Journal of Engineering
Research and Education
Vol. 9 (2017) 11-18

Study of Saccharomyces Cerevisiae Concentration Dependence on the Bioethanol Production Using Rotary Evaporator

E. I. Retnowati[1,]*, H. Zulaika[2] and M. Novita[1]

[1]Faculty of Engineering and Informatics, University of PGRI Semarang.
[2]Chemical Engineering, Institute of Technology Medan.

ABSTRACT

The use of fossil fuel energy has a negative impact on the environment by causing energy pollution and also triggers the global warming problem. Therefore, an alternative of fuel energy sources is highly required. Currently, the alternative fuel energy such as bioethanol has gained a lot of attention. Bioethanol is ethanol resulted from the fermentation of raw materials such as vegetable. The purpose of this work is to investigate the relationship between fermentation time, ratio of organic vegetable waste to water (R) and Saccharomyces Cerevisiae concentration on the quantity of bioethanol production. The vegetable waste collected from one of the markets in Medan was used as the main material in this experiment. The bioethanol produced from samples with various experimental conditions were analyzed by Gas Chromatography (GCMS). The results indicate that the optimum ratio of organic vegetable waste and water to produce the highest bioethanol concentration is 3:1. The samples containing 6% Saccharomyces Cerevisiae with 6 - 10 days fermentation produced ca. 11 - 30% bioethanol. On the other hand, those with 10% Saccharomyces Cerevisiae with 6 - 10 days fermentation produced ca. 13 - 33% bioethanol.

Keywords: Gas chromatography, vegetable waste, fermentation, ethanol, rotav.

INTRODUCTION

Energy resources can be categorized as non-renewable and renewable energy resources. The non-renewable energy resource is a resource that does not renew itself at a sufficient rate for sustainable economic extraction in meaningful human time-frames. Basically, fossil fuel such as gasoline is an example of non-renewable energy resources. On the other hand, the renewable energy resource is those that able renews itself. Biofuel such as ethanol is an example of the renewable energy resource. Since it is an environmentally friendly energy, this material has received increasing attention of Indonesian government as a replacement for gasoline.

* Corresponding author: E-mail address *endangiswahid137@gmail.com1,ZulaikaLubishafni@yahoo.com*

Indonesia has approximately 60 species of plants that have potential as a biofuel energy sources. The use of fossil fuel energy has a negative impact on the environment. Not only causing energy pollution, the used fossil fuel energy also triggers the global warming problem. Therefore, an alternative of fuel energy sources is highly required. Currently, the alternative fuel energy such as bioethanol has gained a lot of attention. Compared to other fuel energies, bioethanol has several advantages such as higher oxygen content which burns more perfectly, higher octane, and environmental friendly. Besides that, the raw material for bioethanol production is relatively abundant in Indonesia. Eventually, raw material has been expected to replace the fossil fuel energy which has been used in the motor vehicles and in the industrial machinery.

The sugar-based-bioethanol can be obtained from molasses, palm sugar, coconut sap, beet, palm, etc. On the other hand, the starch-based-bioethanol can be obtained from cassava, maize, sweet potato, sweet sorghum, grain sorghum, and taro. The fiber-based-bioethanol can be obtained from agricultural waste such as straw, bagasse tapioca, arrowroot, tubers and corn husks, palm empty fruit bunches, bagasse sugar cane, coffee and junk skin of vegetative and fruits (Richana *et al*., 2011).

Initially, Indonesian government focuses on the raw materials for bioethanol production in industrial scale such as cassava and corn drops. However, since they were utilized as human's food, these materials are quite expensive. In addition, since the raw materials as organic waste has not been managed properly, it disturbs the environment, bad smell and affects human health. Generally, these raw organic wastes were thrown away together with those of inorganic waste, and dumped in the landfill. From the total of 4,000 tons garbage per day, the organic waste in Medan is about 70.69% while those of the inorganic waste is about 29.31%, and the amount of waste is continuously increasing.

Therefore, research on the vegetable waste as energy alternative to overcome the above problems is needed. Several investigations on the bioethanol production originating from various types of waste have been reported by several authors. Kusnaedi (2009) investigated the bioethanol fuel originates from the organic waste with 3% tape yeast for 3 - 6 days fermentation. The fermentation successfully produced 31% bioethanol. In the same year, Sari (2009) reported that 20 gr waste of elephant grass with 20% *Saccharomyces Sereviceae* for 6 days fermentation produced 27.7% bioethanol. Later, Papatungan (2013) investigated the bioethanol production originates from the waste of pineapple fruit with 45 gr *Saccharomyces Sereviceae* for 3 days fermentation and the fermentation able to produce 47% bioethanol. In 2015, Jhonatan and Retnowati produced 5.4% bioethanol when performed an experiment using a ratio of household waste and water by 3:1 with 3% *Saccharomyces Sereviceae.* Recently, an experiment using a ratio of vegetable waste and water by 2:1 has been studied by (Jefrinaldi and Gustiawan R, 2016). By

using 7 gr *Saccharomyces Sereviceae* for 15 days fermentation in the rotary evaporator, 35% bioethanol has been produced.

Experimental Procedure

This study used vegetable waste collected from one of the market in Medan (North Sumatra, Indonesia) and Saccharomyces Cerevisiae (called as *S. Cerevisiae* for simplicity). In addition, H_2SO_4, NaOH, glucose, urea, and NPK were also used. The equipment utilized in this study are analytical balance, a set of pads laboratory glassware, pipette volume, measure pipette, a pipette, porcelain dish, pH meter, blender, gauze, thermometer, plastic bottles for fermentation, incubators, hotplate, desiccator, knife, magnetic stirrer, and a set of rotary evaporator (rotav).

The experimental work is designed with two variables; fixed and independent variables. The fixed variables are 300 gr vegetable waste, 10% v/v H_2SO_4, pH 4.5-5, 30^oC fermentation temperature, and 100^oC hydrolysis temperature. On the other hand, the independent variables are the fermentation time symbolized by t, the ratio between vegetable waste and water which is symbolized by R, and the concentration of S. Cerevisiae. The fermentation times indicated by t1, t2, and t3 are 6, 8, and 10 days fermentation respectively. The ratios between vegetable waste and water indicated by R1, R2, and R3 are 1:1, 3:1, and 4:1 respectively. On the other hand, the concentrations of *S. Cerevisiae* are 6% and 10% v/v.

The experimental procedure comprise of several steps where in the first step, the samples were prepared. 300 gr vegetables waste was weighed, washed, and cut. For about 300 gr (R1), 100 gr (R2), or 75 gr (R3) water was added, blended well, and then strained with gauze to obtain vegetable waste concentrate.

Secondly, hydrolysis was performed. Approximately 100 gr vegetable waste concentrate was added with 10% v/v H_2SO_4, preheated at T = 100^oC for about 45 minutes. After the mixture cooled down to room temperature and hydrolysis was stop, sodium hydroxide (NaOH) was added.

Thirdly, the fermentation procedure was performed. *S. Cerevisiae* was activated by adding sugar to it. Let it simmer at 30 - 35^oC for about 24 hours. For each 100 gr of vegetable waste concentrate which has been hydrolyzed and cooled down at room temperature was placed into the fermentation bottles. Then, the *S. Cerevisiae* which has been activated for about ca. 6 and 10 % was added into the bottles. The samples were then fermented for 6, 8, and 10 days at T = 30^oC. After the fermentation process, the samples were then purified using a rotary evaporator (rotav) as shown in Figure 1. It was set to be in the vacuum pressure and the stable temperature (T = 78^o C), so that bioethanol content could be maximized. The bioethanol production from each sample was then analyzed using Gas Chromatography (GCMS).

Figure 1: Rotary evaporator

RESULT AND DISCUSSION

Table 1 and Figure 2 show the bioethanol production from the samples containing 6% *S. Cerevisiae* with various experimental conditions. The fermentation times are indicated by t1, t2, and t3 for 6, 8, and 10 days fermentation respectively. The ratios between vegetable waste and water are indicated by R1 for 1:1, R2 for 3:1, and R3 for 4:1. The result in Table 1 and Figure 2 shows that by 1:1 ratio of vegetable waste and water (R1), ca. 11 - 30% of bioethanol has been produced by the samples with 6 – 10 days fermentation. In the case of 3:1 ratio of vegetable waste and water (R2), ca. 10 - 31% of bioethanol has been produced by samples with the same fermentation time. On the other hand, ca. 11 - 30% of bioethanol has been produced by the samples with 4:1 ratio of vegetable waste and water (R3) and 6 – 10 days fermentation. In this case, R1 which has high water concentration and R3 which has low water concentration, resulted in the low concentration of bioethanol. The maximum bioethanol concentration has been obtained by samples with R2, 3:1 ratio of vegetable waste and water. Basically, the water concentration for fermentation process should not be too low nor too high, otherwise it leads dehydration or hypotonic condition which disturbs the growth of *S. Cerevisiae,* therefore decreases the bioethanol production. Bamforth *et al*. (2005) proposed that the water concentration for fermentation process should not exceed 70%.

The bioethanol production of samples with 6 days fermentation is relatively low. It seems that the *S. Cerevisiae* are still adjusting to environmental condition, thus the microbial activity is not optimum. For longer fermentation time, the cell has passed

the adaptation phase; the cell population increased hence increased the bioethanol production. Therefore, the samples with 10 days fermentation showed the highest production of bioethanol concentration. Results shown in Figure 2 are consistent with the study reported by Kusumaningati *et al*., (2013). The longer the fermentation time the higher the bioethanol production. As the source of nitrogen and fermentation media are still available, the *S. Cerevisiae* grows exponentially until it reaches the maximum number and then continue to grow logarithmically.

Table 1: Bioethanol (%) produced by the samples containing 6% *S. Cerevisiae* with various experimental conditions. The fermentation times are indicated by t1, t2, and t3 for 6, 8, and 10 days fermentation, respectively. The ratios between vegetable waste and water are indicated by R1 for 1:1, R2 for 3:1, and R3 for 4:1

		Fermentation time		
		t1	**t2**	**t3**
Ratio	**R1**	11	30	30
	R2	10	28	31
	R3	11	28	30

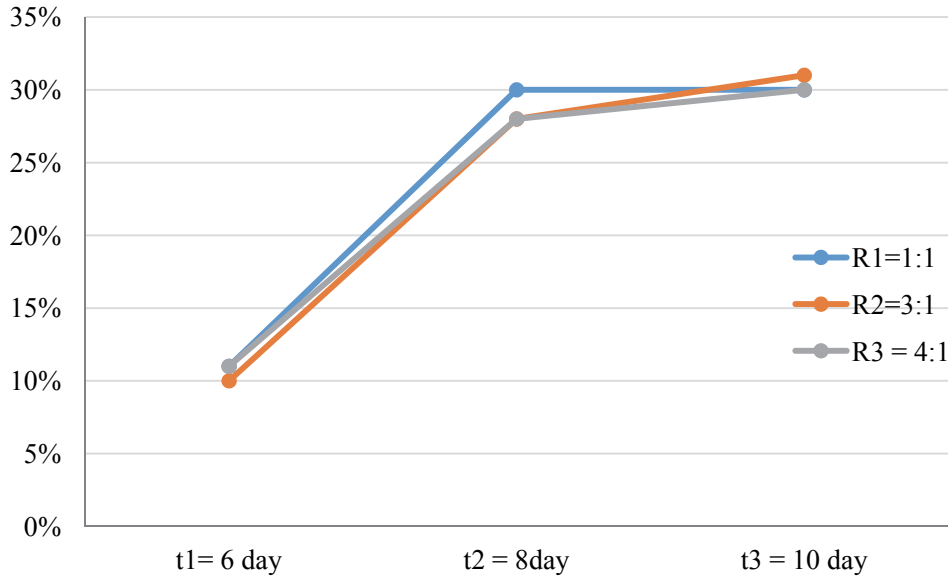

Figure 2: Bioethanol production (%) of samples containing 6% *S. Cerevisiae* with various experimental conditions

Similarly, Table 2 and Figure 3 show the bioethanol production from the samples containing 10% *S. Cerevisiae* with various experimental conditions. Table 2 and Figure 3 show that by 1:1 ratio of vegetable waste and water (R1), ca. 13 - 32% of bioethanol has been produced by the samples with 6 – 10 days fermentation. In the case of 3:1 ratio of vegetable waste and water (R2), ca. 12 - 33% of bioethanol has been produced by samples with the same fermentation time. On the other hand, ca. 12 - 32% of bioethanol has been produced by the samples with 4:1 ratio of vegetable waste and water (R3) and 6 – 10 days fermentation.

Table 2: Bioethanol (%) produced by the samples containing 10% *S. Cerevisiae* with various experimental conditions. The fermentation times are indicated by t1, t2, and t3 for 6, 8, and 10 days fermentation, respectively. The ratios between vegetable waste and water are indicated by R1 for 1:1, R2 for 3:1, and R3 for 4:1

		Fermentation time		
		t1	**t2**	**t3**
Ratio	**R1**	13	32	30
	R2	12	28	33
	R3	12	29	32

Generally, by increasing the concentration of *S. Cerevisiae* (Table 1 and 2) will increase the bioethanol production. This indicates that the higher concentration of *S. Cerevisiae* is more effective to increase the bioethanol production. When 6% *S. Cerevisiae* was considered, the optimum bioethanol production required longer fermentation day. However, comparison among R1, R2, and R3 obtained by samples containing 10% *S. Cerevisiae* is almost similar with those samples containing 6% *S. Cerevisiae*. R2 in both cases produced the highest bioethanol concentration. Nevertheless, with 10 days fermentation, the bioethanol production increased from 31% to 33%, for 6% and 10% *S. Cerevisiae*, respectively. The optimum condition to obtain the highest bioethanol concentration is R2 and t3, 3:1 ratio of vegetable waste and water with 10 days fermentation. In this case, there is no requirement to increase the concentration of *S. Cerevisiae* since it may reduce the cell viability (Kusumaningati, *et al*., 2013). Similarly, the optimum fermentation time has been reached and therefore no extra fermentation time is required.

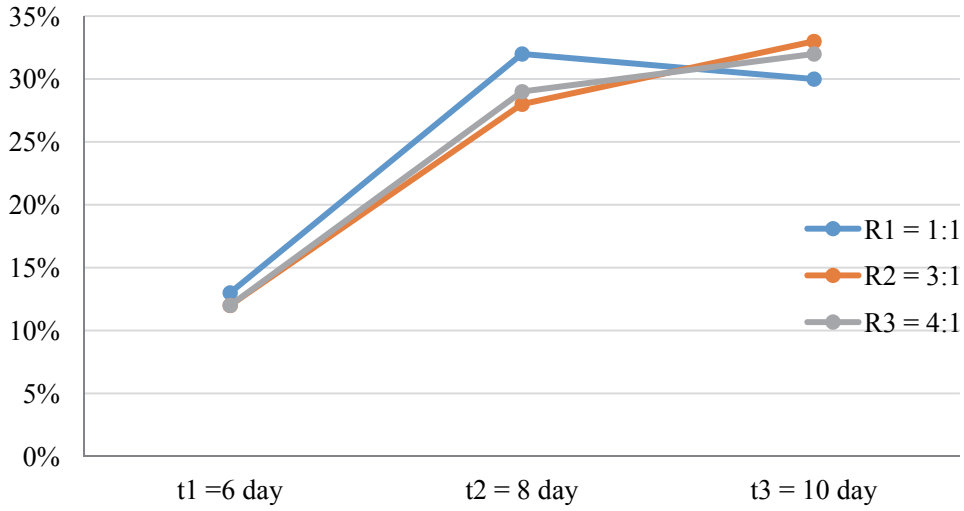

Figure 3: Bioethanol production (%) of samples containing 10% *S. Cerevisiae* with various experimental conditions

CONCLUSION

In this work, the *S. Cerevisiae* concentration dependence on the bioethanol production of vegetable waste using rotary evaporator has been studied. The relationship between the fermentation time, the ratio of organic vegetable waste to water (R) and *S. Cerevisiae* concentration on the quantity of bioethanol production were investigated. First, the samples with several experimental conditions were prepared. The samples mainly consist of the vegetable waste, water, and *S. Cerevisiae*. The ratios of vegetable waste and water were varied from 1:1 (R1), 3:1 (R2), and 4:1 (R3). On the other hand, the *S. Cerevisiae* concentrations were varied from 6 to 10%. Next, these samples were treated carefully and then fermented for 6 days (t1), 8 days (t2), and 10 days (t3). Finally, they were purified using Rotary Evaporator and analyzed using Gas Chromatography. The results show that increasing the fermentation time, increased the bioethanol production. In the case of samples containing of 6% *S. Cerevisiae*, increasing the fermentation time from 6, to 8 and to 10 days increased the bioethanol production from 11 to 31%. In the case of samples that contain 10% *S. Cerevisiae*, the bioethanol production increased from 12 to 32%. Increasing the ratio of vegetable waste and water also increased the bioethanol production. However, the optimum ratio to obtain the highest bioethanol production was 3:1. Last but not the least, the *S. Cerevisiae* plays an important role in increasing the bioethanol production.

ACKNOWLEDGEMENT

The authors would like to convey gratefulness to Directorate of Research and Community Service, strengthening of the Directorate General of Research and Development, Ministry of Research, Technology and Higher Education in financing this competitive grant funding in 2016. This study was made based on competitive research grants of aforementioned state institutions. The authors also grateful for the support from University of PGRI Semarang and Institute of Technology Medan, who have facilitated to ease the procedures so that this research can be carried out satisfactorily.

REFERENCES

[1] Bamfortth, C. W. (2005). "Food, Fermentation and Microorganism", Blackwell Science Ltd. Blackwell Publishing Company, Iowa, USA.
[2] Gozan, M. (2014). "Bioetanol Techcnology Teknologi Second Generaration", Erlangga Publish, Jakarta.
[3] Jefrinaldi and Gustiawan, R. (2016). "Optimize bioethanol Production from vegetable Waste Medan City" Reaserch Report, Chemical Engeneering, Institute of Technology Medan.
[4] Jonathan and Retnowati, E. I. (2015). "Influence Organic Garbage to Water Ratio for Bioethanol Production" Reaserch Report, Chemical Engineering , Institute of Technology Medan.
[5] Kusumaningati. (2013). "Effect Consentration Inoculum Zymomonas Mobilis Bacteri dan fermentation time for etanol Production from vegetable and fruit Pasar Wonokromo Market Surabaya, Jurnal Sains dan Seni Pomits, 2(2), (2013), E 218-223.
[6] Shuler. (1992). "Bioprocess Engineering", Prentice Hall International, Inc., New Jersey.
[7] Riadi, L. (2007). "Fermentation technology", Graha Ilmu, first Edition, Yogyakarta.
[8] Roger, Y. S., *et al*. (1986). "The Microbial World", Prentice Hall International, Inc., New Jersey.
[9] Richana, N. (2011). "Bioetanol: Raw Material, Technology, Production and Quality Control", Nuansa Publish, First Publish, Bandung.
[10] Shuichi, A. (1973). "Biochemical Engineering", University of Tokyo Press, Second Ed., Tokyo.
[11] Speece, R. E. (1996). "Anaerobic Biotechnology", Archae Press, Tennessee, USA.