The Effect of Inoculum Volume on Bioethanol Production from Saba Banana Hump (Musa Paradisiacal. L) Starch by Zymomonas Mobilis using Immobilization Technique

Rifqi Syahbana[1] , Emma Zaidar Nasution[2*] , Rumondang Bulan Nasution[2]
[1]*Postgraduate Chemistry Study Programme, Universitas Sumatera Utara, Jl. Bioteknologi No. 1, Medan, Indonesia*
[2]*Department of Chemistry, Universitas Sumatera Utara, Jl. Bioteknologi No. 1, Medan, Indonesia*

Keywords: Bioethanol, Immobilization, Inoculum volume, Saba Banana Hump, Zymomonas Mobilis.

Abstract: Bioethanol is a liquid produced from the sugar fermentation process from carbohydrate sources by using the microorganism. In present study, Saba banana hump (Musa paradisiacal. L) sample was isolated by precipitating the starch with water for 12 hours. Furthermore, the starch was hydrolyzed using HCl 25%, and 14.01% of glucose solution was obtained. The starch obtained was tested using Fourier-transform infrared spectroscopy. Then the sample was fermented using Zymomonas mobilis microorganism immobilized by Ca-Alginate 4% with variation of inoculums 5%, 10%, 15% (v/v). Bioethanol from fermentation process was tested using gas chromatography (GC). The highest bioethanol content is 79.01% and the highest productivity that obtain was 2.66 g/L.Hours.

1 INTRODUCTION

Banana hump is the bottom of a banana plant that has characteristics that are not too hard, banana hump waste has a starch composition of approximately 70%, 20% water, the rest is protein and vitamins. For the banana hump itself has not been used optimally, where the banana weevil waste is sometimes only as animal feed or left alone to rot. The carbohydrate content of banana hump is very potential as a source of bioethanol, so that it can increase the use value of the banana weevil and can optimize the utilization of the banana hump. As for one type of carbohydrate that is in the banana weevil is starch.

Starch is a carbohydrate polymer consisting of glucose monomers with the molecular formula $(C_6H_{10}O_5)_n$. Starch is known as biocompatible, biodegradable, non-toxic, environmentally friendly and inexpensive natural polysaccharides (Atwell, W.A., Hood, L., Lineback, D., Varriano-Morston, E., Zobel, 1988; Rodrigues and Emeje, 2012). Starch can be a source of glucose in the fermentation process that produces bioethanol by hydrolyzing starch with a dilute acid solution or enzyme.

Bioethanol in the realm of renewable energy is one source of energy that continues to be developed. Bioethanol is the center of attention because this compound can be used as fuel (Setiadji *et al.*, 2017). The raw materials for the bioethanol production process are classified into three groups, namely sugar, starch and cellulose. Sources of sugar derived from cane sugar, beet sugar, molasses and fruits can be directly converted to ethanol. Sources of starchy ingredients such as corn, cassava, potatoes and plant roots must first be hydrolyzed into sugar. Sources of cellulose derived from wood, agricultural waste, pulp and paper mill waste as a whole must be converted to sugar with the help of mineral acids (Lin and Tanaka, 2006).

Z. mobilis is a gram-negative, stem-shaped bacterium, can be found in sugar-rich sap plants, has a length of 2-6 μm and a width of 1-1.4 μm, the optimum temperature for growth is 25-30ºC, and is anaerobic. Z. mobilis can ferment glucose, fructose, but cannot ferment silosa (Gunasekaran and Chandra Raj, 1999; Geeta, 2007). Zymomonas mobilis bacteria can survive on glucose level

Immobilization is the process of wrapping (coating) a core material, in this case bacteria as a core material by using its viability and protecting it from damage due to environmental conditions that are not possible (Wu, Roe and Gimino, 2000). (Pacifico, Wu and Fraley, 2001) states that for sensitive components such as microorganisms, it can be

Syahbana, R., Zaidar Nasution, E. and Bulan Nasution, R.
The Effect of Inoculum Volume on Bioethanol Production from Saba Banana Hump (Musa Paradisiacal. L) Starch by Zymomonas Mobilis using Immobilization Technique.
DOI: 10.5220/0010133200002775
In *Proceedings of the 1st International MIPAnet Conference on Science and Mathematics (IMC-SciMath 2019)*, pages 53-58
ISBN: 978-989-758-556-2

immobilized to increase viability of shelf life. Common ingredients used for immobilization are various types of polysaccharides and proteins such as starch, alginate, arabic gum, gelatin, carrageenan, albumin, and casein.

Research has been carried out on immobilization of zymomonas mobilis cells and saccharomyces cerevisiae to increase the production of bioethanol from sugar from the hydrolysis of sugarcane bagasse waste. The results showed ethanol levels increased using cells immobilized by the addition of sugarcane bagasse to the modification of the calcium alginate matrix, where the efficiency of fermentation in zymomonas mobilis increased by 1.8 times while in S. cerevisiae by 1.6 times (Kusumaningati, Nurhatika and Muhibuddin, 2013).

The purpose of this study was to determine the levels of bioethanol produced from zymomonas mobilis fermentation with immobilization techniques.

2 METHODS

2.1 Isolation of Starch from Kepok Banana Weevil

Kepok banana weevil that is obtained is then peeled and cleaned with clear water, then cut into cubes and then put into a bucket and soaked with 0.2% $Na_2S_2O_5$ for 12 hours. Furthermore, the banana weevil is dried in the sun, then blended until smooth and then filtered using an 80 mesh sieve. Then dissolved with Aquadest and deposited for 24 hours. Furthermore, starch is separated from the solution and roasted at a temperature of 45^{o}C for 24 hours.

2.2 Starch Hydrolysis

The starch obtained was weighed as much as 20 g then put into a 250 ml glass beaker, added as much as 100 ml 25% HCl and covered with alumanium foil. Then it is heated at 80^{o}C while stirring for 30 minutes. After being cooled, the hydrolyzate was adjusted to neutral pH using NaOH 0.7%.

2.3 Making YEPD (Yeast Extract Pepton Dextrose) Media

The making of YEPD media is by dissolving 4 g Yeast extract, 2 g $KH_2PO_{4(s)}$, 3 g $(NH_4)_2SO_{4(s)}$, 1 g $MgSO_4.7H_2O_{(s)}$, 3.6 g Pepton and 2% Bacto agar with 1000 ml Aquadest. Then heated on a hotplate until it is clear yellow.

2.4 Making Cell Immobilization

Nutrient media mixed with 20 gram (NH_4) $2SO_4$ 18 gram starch, 10 gram $NaHPO_4$, 5 gram KH_2PO4, 5 gram $MgSO_4.7H_2O$ and 1 gram yeast extract added to culture. Put in the shaker incubator for 24 hours. Then mixed 50 ml of nutrient media with 50 ml of 4% alginate solution. Next 100 ml of Alginate-cell mixture was added into 1000 ml of 2% $CaCl_2$ solution until the solution was in the form of solids and allowed to stand to harden for 30 minutes. Subsequently washed solids with 0.85% NaCl and incubated into the production medium with shaking for 24 hours. Then the solid is stored in the yeast extract at 4^{o}C until the cell is used.

2.5 Hydrolyzed Fermentation using Inoculum Z. Mobilis

Fermentation using inoculum Z. Mobilis by dissolving 4 g Yeast extract, 2 g $KH_2PO_{4(s)}$, 3 g (NH_4) $2SO_4$ (s), 1 g $MgSO_4.7H_2O_{(s)}$, 3.6 g Pepton and 2% agar Bacto with 1000 ml of hydrolyzate. Then sterilized using an autoclave for 2 hours at 121^{o}C. After the cold hydrolyzate was added inoculum Z. Mobilis were 5%, 10%, 15% with the amount of bacteria 9.0 x 10^8 (McFarland 3). Then it is tightly closed using alumanium foil and plastic wrap and put in a shaker incubator for 21 hours at 30^{o}C while the dishaker is at 100 rpm.

2.6 Separation of Bioethanol from Fermentation Solutions

500 ml fermentation solution was put into 1000 ml rotav flask then CaO was added to the fermentation solution at a ratio of 1:2 (g / ml) and then in the rotary evaporator at 78^{o}C for 1 hour. Then the ethanol qualitative test on the distillate obtained.

2.7 Quantitative Analysis of Bioethanol using GC

Fermentation solution as much 500 ml was put into a 1000 ml rotav flask then CaO was added to the fermentation solution at a ratio of 1:2 (g / ml) and then in the rotary evaporator at 78^{o}C for 1 hour. Then the ethanol qualitative test on the distillate obtained.

3 RESULTS AND DISCUSSION

3.1 Isolation of Starch from Saba Banana Hump

The results of isolation of starch from Saba Banana Hump by stopping the browning process using 0.2% Na_2S_2O for 12 hours, so that the squeeze process will produce a white milk solution and deposited for 24 hours. From 1000 grams of saba banana hump obtained 157 grams of starch banana weights that are milky white. The results of Saba Banana Hump starch can be seen in Figure 1

Figure 1: Results of Isolation of Starch from Saba Banana Hump.

Starch isolation aims to be hydrolyzed into sugar. The sample as a source of starch used is banana weevil. From 1000 grams of Saba Banana Hump obtained 157 grams of starch banana weights that are milky white. Then a qualitative test was carried out on the starch that had been obtained using iodine solution. Iodine test is used to detect the presence of starch.

The iodine test is carried out by means of a starch sample inserted into the test board, adding one drop of aqueous iodine solution (Widyaningsih, Kartika and Tri Nurhayati, 2012). Then mixed evenly. Test positive iodine for starch with the appearance of blue after adding iodine to the sample. Starch that binds to iodine will produce a blue color and this property can be used to analyze the presence of starch. This is caused by the structure of the spiral starch molecule so that it will bind to the iodine molecule and form a blue color (Winarno, 2004).

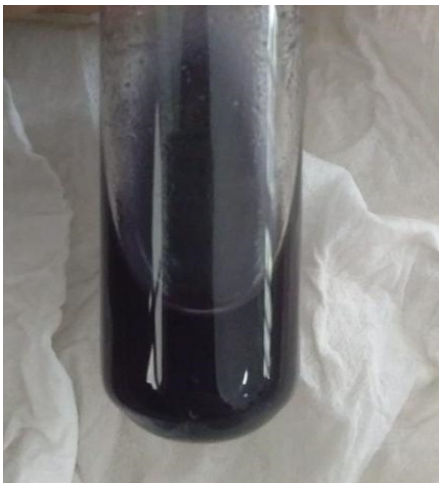

Figure 2: Qualitative Test of Saba Banana Hump.

3.2 Analysis of Saba Banana Hump Starch using FT-IR Spectroscopy

Functional group analysis in Saba Banana Hump starch and commercial starch using FT-IR spectrocopy showed almost the same wave number and spectrum shape. Spectrum results and wave numbers can be seen in Figure 3.

Observed at a glance, the FT-IR spectra pattern of saba banana hump starch and commercial starch above shows the number and location of peaks that are relatively the same. Thus it can be interpreted that the results of insulation are starches.

From FT-IR spectra of saba banana hump starch and commercial starch, each absorption peak at wave numbers 3336 cm^{-1} and 3286 cm^{-1} is associated as OH strain vibrations of alcohol in starch molecules, followed by absorption peaks at wave numbers of 2931 cm^{-1} is associated as the CH stretch vibration of the alkane chain. Whereas absorption at wave number 1639 cm^{-1} is associated as O-H (H_2O), then absorption at wave numbers 1408 cm^{-1} and 1350 cm^{-1} indicates the presence of C-H groups (Wijaya *et al.*, 2019). In addition, uptake at wave numbers of 1149 cm^{-1} and 1145 cm^{-1} is associated as vibrational strain of C-O-C in the starch ring (Leon, 2016).

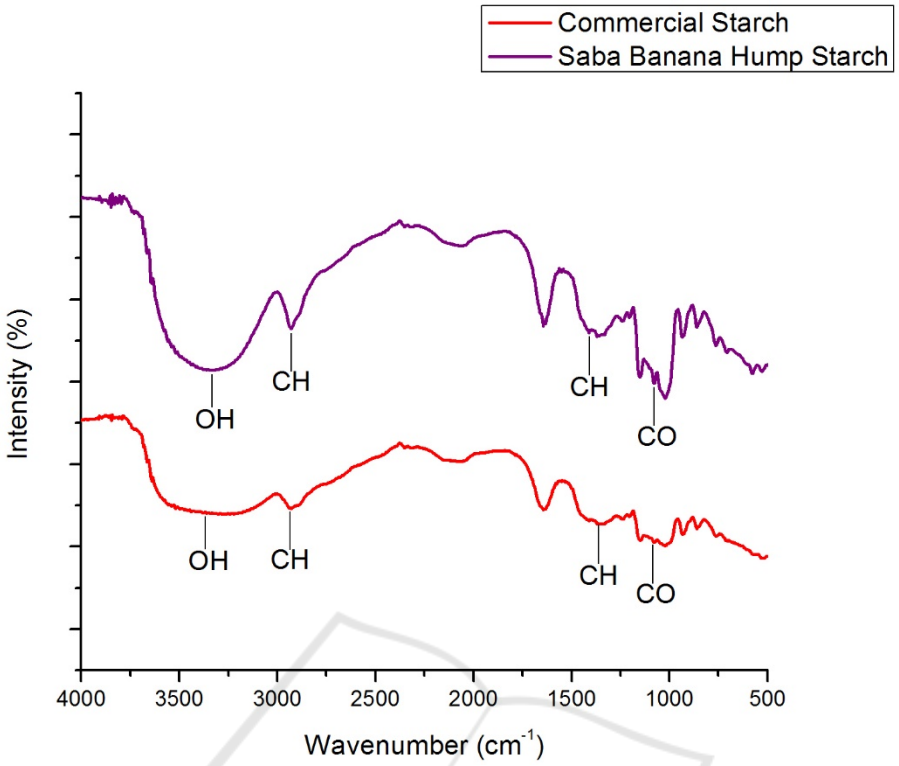

Figure 3: Results of FT-IR Spectrum of Saba Banana Hump Starch and Commercial Starch.

3.3 Starch Hydrolysis

Hydrolysis of 20 gram saba banana starch using 25% HCl solution and 30% NaOH produced 14.01% glucose solution. Hydrolysis of saba banana starch using 25% HCl solution at 90°C produces glucose solution. The purpose of hydrolysis is to break down carbohydrates into simple sugars so that they can be used in the fermentation process. In this research, HCl acid is used as a catalyst in the hydrolysis process. HCl catalysts produce higher glucose when compared with H_2SO_4 catalysts. This is due to H_2SO_4 being combustible while HCl is not so that the use of HCl catalyst is more optimal in producing reducing sugars. The acid produces H^+ ions and binds with H_2O to form H_3O^+ will break the gilosidic bonds in amylose and amylopectin to form simple monomers (Balat, Balat and Öz, 2008).

3.4 Cell Immobilization Process

The process of immobilization of zymomonas mobilis bacteria is to add a bacterial inoculum into the nutrient media with variations in the bacterial inoculum 5%, 10%, and 15%. After the next incubation process 4% alginate solution will be added in a ratio of 1:1 to a 2% CaCl solution which results in a compact and round solid beads. Various studies have shown that calcium alginate protects culture cell immobilization better by increasing bacterial resistance than without immobilization (Anal and Singh, 2007). Alginates can form gels (egg-box formations), films, manic (beads), pellets, microparticles, and nanoparticles (Natalia, 2015).

Table 1: Bioethanol results from the fermentation of Saba banana hump starch.

No	Zymomonas mobilis Inoculum (%)	Bioethanol Content (%)	Productivity (g/L.Hours)
1	5	68.93	2.49
2	10	79.01	2.60
3	15	77	2.66

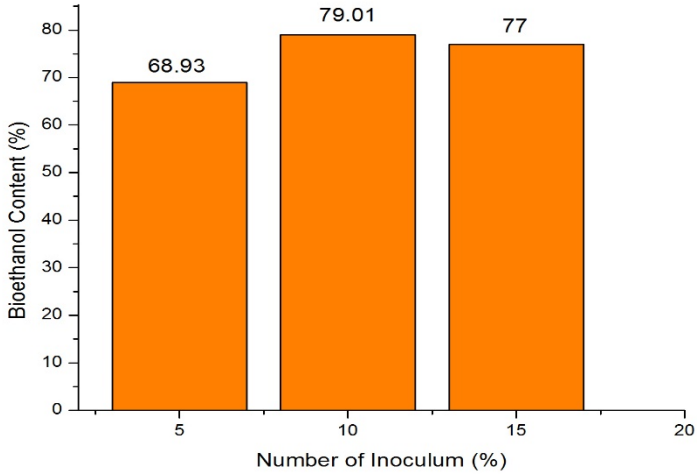

Figure 4: Graph of Bioethanol Levels.

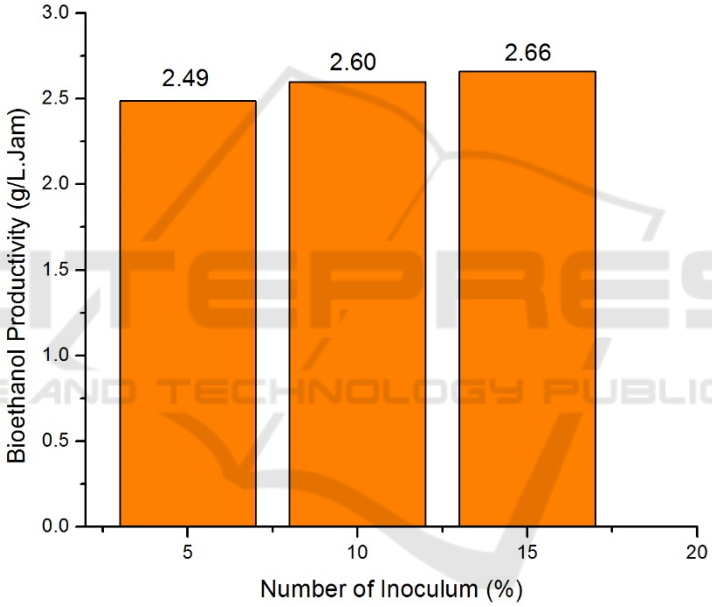

Figure 5: Graph of Bioethanol Productivity.

3.5 Fermented Banana Starch Results Kepok Banana

The fermentation process is carried out using an immobilized Zymomonas Mobilis inoculum. Where with the treatment of variation inoculum Zymomonas Mobilis as much as 5%, 10%, and 15%. Furthermore, the separation of bioethanol from the fermentation solution using a rotary evaporator. Then the fermentation results are tested using Gas Chromatography. In the variation of zymomonas mobilis inoculum as much as 5%, 10%, and 15%, the results were fluactative yield. The results of bioethanol can be seen in table 1.

From Figure 4 the diagram of the effect of inoculum volume on bioethanol levels can be explained that at 5% inocolum volume of bioethanol produced is 68.93%, whereas an increase in bioethanol levels in 10% inoculum volume with a duration of 79.01%. However, there was a decrease in levels of bioethanol at 15% inoculum volume treatment by 77%. Hisreidi, (2016) said the higher inoculum volume indicates that more and more populations of Zymomonas mobilis bacteria are fermenting and as a result the higher levels of bioethanol produced (Hisreidi, 2016).

Figure 5 is a diagram of bioethanol productivity with Zymomonas Mobilis inoculum variations of 5%,

10%, and 15%. Bioethanol productivity in zymomonas mobilis inoculum variation of 5%, 10%, and 15% showed increased results, namely 2.49%, 2.6%, and 2.66%. The highest results were obtained in the zymomonas mobilis inoculum variation 15% by 2.66%, while the lowest results were obtained in the zymomonas mobilis inoculum variation 5% by 2.49%. It can be concluded that the higher the level of bioethanol, the higher the value of bioethanol productivity

4 CONCLUSIONS

Saba banana hump can produce starch that has a white color. Starch from kepok banana weevil hydrolyzed using acid to produce glucose as much as 14.01% with 20 grams of starch content. The highest bioethanol content is 79.01% and the highest productivity that obtain was 2.66 g/L.Hours.

REFERENCES

Anal, A. K. and Singh, H. (2007) 'Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery', *Trends in Food Science and Technology*, pp. 240–251. doi: 10.1016/j.tifs.2007.01.004.

Atwell, W.A., Hood, L., Lineback, D., Varriano-Morston, E., Zobel, H. (1988) 'The terminology and methodology associated with basic starch phenomena', *Cereal Food World*, 33, pp. 306–311.

Balat, M., Balat, H. and Öz, C. (2008) 'Progress in bioethanol processing', *Progress in Energy and Combustion Science*, pp. 551–573. doi: 10.1016/j.pecs.2007.11.001.

Geeta, M. S. G. & G. S. (2007) 'Effectivenes of Fungal Pretreament of Agro Residues on Etanol Production by Yeast and Zymomonas mobilis', 2, pp. 301–304.

Gunasekaran, P. and Chandra Raj, K. (1999) 'Ethanol fermentation technology - Zymomonas mobilis', *Current Science*.

Hisreidi (2016) *Pengaruh Volume Inokulum Pada Produksi Bioetanol Dari Kulit Pisang Kepok Kuning (Musa paradisiaca L. var. Kepok Kuning) Menggunakan Zymomonas mobilis Dengan Metode Solid State Fermentation (SSF)*. Universitas Sanata Dharma.

Kusumaningati, M. A., Nurhatika, S. and Muhibuddin, A. (2013) 'Potensi Kapang Aspergillus sp. dalam Proses Hidrolisis untuk Produksi Etanol dari Sampah Sayur dan Buah Pasar Wonokromo Surabaya', *Makalah Orasi Ilmiah*.

Leon (2016) *AC SC*, *Food Hydrocolloids*. Elsevier Ltd. doi: 10.1016/j.foodhyd.2016.01.008.

Lin, Y. and Tanaka, S. (2006) 'Ethanol fermentation from biomass resources: Current state and prospects', *Applied Microbiology and Biotechnology*, pp. 627–642. doi: 10.1007/s00253-005-0229-x.

Natalia, S. . (2015) *Viabilitas enkapsulasi sinbiotik isolat BAL dengan berbagai bahan enkapsulasi selama masa simpan dan simulasi asam lambung*. Universitas Sumatera Utara.

Pacifico, C., Wu, W. and Fraley, M. (2001) 'Sensitive substance encapsulation', *US Patent 6,251,478*, 1(12), pp. 1–8.

Rodrigues, A. and Emeje, M. (2012) 'Recent applications of starch derivatives in nanodrug delivery', *Carbohydrate Polymers*, pp. 987–994. doi: 10.1016/j.carbpol.2011.09.044.

Setiadji, S. *et al.* (2017) 'Alternatif Pembuatan Biodiesel Melalui Transesterifikasi Minyak Castor (Ricinus communis) Menggunakan Katalis Campuran Cangkang Telur Ayam dan Kaolin', *Jurnal Kimia VALENSI*, 3(1), pp. 1–10. doi: 10.15408/jkv.v3i1.4778.

Widyaningsih, S., Kartika, D. and Tri Nurhayati, Y. (2012) 'PENGARUH PENAMBAHAN SORBITOL DAN KALSIUM KARBONAT TERHADAP KARAKTERISTIK DAN SIFAT BIODEGRADASI FILM DARI PATI KULIT PISANG', *Molekul*, 7(1), p. 69. doi: 10.20884/1.jm.2012.7.1.108.

Wijaya, C. *et al.* (2019) 'Heliyon Isolation and characterization of starch from Limnophila aromatica', *Heliyon*. Elsevier Ltd, 5(October 2018), p. e01622. doi: 10.1016/j.heliyon.2019.e01622.

Winarno, F. G. (2004) *Kimia Pangan dan Gizi*. Jakarta: PT. Gramedia.

Wu, W., Roe, W. and Gimino, V. (2000) 'Low melt encapsulation with high laurate canola oil', *US Patent*