



KEMENTERIAN PENDIDIKAN, KEBUDAYAAN, RISET DAN TEKNOLOGI
UNIVERSITAS PEMBANGUNAN NASIONAL "VETERAN" JAWA TIMUR

UPT TEKNOLOGI INFORMASI DAN KOMUNIKASI

Jl. Raya Rungkut Madya Gunung Anyar Surabaya Telp.(031) 8793589
Laman: <http://upttik.upnjatim.ac.id>, Email: upttik@upnjatim.ac.id

SURAT KETERANGAN
HASIL PEMERIKSAAN TINGKAT PLAGIARISME
Nomor : 244/UN63/UPTTIK/VII/2022

Yang bertanda-tangan di bawah ini,

Nama : Mohamad Irwan Afandi, ST, M.Sc.M.Sc.

NI P3K : 197607182021211003

Jabatan : Kepala UPT TIK – UPN “Veteran” Jawa Timur

dengan ini menerangkan bahwa Penulis telah melakukan pemeriksaan tingkat kesamaan (plagiarisme) menggunakan *software Turnitin* secara mandiri terhadap dokumen dalam daftar di bawah ini:

Judul Karya Tulis : Synthesis of Bioethanol from Cocoa Pod Husk Using
Zymomonas Mobilis

Jenis Publikasi : Jurnal

Penulis : NOVE KARTIKA ERLIYANTI , ST., MT.

Tingkat Kesamaan (%) : 9%

Demikian Surat Keterangan ini dibuat dengan sebenarnya, agar dapat digunakan sebagaimana mestinya.

Surabaya, 18 Juli 2022

Kepala UPT TIK

Mohamad Irwan Afandi, ST, MSc.

NI P3K 197607182021211003

17. Synthesis of Bioethanol from Cocoa Pod Husk Using Zymomonas Mobilis

by Nove Kartika Erliyanti

Submission date: 21-Apr-2021 09:04AM (UTC+0700)

Submission ID: 1565218741

File name: esis_of_Bioethanolfrom_Cocoa_Pod_Husk_Using_ZymomonasMobilis.pdf (277.71K)

Word count: 2695

Character count: 13667



Article

Synthesis of Bioethanol from Cocoa Pod Husk Using *Zymomonas Mobilis*

Mu'tasim Billah¹, Tikat Deri Agratiyan¹, Dhining Ayu¹, Nove Kartika Erliyanti¹, Erwan Adi Saputro¹, Rachmad Ramadhan Yogaswara^{1,a*}

¹Chemical Engineering, Engineering Faculty, Universitas Pembangunan Nasional "Veteran" Jawa Timur. Jalan Raya Rungkut Madya Gunung Anyar, Surabaya, Indonesia, 60249
E-mail: ^{a*}r.yogaswara.tk@upnjatim.ac.id

Abstract. Nowadays, energy issue is one of the interesting discussions among the researchers and energy stakeholders. This is due to the reducing of the main source of world energy which consists mostly of non-renewable energy derived from fossils of living things millions of years ago. Alternatively, there are some alternative energy that promising in the future such as bioethanol from plant or fruits. For that reasons, the aim of this research is to make bioethanol from the peel of cocoa fruit. Cocoa pod husk can be used as source of bioethanol through a fermentation process with the help of bacteria. This study will examine the ethanol content in fermented cocoa pod husks. The fermentation time was 0 days, 2 days, 4 days, 6 days, 8 days. The *Zymomonas mobilis* bacteria is used in the fermentation process and their percentage is 8%v/v, 10%v/v, 12%v/v, 14%v/v, 16%v/v. In the process of fermentation of cocoa pod husk with *Zymomonas mobilis* inoculum on the 3rd day to 9th showed an increase. On the eleventh day in all conditions the addition of a starter was decreased. The best condition when fermentation takes place on the 8th day with a starter dose of 14% and obtained an alcohol content of 10.62%. The results of this study indicate that the addition of *Zymomonas mobilis* starter and fermentation time affect the levels of bioethanol produced. The addition of a starter as much as 14% and the fermentation time for 8 days are the best condition in this study.

Keywords: Bioethanol, *Zymomonas mobilis*, Cocoa pod husk, Fermentation

International Journal of Eco-Innovation in Science and Engineering Volume 01 No. 01

Received Date 14th April 2020

Accepted Date 9th May 2020

Published Date 15th May 2020

Online at <https://ijeise.upnjatim.ac.id/>

1. Introduction

The depletion of fossil fuel reserves and the increasing human population is very contractive with the energy needs for human survival, economic and social activities. In the last five years, Indonesia has experienced a decline in national oil production as a result of a decline in natural oil reserves in production wells, despite the increase in population, the need for transportation facilities and industrial activities [1]. This result in an increase in demand and consumption of fuel oil which is a non-renewable natural resource. The Indonesian government still imports part of the fuel to meet domestic needs [2]. Up to now there have been quite a lot of biofuels developed from cassava, sugar cane, sugar palm, sorghum and other materials [3]. However, this plant has another use value as food. If the plant is used as raw material in producing ethanol commercially, it will result in the change of food functionality [4].

One of the most potential plants in Indonesia due to its abundant availability is cocoa. According to plantation statistics, the total area of cocoa in Indonesia in 2015 was recorded at 1,622,600 Ha with a production of 641,997 tons of cocoa and a productivity level reaching 800 kg/ha. The weight of the cocoa fruit harvested in Indonesia per Ha around 7751 kg of fruit peels and 2733 kg of wet seeds [2]. If the waste of the husk is not handled properly, it can cause environmental problems. On the other hand, the process of making bioethanol requires raw materials with less economic and do not compete with the food material [5]. Furthermore, the cocoa pod husk has a lot of cellulose allowing it to be used as raw material for bioethanol synthesis [6].

Several studies have been conducted relating to the utilization of organic waste to be used as raw material for making fuel, namely bioethanol. In bioethanol research, most people use *Jathropa* plant. The yields of the saccharification and fermentation processes could reach a level more than 39% [7]. Besides that, the same enzymatic method can be applied to synthesize biofuel from papaya seed. The vegetable oil from papaya seed that can be converted to biofuel is around 30% [8].

There are various types of microorganisms that are used to produce bioethanol, one of the most widely used microorganisms in the bioethanol company in the world is *Zymomonas mobilis*. This bacterium can surpass *Saccharomyces cerevisiae* within this process. The advantages of bacterium are including the growth of facultative anaerobes and having a high temperature tolerance, the ability to achieve higher conversion, resistant to high ethanol levels and low pH [9]. In fact, there are not many studies that reveal the ability of

Zymomonas mobilis bacteria to convert cocoa pod husk into bioethanol. For that reason, this investigation will use the waste of cocoa pod husk as a raw material to produce bioethanol. The research aims are to gain the best composition of the volume of *Zymomonas mobilis* starter and the optimum day of bioethanol production.

2. Materials and method

The cocoa pod husk that has a yellowish-brown color and medium texture with water content around 60 – 80% was used as raw material in this study. This cocoa pod husk was obtained from *Kampung Cokelat, Kademangan, Blitar, Indonesia*. Bacteria used *Zymomonas mobilis* included the nutrient broth. And, the supporting materials for analysis are 97% concentrated H_2SO_4 , NaOH, and glucose. The equipment used are a set of hydrolysis, one set of fermentation unit, and a set of distillation apparatus. Moreover, other supporting equipment such as asthermometers, ovens, analytical balance, autoclaves, incubators and other laboratory tools are utilized in this study.

2.1. Preparation

There are two processes that carried out to prepare the cocoa pod husk before fermentation. These two processes are delignification and hydrolysis process. First, 100 grams of the cocoa pod husk were boiled at 70°C for 1 hour and then were dried at the same temperature for 1 hour. After that, delignification of the cocoa pod husk was done by immersing and mixing this dried pod husk into 500 ml of 10% NaOH solution for 1 hour. Then, this cocoa pod husk was neutralized with distilled water to pH 8.

The cocoa pod husk as much as 100 grams from delignification process before were mixed with H_2SO_4 10% (v/v) of the total solution. Then, distilled water was added to the volume of 500 ml. Then the hydrolysis process was conducted with a temperature of 100°C for 3 hours at 300 rpm. Finally, the solution of hydrolysis was filtered and the filtrate was taken for analyzing the glucose level.

2.2. Fermentation process

Zymomonas mobilis bacteria starter was prepared by this following procedure. There are several materials used for bacterial growth media including 6 grams of nutrient broth, 1 gram of glucose, and 500 ml of distilled water. *Zymomonas mobilis* was inoculated on the media and incubated at 30°C for 1 day. Then, 250 ml of filtrate resulting from the hydrolysis process were put in a bottle to be fermented, covered tightly with cotton and aluminum foil. The filtrate was sterilized from the hydrolysis process into the auto clave at 120°C for 15

minutes and cooled to 30°C. After wards, *Zymomonas mobilis* bacteria starter was added with the variables carried out of 8, 10, 12, 14, and 16 (%v/v). Then it was fermented with the run time variable of 0, 2, 4, 6 and 8 days. The fermented solution was filtered and distilled in order to purify the ethanol product.

2.3 Analysis

There are two compounds analyzed in this study that is glucose as a fermentation reactant and bioethanol product as well. Distillate product that contains ethanol compound was measured its density using pycnometer. Then, its specific gravity was calculated and the ethanol content was obtained from AOAC (Association of Official Agricultural Chemists) table based on the specific gravity value of product.

Otherwise, glucose that was a product from hydrolysis process was measured using Nelson-Somogyi method. 1 ml of sample was taken from hydrolysis filtrate and mixed with 1 ml of Nelson solution. This mixture, then was heated at 100°C for 2 hours. After that, the mixture was cooled into room temperature and was added by 1 ml of arsenomolybdate reagent and 7 ml of distilled water. The final mixture was measured its absorbance by Spectrophotometry UV-Vis with 510 nm of wave length. This absorbance value can be converted into glucose concentration.

3. Results and discussion

Cocoa pod husk is one of biomass waste having good prospect to be utilized as a feedstock for bioenergy production due to its abundant availability. Cocoa pod husk contains high carbohydrate and cellulose components as shown in table 1, so that it must be converted first into glucose via hydrolysis process [6]. This hydrolysis process using H₂SO₄ solution with 10% (v/v) of concentration yields the product with 9.89% (v/v) of glucose content. This result is quite smaller than Nazir et al. study which cocoa pod husk was treated by the same acid solution yet with different concentration (1.5%w/v) resulting 15.59% of glucose content as reducing sugar [10].

Table 1. Composition of cocoa pod husk (g/100 g of dry matter) [6]

Compound	Value
Carbohydrates	29.04-32.2
Cellulose	24.24-35.0
Hemicellulose	8.72-11.0
Lignin	14.6-26.38
Total proteins	4.21-10.74
Lipids	1.5-2.24
Pectin	6.1-9.2
Ash	6.7-10.02
Total dietary fiber	36.6-56.10

In the fermentation process, there is not only enough glucose levels that influences the reaction kinetics, but also other factors such as fermentation temperature, optimal pH, microbes, nutrients needed by microbes and reaction time [11]. Temperature affects the fermentation process as product formation depends on temperature. The temperature used in this study ranges from 31-33°C or room temperature. While the pH used for the growth of *Zymomonas mobilis* was around 5 [9]. Fermentation was carried out for 192 hours, and with measurements of ethanol levels once every 48 hours. The starting time of reaction at 0 hour were observed at different concentrations of inoculum as a control.

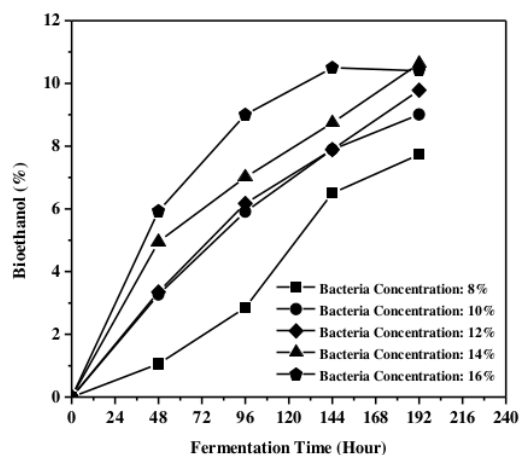


Fig. 1. The concentration of bioethanol vs fermentation time at various bacteria concentration

Bioethanol level in the distillate product was measured and analyzed using specific gravity and AOAC table. The results of the ethanol sample analysis are presented in the form of a curve in Figure 1. Based on figure 1, it can be seen that the highest bioethanol content reached at 14% of inoculum concentration with 192 hours fermentation time. This distillate sample has 10.62% (v/v) ethanol content from 25 ml of the sample from the purification process.

The levels of ethanol had been raised along with increasing the fermentation time. For example, in the concentration of inoculum 8%, 10%, 12% and 14%, until the 192-hour fermentation shows a graph that continues to increase. It is suspected that until the 192nd hour, the bacteria are still experiencing a log phase (exponential) to stationary or there are still nutrients added to the fermentation medium such as yeast extract which is a source of nitrogen for the growth of *Zymomonas mobilis* [9]. So that bacterial cells will grow and divide exponentially to the maximum amount. Conversely at an inoculum concentration of 16%, fer-

mentation at 192 hours decreases in ethanol levels because ethanol accumulates quite a lot in the medium, then *Zymomonas mobilis* cell growth will be inhibited.

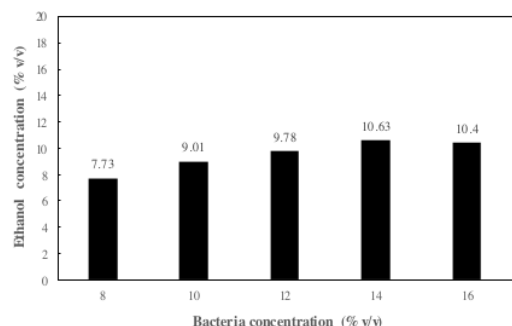


Fig. 2. Effect of bacteria concentration (%v/v) enhancement towards ethanol concentration (%v/v) at 192 hours of fermentation

In the starter 14 hour to 192 hours had an ethanol content of 10.62%. Bioethanol levels that are formed in the fermentation process of cocoa pod husks are quite small on average, this is influenced by several factors, one of them is sugar content. The level of sugar produced in the hydrolysis process of cocoa peel using H_2SO_4 10% at 100 °C for 3 hours was 9.89%. However, several factors affected the bioethanol synthesis including the concentration of bacteria are not the same and the starter extraction when analyzed must be concurrent. Moreover, the rate of microorganism growth and the rate of production should be in line. And, after delignification, the lignin levels should be analyzed so that, we can find out what percentage of residual lignin levels that can interfere during fermentation. A few bioethanol contents in the product gives a low value of process yield as shown in figure 3. The maximum yield was achieved at 5.45% when 14% (v/v) of bacteria concentration was applied for 192 hours of fermentation.

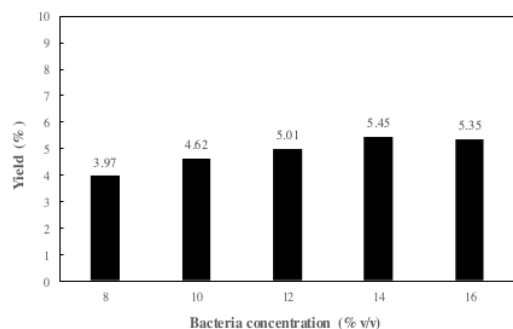


Fig. 3. Yield of bioethanol product with several bacteria concentration (%v/v) at 192 hours of fermentation

4. Conclusions

Bioethanol levels that are formed in the fermentation process are influenced by several factors one of which is reducing sugar levels. Reducing sugar levels produced in the hydrolysis process of cocoa peel using H_2SO_4 30% at 100 °C for 3 hours around 9.88%. Optimal fermentation conditions were obtained at 14% *Zymomonas mobilis* bacteria with 192 hours fermentation time at 30 °C which produced the highest ethanol content of 10.62% from 25 ml of samples obtained after the distillation process.

References

- [1]. Khatiwada, D. and Silveira, S. 2017. Scenarios for bioethanol production in Indonesia: How can we meet mandatory blending targets? *Energy* 119: 351-361.
- [2]. Ditjenbun.2016.http://ditjenbun.pertanian.go.id/tiny_mcpuk/gambar/file/statistik/2016/KAKAO%202016-2016.pdf[accessed on 22 February 2016].
- [3]. Suryaningsih, R. and Irhas. 2014. Bioenergy plants in Indonesia: Sorghum for producing bioethanol as an alternative energy substitute of fossil fuels. *Energy Procedia* 47: 211-216.
- [4]. Mood, H.S., Golfeshan, A.H., Tabatabaei, M., Jouzani, G.S., Najafi, G.H., Gholami, M., Ardjmand, M. 2013. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. *Renewable and Sustainable Energy Reviews* 27: 77-93.
- [5]. Badger, PC. 2002. Ethanol from Cellulose: A General Review. In *Treed in New Crop and new Uses*, J. Jannick and A. Whipkey (eds). Alexandria,VA: ASHS Press.
- [6]. Vasquez, Z.S., et.al. 2019. Biotechnological approaches for cocoa waste management: A review. *Waste Management* 90: 72-83.
- [7]. Macedo, RSDS., Pantoja, LA., Santos, AS., Bioethanol from Jatropha Seed Cakes Produced by Acid Hydrolysis Followed by Fermentation with Baker's Yeast, *International Journal of Applied Science and Technology*, Vol. 4, No. 4; July 2014.
- [8]. Puangsri, T., Abdul karim, S.M. and Ghazali, H.M., 2005. Properties of Carica Papaya L. (Papaya) Seed Oil Following Extractions Using Solvent and Aqueous Enzymatic Methods, *Journal of Food Lipids*, 12, pp. 62-76.
- [9]. Gunasekaran, P., Karunakaran, T., Kasthuribia, M. 1986. Fermentation Pattern of *Zymomonas mobilis* strains of different substrate-a comparative study. Department of Microbial Technology, School of Biological Sciences, Madurai Kamaraj University, India.

- [10].³ Nazir, N., Novelina, Juita, E., Amelia, C., Fatli, R. 2016. Optimization of pre-treatment process of cocoa pod husk using various chemical solvents. *International Journal on Advanced Science Engineering Information Technology*, Vol. 6 No. 3: 403-409.
- [11].² Baeyens, J., Kang, Q., Appels, L., Dewil, R., Lv, Y., Tan, T. 2015. Challenges and opportunities in improving the production of bio-ethanol. *Progress in Energy and Combustion Science* 47: 60-88.

17. Synthesis of Bioethanol from Cocoa Pod Husk Using Zymomonas Mobilis

ORIGINALITY REPORT

9%

SIMILARITY INDEX

%

INTERNET SOURCES

%

PUBLICATIONS

9%

STUDENT PAPERS

PRIMARY SOURCES

1 Submitted to Chungnam National University 1%
Student Paper

2 Submitted to University of Strathclyde 1%
Student Paper

3 Submitted to Universiti Malaysia Sabah 1%
Student Paper

4 Submitted to Institute of Research & 1%
Postgraduate Studies, Universiti Kuala
Lumpur
Student Paper

5 Submitted to University of the Philippines Los 1%
Banos
Student Paper

6 Submitted to Universiti Malaysia Terengganu 1%
UMT
Student Paper

7 Submitted to Coventry University 1%
Student Paper

Submitted to UPN Veteran Jawa Timur

8

Student Paper

1 %

9

Submitted to Imperial College of Science,
Technology and Medicine

Student Paper

<1 %

10

Submitted to Lambung Mangkurat University

Student Paper

<1 %

11

Submitted to University of Sheffield

Student Paper

<1 %

Exclude quotes Off

Exclude matches Off

Exclude bibliography Off