yeast (yeast, mold and bacteria) make a brief adaptation to the fermentor environment (lag phase). This adaptation includes producing enzymes that are compatible with the substrate. According to Elevri and Putra (2006)[13], S. *cerevisiae* adapts (lag phase) within the first 20 hours of its growth. Meanwhile, Kurniawan *et al.* (2014)[11] stated that in the span of 24 - 72 hours, yeast cells are in the exponential phase. Yeast cells experience an increase in the number of cells and alcohol compounds and other compounds begin to form as a result of their metabolites. It is proven by the presence of CO_2 gas bubbles during the fermentation period takes place in a beaker glass containing aquades. In addition, lactic acid bacteria in yeast tape oxidize acid until the pH of the environment decreases. The results of pH measurements after the fermentation period ends, the pH value decreases from the initial pH 5 to the average 3. According to Ristiarini *et al.* (2001) in Azizah *et al.* (2012)[7], during 72 hours of fermentation there is a decrease in reducing sugar and pH of the environment. This is because the reducing sugar has been used as a substrate by yeast and lactic acid bacteria for its activity to produce alcohol and organic acids.

After fermentation for 3 days (72 hours) the distillation process was carried out at a temperature of 70 - 80 °C and obtained by distillate. Distillation is done to separate ethanol from other compounds to obtain high levels of ethanol. This separation is based on the difference in boiling points. Destilat is then tested for its ethanol content in the Primary Chem-Mix Laboratory using a UV-vis spectrophotometer at a wavelength of 470 nm. The test results can be seen in table 4 where two replications were carried out so that the results obtained can be more accurate.

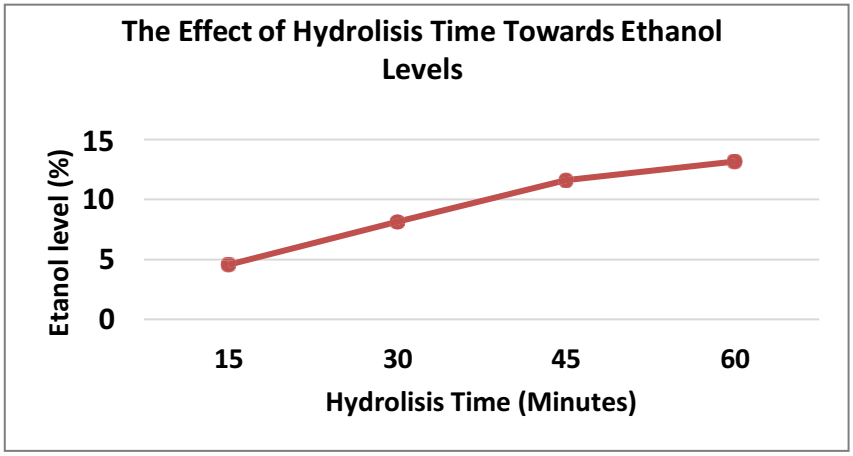

Figure 1. The effect of hydrolisis time towards ethanol levels

Overall, the ethanol content in each treatment with the hydrolysis time of 15 minutes (A), 30 minutes (B), 45 minutes (C), and 60 minutes (D) respectively were 4.55%, 8.15%, 11.62%, and 13.17%. In other words, it can be concluded that the ethanol levels increase with the increasing of hydrolysis time. The highest ethanol content was produced at 60 minute of hydrolysis time which was 13.17%. The relationship between hydrolysis time and the increasing of ethanol levels is also presented in Figure 1. From the graph in the figure it can be seen that the ethanol level curve moves up as the hyrolysis time increasing.

To determine the effect of hydrolysis time on bioethanol levels, statistical tests were performed using one-way Anova Test (One way Anova). The results of the research data were processed using SPSS ver 20.0. Before analyzing using one-way ANOVA Test, the Normality Test and Homogeneity Test were first carried out. The first step is testing whether the data is normally distributed or not using the Normality Test. From the results of the analysis, it is known that the value of Sig = 0.552, this value is greater than (α) = 0.05 so it can be concluded that the data is normally distributed. Furthermore, one-way ANOVA test is performed to find out whether the data is homogeneous or not, and whether there are significant differences in the results of the average ethanol content in each treatment sample.In this analysis, the significance level (α) is 0.01 so the results are more accurate. From the Homogenity Test results, it is known that the data is homogeneous so that it meets the Anova Test requirements. Meanwhile, from the results of one-way ANOVA Test, it is known that the value of sig = 0,000 (attached). This value is smaller than (α) 0.01 so it can be concluded that there is an average significant difference or hydrolysis time has a significant effect on ethanol levels. In addition, Post Hoc analysis was also done using the Tukey Test to find out more details about the real differences between each sample. The Tukey test results showed a significant difference in the length of hydrolysis time to bioethanol levels.

4. Conclusion

From the present study it can be concluded that the hydrolysis time has a significant effect to the ethanol levels produced through the fermentation of *Ulva lactuca*. The studies also conclude that the most optimum time for hydrolisis stage in this research was 60 minutes, which produced the highest levels of ethanol which is 13,17%.

References

[1] Patil V, Khanh-Quang Tran and Hans R 2008 Towards Sustainable Production of Biofuels from Microalgae. *Int. J. of Mol. Sci.* ISSN 1422-0067
[2] Asriyana and Yuliana 2012 *Produktivitas Perairan* (Jakarta: Bumi Aksara)
[3] Santi R A, Sunarti, T C, Santoso D and Triwisari D A 2012 Komposisi Kimia dan Profil Polisakarida Rumput Laut Hijau. *Jurnal Akuatika Vol. **3**,* No. 2
[4] John 2004 *Biofuels for Transport*. Downloaded from www.task39.org on February 7, 2018 at 17.50
[5] Hening and Zeddies 2006 *Bioengineering and Agriculture* Promises and Challenges International Food Policy Institute
[6] Susilawati, R 2012 *Bisnis Etanol Medco Tersandung Mahalnya Singkong*. Downloaded from http://www.beritajatim.com/detailnews.php/1/Ekonomi/2012-04-2/133684Bisnis_Bio_EtanolMedco_Tersandung_Mahalnya_Singkong on January 27, 2018.
[7] Azizah N, Al-Baarri A N and Mulyani S 2012 Pengaruh Lama Fermentasi terhadap Kadar Alkohol, pH, dan Produksi Gas Pada Proses Fermentasi Bioetanol dari Whey dengan Substitusi Kulit Nanas. *J. Ind. Food Tech.* **1** : 4 – 6
[8] Purwoko 2007 *Fisiologi Mikrobia* (Jakarta: Bumi Aksara)
[9] Pelczar M J and Chan E C S 1998 *Dasar-dasar Mikrobiologi Jilid 1* Translated by RS Hadioetomo, T Imas, SS Tjitrosomo and SL Angka (Jakarta: UI Press)
[10] Dwidjoseputro D 2005 *Dasar-dasar Mikrobiologi* (Jakarta: Djambatan)
[11] Kurniawan T B, Siti H B and R Susanti 2014 Efek Interaksi Ragi Tape dan Ragi Roti terhadap Kadar Bioetanol Ketela Pohon (*Manihot utilissima* Pohl) Varietas Mukibat *Biosaintifika* **6** 152-160
[12] Utami R S, Eva P S and Inayati 2014 Pengaruh Waktu Hidrolisa dan Konsentrasi Asam pada Hidrolisa Pati Kentang dengan Katalis Asam *Ekuilibrium **13** No. 2* 45-49
[13] Elevri P A and Putra S R 2006 Produksi Etanol menggunakan *Saccharomyces cerevisiae* yang diamobilisasi dengan Agar Batang *Akta Kimindo* **1** (2) 105-114
[14] Chairul and Silvia R N 2013 *Pembuatan Bioetanol dari Nira Nipah Menggunakan Saccharomyces cerevisiae* (Pekanbaru: Department of Chemical Engineering, Riau University)
[15] Yuniarsih F N 2009 Pembuatan Bioetanol dari Dekstrin dan Sirup Glukosa Sagu (*Metroxylen* sp.) menggunakan *Saccharomyces cerevisiae* var Ellipsoideus (Bogor: Department of Industrial Engineering, Bogor Agricultural University) *Thesis*
[16] Kartohardjono S, Anggara, Subihi and Yuliusman 2007 Absorbsi CO2 dari campurannya dengan CH4 atau N2 melalui kontraktor membran serat berongga menggunakan pelaruh air *Jurnal Teknologi **11*** (2) 97-102
[17] Matthews C K, van Holde, K E, and Ahrn K G 2000 *Biochemistry 3rd Ed* (San Francisco: Addison-Wesley Pub. Com)
[18] Balat M, Balat H and Oz C 2008. Progress in Bioethanol Processing. *Progress in Energy and Combustion Science* (34): 551-573
[19] Sari I M, Noverita and Yulneriwarni 2008 Pemanfaatan jerami padi dan alang-alang dalam fermentasi etanol menggunakan kapang *Trichoderma viride* dan khamir *Saccharomyces cerevisiae* Vis Vitalis **5** (2) 55-62