J. Appl. Environ. Biol. Sci., 1(10)420-425, 2011

ISSN: 2090-4215
Journal of Applied Environmental and Biological Sciences
www.textroad.com

AEROBIC AND ANAEROBIC PROCESSES OF SPIROGYRA EXTRACT USING DIFFERENT DOSES OF *Zymomonas mobilis*

Sulfahri*, Sri Nurhatika, Tutik Nurhidayati

Department of Biological Science,
Sepuluh Nopember Institute of Technology (ITS), Surabaya, 60111, Indonesia

ABSTRACT

This study investigated *Zymomonas mobilis* bacteria for fermenting carbohydrates of algae Spirogyra extract into ethanol. Process variables consisted of inoculum dose, aerobic and anaerobic conditions. Results showed that the most effective inoculum dose was 5% in aerobic condition for 96 hours retention time. While in the anaerobic condition, the most effective inoculum dose was 10% for 48 hours retention time. It revealed that Spirogyra extract fermentation into ethanol in anaerobic condition was more effective than aerobic condition.

Key words: algae Spirogyra, ethanol, aerobic, anaerobic, *Zymomonas mobilis*.

INTRODUCTION

Bioethanol made from plants, require vast land to meet the needs of bioethanol in Indonesia. Therefore, Indonesia still requires a fuel source bioethanol is more effective. Bioethanol production from algae can be a realistic solution to replace gasoline. This is because the carbohydrate content of the algae can reach 64% and also reproduction is very rapidly by fragmentation (Becker, 2004). One of the algae with the potential to be developed as the raw material of bioethanol is the algae Spirogyra (Sulfahri et al., 2010).

The carbohydrate type of the algae spirogyra is starch (Mitova et al., 1999). Starch is a substance that must first be converted into simpler molecules that can be absorbed by cells. Amylase enzyme has the ability to break down the molecules of starch and glycogen. Starch molecule which is a polymer of 1,4-α-bond will be broken down by glycoside α-amylase enzyme in the bond α-1,4 produce glucose, maltose, and dextrin (Bascar et al., 2008).

Spirogyra algae fermentation by the yeast using *Saccharomyces cerevisiae* can produce ethanol at 9.245% with the addition of α-amylase enzyme 0.12% with 10 days fermentation duration (Sulfahri et al., 2010). However, *Saccharomyces cerevisiae* appears to have some shortcomings, such is not resistant to high concentrations of ethanol produced. *Zymomonas mobilis* has several advantages compared to *Saccharomyces cerevisiae*, among them conversion into ethanol faster (Sulfahri *et al*., 2011), more tolerant of temperature, low pH (Nowak, 200), and resistant to high concentrations of ethanol (Busche et al., 1992). In addition, *Saccharomyces cerevisiae*, requires a long time (240 hours) to produce ethanol with raw material algae Spirogyra (Sulfahri et al., 2010), While *Zymomonas mobilis* only takes 24 hours to produce ethanol using sweet potato (carbohydrate) raw materials (Zhang et al., 2010). Therefore this study aims to determine the inoculum size of bacteria *Zymomonas mobilis* and long fermentation time is effective for fermenting carbohydrates into ethanol extract of Spirogyra in aerobic and anaerobic conditions.

MATERIALS AND METHODS

Extracts of Spirogyra

Spirogyra samples washed with water to clean the dirt, then dried for three days in the sun. Spirogyra which had been dried weighed and added aquadest with 20% of substrate concentration, mashed with a blender, put into 250 ml erlenmeyer (Zhang *et al*., 2010). Spirogyra extract sterilized in autoclave 1,5 atm 121°C. Further Spirogyra extracts will be used to process the growth curve of *Z. mobilis*, strarter preparation, and substrate of fermentation.

Stock Cultures and Work Culture

Z. mobilis was grown at 30°C for 24 h by regularly streaking on the agar plates of the rich medium (RM) containing (per liter): glucose, 20 g; yeast extract, 10 g; $(NH_4)_2SO_4$, 1 g; K_2HPO_4, 1 g; $MgSO_4.7H_2O$, 0.5 g (Struch *et al*., 1991).

Measurement of Growth

Z. mobilis was inoculated into a 50 ml erlenmeyer containing 5 ml of sterile extract of Spirogyra which

***Correspondence Author:** Sulfahri, Department of Biological Science, Sepuluh Nopember Institute of Technology (ITS), Surabaya, 60111, Indonesia. Phone, +6282140839288, e-mail: mynameisfahri@gmail.com

had governed the pH to 4 by adding 30% HCl solution (Zhang *et al*., 2010). Then it was incubated in a rotary shaker with agitation speed of 15 rpm at a temperature of 30 °C for 24 hours (Activation I). A total of 1 ml of activation I inoculated again into 50 ml erlenmeyer containing 9 ml of Spirogyra extract, incubated in a rotary shaker with agitation speed of 15 rpm at a temperature of 30 °C for 24 hours (Activation II). A total of 5 ml of activation inoculated again into 100 ml erlenmeyer containing 50 ml of extract Spirogyra, were incubated in rotary shaker with agitation speed of 15 rpm at 30°C temperature for 24 hours is referred to as fermentation cultures (Cazetta *et al*., 2007; Zhang *et al*., 2010).

Performed dilutions were from 10^{-1} to 10^{-9}. The growth curve was made by measuring the absorbance of cultures of *Z. mobilis* on Spirogyra extract. *Z. mobilis* was measured at a wavelength of 600 nm at intervals of once every one hour during 24 hours. Growth curve of *Z. mobilis* measured by absorbance values and the fermentation time (Obire, 2005).

Starter Preparation of *Z.mobilis*

Z. mobilis was inoculated into 50 ml erlenmeyer containing 5 ml of sterile Spirogyra extract that has been set pH to 4 by adding 30% HCl solution, then incubated in a rotary shaker with agitation speed of 15 rpm at 30 °C for 24 hours (Activation I). A total of 1 ml of activation I inoculated again into 50 ml erlenmeyer containing 9 ml of Spirogyra extract, incubated in a rotary shaker with agitation speed of 15 rpm at a temperature of 30 ° C for 24 hours (Activation II). A total of 5 ml of of activation II inoculated again into 100 ml erlenmeyer containing 50 ml of extract of Spirogyra, were incubated in rotary shaker with agitation speed of 15 rpm at a temperature of 30 °C and incubated until the hour in which log phase of *Z. mobilis* occur (in accordance with the growth curve) (Activation III) (Cazetta *et al*., 2007; Zhang *et al*., 2010).

Hydrolysis Process

Spirogyra extract as much as 50 ml incorporated into the erlenmeyer. Erlenmeyer was heated on a hot plate for 2 hours in with the temperature 100°C, stirring occasionally open funnel-flops (Mosier *et al*., 2006). The addition of the α-amylase enzyme with each concentration 0.06 grams/50 ml substrate with the temperature 40°C for 80 minutes (Bascar *et al*., 2008; Sulfahri *et al*., 2010).

The Fermentation Process and Measurement of Ethanol Concentration

Starter is added with a concentration in accordance with the study design (0%, 5%, 7.5%, and 10%) into the fermenter bottle (100 ml) containing 50 ml of Spirogyra extract, incubated with the duration according to the study design (0 hours, 24 hours , 48 hours, 72 hours and 96 hours) at room temperature (30°C-32°C). If the ethanol content in 96 hours have not decreased, the fermentation process continues. Fermentation process performed on aerobic and anaerobic conditions. Conditioned by aerobic fermentation using fermenters from cotton cap fat. While the anaerobic fermentation is done by using Hungate techniques, namely by passing nitrogen gas into the fermenter. Fermenter was closed with rubber stoppers and then closing the gas flowing nitrogen for 2 minutes. After fermentation is complete, the bottle cap is removed, covered with cotton fat and pasteurized at a temperature of ± 80 °C for 10 minutes (Puspita et al., 2010). Ethanol concentration measured by using specific gravity method (Purwanto, 2004).

RESULTS AND DISCUSSION

Age Determination in the Medium Starter *Zymomonas mobilis* Fermentation

Each microorganism has a specific growth curve. It is also seen in *Zymomonas mobilis* growth curve (Figure 1). Starter is a collection of microorganisms that are ready inoculated into the fermentation medium. Basically, the growth of microbial cells held indefinitely. However, because the growth takes place by consuming nutrients at once issued (excretion) of metabolism products formed, then after a certain time the growth rate will decline and eventually cease altogether (Hutkins, 2006).

The growth curve gives an overview of the environmental factors that influence the growth of a microorganism such as substrate, ambient temperature, and pH (Hogg, 2005). Age starter is either used as inoculum fermentation medium is along the logarithmic phase, because at this phase of cells of microorganisms have the ability to divide the maximum growth rate and metabolic activity constant. According to Hogg (2005) age starter used as inoculum, determined by calculating the specific growth rate (μ) and the doubling time (tg) based on cell number and incubation time on the growth curve. Age of the starter *Zymomonas mobilis* which is used by a growth curve that is an 6.5 h, at μ=0.592 generations/hour with a time of doubling time (double time) the fastest 70 minutes.

Commonly used culture age was taken at mid exponential phase. Hogg (2005) explains that in the exponential phase cells of microorganisms in a stable condition, new cells are formed at a constant rate of microorganisms and cells divide at its optimum when the doubling time (double time), which is usually reached in the middle of logarithmic phase.

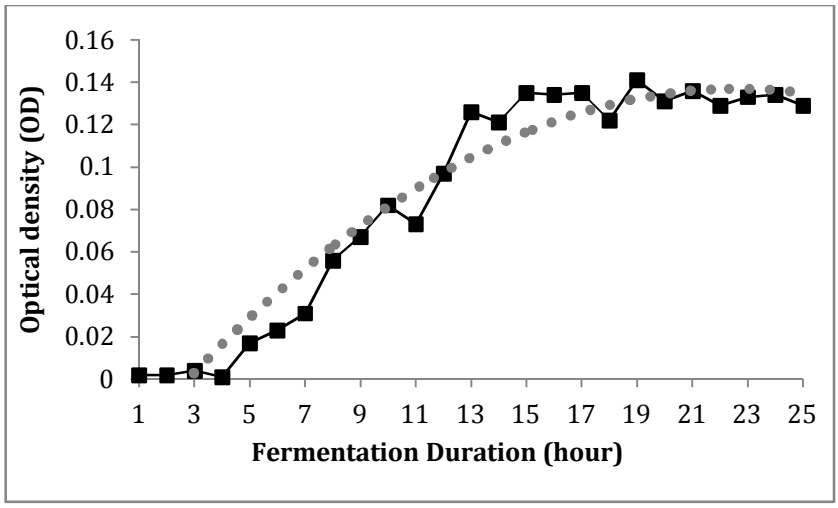

Figure 1. Growth curves of *Z. mobilis*

Ethanol Fermentation

Ethanol fermentation is influenced by several factors, one of which is oxygen. Therefore, the fermentation of ethanol from algae Spirogyra using the bacteria *Zymomonas mobilis* performed on aerobic and anaerobic conditions. Besides oxygen, other factors affecting of the fermentation process is inoculum size. Inoculum is microorganism that was inoculated into the fermentation medium. Inoculum has a most important role in supporting the success of the fermentation process. In the fermentation of algae Spirogyra, inoculum was used *Zymomonas mobilis*. In this study, the use of *Zymomonas mobilis* bacteria, as it has many advantages of which are more tolerant of temperature, low pH (pH 3.5 - pH 7.5) (Nowak, 2000), and resistant to high concentrations of sugar (40%) and high concentrations of ethanol (15%) (Busche et al., 1992).

Fermentation of algae Spirogyra conducted for 120 hours with *Zymomonas mobilis* inoculum size variations are added 0% (control), 5%, 7.5% and 10% in aerobic and anaerobic conditions.

Effect of Inoculum Size and Fermentation Duration in aerobic condition Against Ethanol Production

Ethanol fermentation was carried out on a variety of different inoculum sizes of 0% (control), 5%, 7.5% and 10%. Fermentation carried out for 120 hours, and by measuring levels of ethanol every 24 hours. Graph of ethanol content in Figure 2 below.

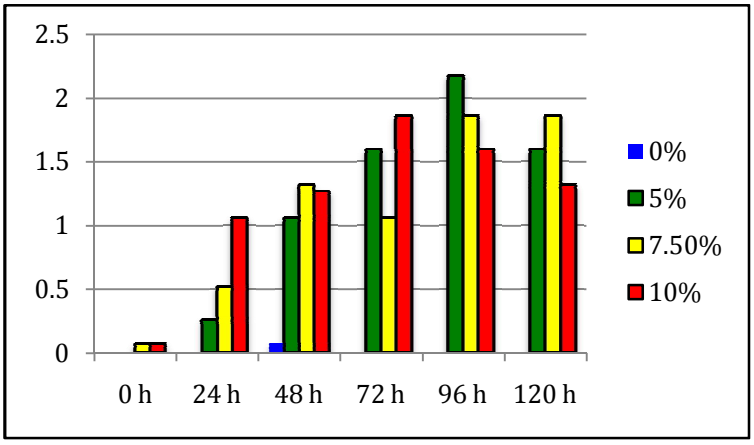

Figure 2. Ethanol production in aerobic condition

Based on Figure 2, it is known that in the 0 h, yet there is ethanol, except at 7.5% inoculum and 10% respectively at 0.07%. But based on results of ANOVA test followed by Tukey test with 95% confidence interval, it is known that levels of ethanol between 0% and 0.07% is not significantly different. Based on the results of ANOVA test followed by Tukey test with 95% confidence interval, it is known that the 0% of inoculum size has no effect on levels of ethanol. While at a inoculum size of 5%, 7.5% and 10% influence on levels of ethanol produced. The highest ethanol content in the 5% of inoculum size with ethanol content of 2.18% (v/v).

At 0% of inoculum sizes, there is no ethanol in all samples, except at the 48 h as much as 0.07% (v/v). But based on results of ANOVA test followed by Tukey test with 95% confidence interval, it is known that levels of ethanol between 0% and 0.07% is not significantly different. So despite the ethanol content in the inoculum 0% of 0.07% which is at 48 h, is considered not significantly different during the hours the other inoculum levels of

0% to 0% ethanol. This is due to the absence of inoculum was inoculated into fermentation samples so that no microbes perform fermentation and fermentation products.

The highest levels of ethanol at 5% of inoculum size. Inoculum has a most important role in supporting the success of the fermentation process. If the cell count is too high, namely with the addition of inoculum size which can lead to more competition between the cells in utilizing nutrients (substrate) which causes the microorganism activity in fermenting reducing sugar to ethanol decreases. According Gibbson, et al. (1986), the use of inoculum size that are too high cause the reduction in cell viability.

Based on the fermentation duration, it is known that ethanol levels continued to increase over time. The highest levels of ethanol that is at the 96 with ethanol content of 2.18% (v/v). After that, ethanol content tends to decrease. Due to decreased levels of ethanol during aerobic respiration, in addition to ethanol produced in very small amounts, also produced a variety of organic acids that may be inhibiting the growth of *Z.mobilis*.

Ethanol produced in the aerobic fermentation process shows low yield, the maximum ethanol content of 2.18% (v / v). Low levels of ethanol are due to aerobic conditions, reduction of sugar tend to be used for energy and cell mass formation. In aerobic conditions, pyruvic acid is converted into acetaldehyde and then ethanol, but is converted into acetyl-CoA and entry into the Krebs cycle. Therefore, the levels of ethanol produced by *Z.mobilis* in aerobic conditions tend to be lower.

Effect of Inoculum Size and Long Fermentation on the Production of Ethanol Against Anaerobic Conditions

Anaerobic fermentation is done by using the Hungate technique, namely by passing nitrogen gas into the fermenter. Fermenter was closed with rubber stoppers and then closing the gas flowing nitrogen for 2 minutes. Ethanol fermentation in anaerobic conditions was performed on a variety of different inoculum Sizes. The mean levels of ethanol produced from the fermentation process during the 120 hours can be seen in Figure 3.

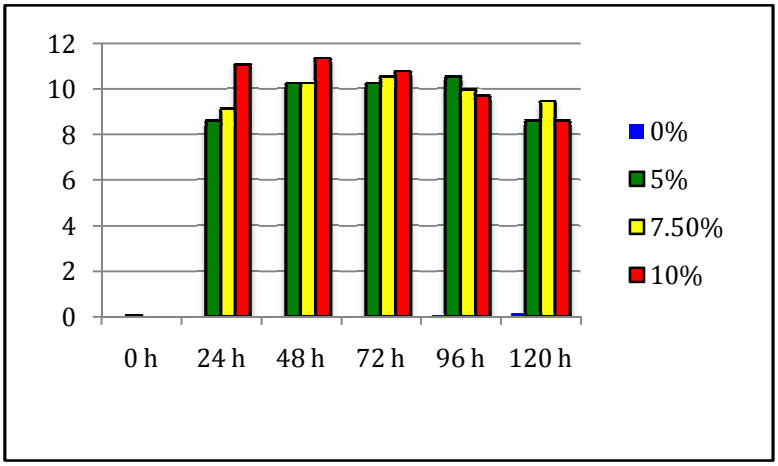

Figure 3. Ethanol production in anaerobic condition

In anaerobic conditions, by ANOVA test followed by Tukey test with 95% confidence interval, it is known that the inoculum size give a noticeable effect on levels of ethanol produced. Inoculum Size of 0% (control), 5%, 7.5% and 10%, respectively significantly different. This suggests that different inoculum Sizes of ethanol showed different results. The highest levels of ethanol produced by 10% of inoculum Size, with ethanol content by 11.36% (v/v). Subsequently followed by 7.5% and 5% with each level of 10.56% ethanol (v/v). Lowest levels of ethanol occurs in the 0 % of inoculum Size (control), which is the highest is only 0.13% (v/v).

Based on ANOVA test followed by Tukey test with 95% confidence interval, it is known that an increase in ethanol content equivalent with the fermentation duration up to 120 hours in all treatments except the control. This is due to the need to form nutrient ethanol at the 0 h to 120 h, nutrients are still available and the process of fermentation is influenced by time. However, ethanol significantly increases occurred at the 24 h of 0.00-0.07% (v/v) to 0.00-11.08% (v/v). It is also reported by Zhang et al. (2010) that the fermentation of carbohydrates from sweet potato optimal at 24 hours. The highest ethanol levels at the 48 h on 10% of inoculum size , with ethanol content 11.36% (v/v). But in the 72 h, ethanol content decreased from11.36% (v / v) to 10.80% (v/v). Decreased levels of ethanol is due to the nutrients that will be used for ethanol formation is limited, so there is no formation of ethanol significantly.

On treatment with using *S.cerevisiae* show the results tend to be lower when using *Z. mobilis*, where the highest levels of ethanol produced by *S.cerevisiae* is at the 120 with the ethanol content of 6.41% (v / v). This is in accordance with the opinion of Nowak (2000), which says that the *S.cerevisiae* describe the source of carbon through the Embden-Mayerhoff, where the metabolism of this produces as many as 2 moles of ATP per mole of glucose. Meanwhile, according to Tsantili et al. (2007), *Z. mobilis* will elaborate on glucose, fructose or sucrose

(carbon source) via the Entner-Doudoroff metabolic pathway. This metabolic pathway produces only 1 mol ATP per mole of glucose or fructose, so that *Z. mobilis* elaborate sugar at high speed to generate enough energy for growth. Therefore only produce one molecule of ATP, it must describe *Z.mobilis* glucose quickly to meet the needs ATP. This causes *Z.mobilis* produce ethanol much faster if compared with *S.cerevisiae.*

The ability of cells of *Z.mobilis* is limited by the tolerance to ethanol. When ethanol accumulated quite a lot in the medium, the cell growth of *Z. mobilis* is inhibited, so that the cell *Z.mobilis* will die. Increasing concentration of ethanol in the medium also causes the membrane structure changed. Toxicity of ethanol affects the cell through changes in membrane phospholipids and weaken the membrane structure. This causes the cell contents leak out and fermentation ability of cells to be damaged (Sturch at al., 1991).

Reducing Sugar

In addition to oxygen and inoculum concentration, other factors that influence the fermentation of ethanol is reducing sugar concentration in the fermentation medium. This is because sugar is an important factors for the reduction of cell *Z. mobilis* as a source of energy for metabolism. Reducing sugar has an effect on the levels of ethanol produced. Reducing sugar measurements performed using Luff-Schoorl method. According to Lehninger (1982), reduction sugar is a type of sugar which can reduce electron-accepting compounds, or sugars can be oxidized by the oxidizing agent for oxidizing agent is reduced in the reaction. Therefore, all monosaccharide (glucose, fructose, galactose) and disaccharides (lactose, maltose), except for sucrose and starch (polysaccharides), including as a reducing sugar. Below is a table of reduction in sugar fermentation medium:

Table 1. Reducing Sugar Average in Early and Late Fermentation Medium

No.	Fermentation Medium	Condition	Initial reducing sugar (%)	Final Reducing sugar (%)	Conversion (%)	Ethanol levels at the 120 hours (%)
1.	Inoculum 0%	Aerob	10,05	9,95	0,99	0,00
		Anaerob	10,00	9,84	1,60	0,13
2.	Inoculum 5%	Aerob	9,90	1,44	85,45	1,59
		Anaerob	10,05	1,25	87,56	8,61
3.	Inoculum 7,5%	Aerob	9,95	2,68	73,06	1,86
		Anaerob	10,05	1,34	86,67	9,51
4.	Inoculum 10%	Aerob	10,05	2,84	71,74	1,35
		Anaerob	9,90	1,20	87,88	8,61

Based on Table 1 above, can be seen that the general decline in levels of reducing sugars during fermentation. This is because during the fermentation process, reduction of the conversion of sugar into ethanol and carbon dioxide. In aerobic conditions with 0% of inoculum size, reducing sugars did not decrease significantly, from 10.05% to 9.95% with a conversion value of 0.99%. Whereas in anaerobic conditions with 0% of inoculum size, reducing sugars did not decrease significantly, ie from 10.00% to 9.84% with a conversion value of 1.60%. This is because the o% inoculum, not the addition of bacteria, so there is no fermentation process that resulted in the reduction of the conversion of sugar tend to exebe lower.

In aerobic conditions with 5% of inoculum size, reducing sugars decreased from 9.90% to 1.44% with a conversion value of 85.45%. While in the 7.5% and 10% of inoculum size, also a decline in glucose reduction with conversion values respectively 73.06% and 71.74%. In anaerobic conditions with inoculum size of 5%, 7.5% and 10%, indicating the average conversion value above 80%. This shows the process of fermentation, because the reduction of sugar has been converted into ethanol and carbon dioxide. According to Yang et al. (2009), more sugar reduction that can be utilized by the cell *Z. mobilis*, the higher the levels of ethanol produced.

In aerobic and anaerobic conditions, a decline in sugar reduction with conversion values ranged between 71.74% -81.88%. This suggests that both aerobic and anaerobic conditions, the conversion of sugar reduction. But the ethanol content in aerobic conditions tend to be lower, at maximum 2.18% (v/v) at the 96 h. This is because in aerobic conditions, reduction of sugar tend to be used for energy and cell mass formation, so that despite the reduction of sugar has been converted to a value above 70%, does not produce ethanol in large numbers.

CONCLUSIONS

The most effective inoculum size of *Zymomonas mobilis* bacteria to produce ethanol from algae Spirogyra in aerobic conditions was ethanol content of 2.16% (v/v) at 5% of inoculum size and 96 hours of fermentation duration. Whereas in the anaerobic conditions was ethanol content of 11.36% (v/v) at 10% of inoculum size and 48 hours of fermentation duration. Spirogyra extract fermentation into ethanol in anaerobic conditions was more effective than aerobic conditions.

ACKNOWLEDGMENT

The authors gratefully acknowledge scholarship (beasiswa unggulan) support from Directorate General of Higher Education, the Ministry of National Education of Indonesia. The authors also wish to thanks Siti Mushlihah, Prof. Dr. Sarwoko Mangkoedihardjo, Nengah Dwianita Kuswitasary, S.Si.,M.Si., Dr.rer.nat.Ir. Maya Shovitri, M.Si. and Mukhammad Muryono, S.Si., M.Si. (Sepuluh Nopember Institute of Technology)

REFERENCES

Bascar, G., Muthukumaran, C., Renganathan, S. (2008). Optimization of Enzymatic Hydrolysis of *Manihot Esculenta* Root Starch by Immobilize α-Amylase Using Response Surface Methodology. International Journal of Natural Sciences and Engineering. 1(3) : 156-160.

Becker, E.W. (2006). Microalgae as A Source of Protein. Journal of Biotechnology Advances. 25 : 207-210.

Busche, R. M., Scott C. D., Davison B. H., Lynd L. R. (1992). Etanol, the ultimate feedstock. A technoeconomic evaluation of etanol manufacture in fluidized bed bioreactors operating with immobilized cells. Journal Application of Biochemistry and Biotechnology. 34/35 : 395-415.

Cazetta, M.L., Celligoi M.A.P.C., Buzato J.B., Scarmino I.S. (2007). Fermentation of molasses by *Zymomonas mobilis*: Effect of temperature and sugar concentration on etanol production. Journal Bioresource and Technology. 98 : 2824-2828.

Gibbons, W.R dan C.A Westby. (1986). Effects of Inoculum Size on Solid-Phase Fermentation of Fodder Beets for Fuel Etanol Production. Journal Applied and Environmental Microbiology. 52 : 960-962

Hogg, S. (2005). Essential Microbiology. John Wiley & Sons Ltd : England.

Hutkins, R.W. (2006). Microbiology and Technology of Fermented Food. Blackwell Publishing Ltd : USA

Mitova M.I, Usov A.I , Bilan M.I , Stefanov K.L, Dimitrova-Konaklieva S.D., Tonov D.P, Popov S.S.(1999). Sterols and Polysaccharides in Freshwater Algae *Spirogyra* and *Mougeotia*. Journal Naturforsch. 54c : 1016.ETH.1020.

Mosier, N.S and Lleji, K. (2006). How Fuel Etanol Is Made from Corn. Department of Agricultural and Biological Engineering : Purdue University.

Nowak, J. (2000). Etanol Yield And Productivity of *Zymomonas Mobilis* In Various Fermentation Methods. Electronic Journal of Polish Agricultural Universities. 3 : 2.

Obire, O. (2005). Activity of *Zymomonas* species in palm-sap obtained from three areas in Edo State, Nigeria. Journal of Applied Science and Environment Management. 9 (1)25-30

Purwanto (2004). Aktivitas Fermentasi Alkoholik Cairan Buah. Jurnal Universitas Widya Mandala Madiun. No. 1 th. XXXII/ISSN 0854-1981

Puspita E. M., Silviana, H., Ismail T. (2010). Fermentasi Etanol dari Molasses dengan *Zymomonas mobilis A3* yang diamobilisasi pada κ-karaginan. Seminar Rekayasa Kimia dan Proses. 2010 ISSN: 1411-4216

Sulfahri, S. Mushlihah, E. Sunarto, Y. Irvansyah, R.S. Utami, and S. Mangkoedihardjo. (2011). Ethanol Production from Algae Spirogyra with Fermentation by *Zymomonas mobilis* and *Saccharomyces cerevisiae*. Journal of Basic and Applied Scientific Research. 1(7): 589-593.

Sulfahri, S. Mushlihah, E. Sunarto, and R.S. Utami. (2010). Pemanfaatan Algae *Spirogyra* Sebagai Bahan Baku Bioetanol dengan Penambahan Enzim α-Amilase. Jurnal Purifikasi. 11 : 2.

Struch T., B. Neuss, S. Bringer-Mayer, and H. Sahm. (1991). Osmotic adjustment of *Zymomonas mobilis* to concentrated glucose solutions. Journal Application of Microbiology and Biotechnology. 34 : 518-523.

Tsantili, I.C., K. Nazmul, K.I. Maria. (2007). Quantifying the Metabolic Capabilities of Engineered *Zymomonas mobilis* Using Linear Programming Analysis. Journal of Microbial Cell Factories. 6 : 8

Tze, P. (1998). A Biology of The Algae 3rd edition. Georgetown University : United States of America.

Yang, S., Timothy, J.T., Nancy, L.E., Sue, L.C., Stanton, L.M., Brian, H.D., Anthony, V.P., Miguel, R., and Steven, D.B. (2009). Transcriptomic and Metabolomic Profiling of *Zymomonas mobilis* During Aerobic and Anaerobic Fermentations. Journal of Bioedical Central Genomics. 10:34**.**

Zhang, K and Feng, H. (2010). Fermentation Potentials of *Zymomonas mobilis* and its Application in Etanol Production from Low-cost Raw Sweet Potato. African Journal of Biotechnology. 9(37) : 6122-6128.