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Fermentation of Glycerol from Biodiesel Waste to 1,3-Propanediol by *Klebsiella Pneumoniae*

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ABSTRACT

One of the oldest products of glycerol fermentation is 1,3-Propanediol. As a glycol, 1,3-propanediol can principally be utilized as a monomer for the synthesis of polyesters and polyurethanes. In this study, 1,3-Propanediol is produced by fermentation of glycerol which is the result of purification of crude glycerol from biodiesel plants. Crude glycerol and glycerol of purification analyzed the content of compound. Glycerol is fermented by *klebsiella pneumoniae*. Glycerol is fermented with the addition of varying the volume of inoculum was 5, 7, and 10 % (v/v), the fermentation time was 1, 2 and 3 days, and the fermentation temperature was 25 and 37 ^{o}C. The results of fermentation of glycerol was analyzed using gas chromatography with column DB 5 HT to obtain a purity of 1,3-Propanediol is generated based on retention time of the standard solution. Retention time of 1,3-Propanediol is 4.846 minute on gas chromatographic analysis. The purity of 1,3-Propanediol at 1, 2 and 3 days is 59.444; 60.7145; and 73.7002 %. The purity of 1,3-Propanediol the addition of bacterial volume 5, 7, and 10 % (v/v) is 63.5320; 72.9740; and 73.7002 %. The purity of 1,3-Propanediol at 25 and 37 ^{o}C is 62.4343 and 73.7002 %.

Keywords: 1,3-propanediol, glycerol, fermentation, klebsiella pneumoniae, gas chromatography

1. INTRODUCTION

In the past, 1,3-Propanediol (1,3-PD) was produced only chemically by two methods: the hydration of acrolein or the hydroformylation of ethylene. The chemical synthesis, however, has many disadvantages – it requires high pressure, high temperature and catalysts. Consequently, the costs of 1,3-PD production are very high [1]. An attractive alternative for chemical synthesis is a microbial conversion of raw materials to 1,3-PD. This method is easy and does not generate toxic by-products. Nevertheless, the major limitation for industrial microbial production of 1,3-PD is the relatively high cost of the typical substrate such as glucose. The economically attractive solution to this problem might be the use of crude glycerol as a fermentative substrate [2; 3].

Biodiesel production increased exponentially in past several years. The principal byproduct of its production is glycerol, also known as glycerin. As the demand and production of biodiesel grows, the quantity of crude glycerol generated will be considerable, and the utilization of it will become an urgent topic [4]. The rest of crude glycerin consists of unconverted triglycerides, unconverted methanol, biodiesel, soaps and contamination. Therefore, this crude glycerol contains too many contaminants for a useful application in chemistry or pharmacy without treatment and the high purification cost of glycerin makes its applications, in pharmaceutical and chemical applications, limited [5]. So, many bioprocesses using glycerol are being studied. In the present work, *Klebsiella pneumoniae* was grown in medium containing crude glycerol from biodiesel production. The objective was to evaluate *Klebsiella pneumoniae* capacity of crude glycerol consumption as principal carbon source

and produce 1,3-PD. *Klebsiella pneumoniae* was chosen to be a promising strain to convert crude glycerol to 1,3-PDO since literature reports 1,3-PD production from pure glycerol by this strain [6]. Glycerol conversion to 1,3-Propanediol can be carried out by Klebsiella as well as Enterobacteriaceae [7]. In the actual fermentation a number of other byproducts are formed, i.e., ethanol, lactic acid, succinic acid, and 2,3-butanediol, by the enterobacteria *Klebsiella pneumoniae*, *Citrobacter freundii* and *Enterobacter agglomerans*, butyric acid by *Clostridium butyricum*, and butanol by *Clostridium pasterianum* [8].

In the metabolic reactions, glycerol is dissimilated through coupled oxidative and reductive pathways [9]. The reductive pathway is carried out in two enzymatic steps. The first enzyme vitamin B12-dependent glycerol dehydratase (GDHt) removes a water molecule from glycerol to form 3-hydroxypropionaldehyde (3-HPA), which is then reduced to 1,3-PD by second enzyme, NADH linked 1,3-propanediol oxidoreductase (PDOR).

1,3-Propanediol (1,3-PD) or trimetylene glycol) has traditionally been considered as a 'specialty chemical' is now undergoing a transition into a 'commodity chemical' [10]. It has been gaining great importance recently due to its role as a monomer in the synthesis of polyesters for fabric and textile applications. 1,3-PD also finds application in making transparent ballistic polymer which is a lifesaving technology, and the war fighter is the primary beneficiary. Ballistic polymer is based on a family of transparent materials whose composition can be tailored to enhance properties such as transparency, impact resistance, and UV stability [11].

2. METHODS

2.1 Crude glycerol

Crude glycerol was obtained from one of Biodiesel Plant at Dumai, Riau, Indonesia. Crude Palm Oil (CPO) was used as a raw material and the process employed an alkali-catalyzed transesterification reaction.

2.2 Microorganism

Klebsiella pneumoniae was obtained from Microbiology Laboratorium at Badan Pengawasan Obat dan Makanan (BPOM), North Sumatera, Indonesia. *Klebsiella pneumonia* was cultured and kept in an agar slant.

2.3 Purification of Crude Glycerol

Crude glycerol 150 ml was prepared in glass beaker, then heated at 60 ℃ for 1 hour to evaporated all the alcohol contents in crude glycerol. Then, 50 ml H_2SO_4 5% was added into it to the desired pH (pH=2), formed two separate layers, where the top layer is fatty acid phase, and the bottom layer is glycerol rich phase. Glycerol has been obtained from separation was added with NaOH solution until the pH of glycerol was neutral (pH=7). And then, glycerol was heated to formed Na_3PO_4 salts and was filtered to separated salts with glycerol. Glycerol was added with activated carbon 2% of total weight of glycerol, to removed the colour of glycerol. The process was heated at 80 ℃ and left for 12 hours. After that, glycerol was filtered to separated activated carbon from it. Glycerol form purification still contained water which affected the purity of glycerol. Therefore, the evaporation was carried out at 110 ℃ for 2 hours in oven, to get glycerol with a higher purity.

2.4 Stock culture preparation

Macro elements: 20 gr $FeCl_3.6H_2O$; 10 gr $CaCl_2H_2O$; 0.03 gr $CuSO_4.5H_2O$; 0.05 gr $MnSO_4.4H_2O$; 0.1 gr $ZnSO_4.7H_2O$ were dissolved into 1 L distilled water in erlenmeyer. Micro elements such 0.5 gr $(NH_4)_2SO_4$; 0.4 gr $MgSO_4.7H_2O$; 9.65 gr Na_2SO_4; 2.65 gr KH_2PO_4 were added into the solution. Extra nutritions such 3 gr peptone; 3 gr margarine; 10 gr glucose were added too. The pH of solution was measured by indicator pH. The pH of solution controlled in the range of 6.4-7.4. If the solution was very acid, NaOH solution was added drop by drop to the desired pH. If the solution was very base, HCl solution was added drop by drop to the desired pH. Solution was heated until it boiled and left for several minutes. After that, the solution in erlenmeyer was closed by cotton and wrapped by paper and bounded by rubber. Then it was sterilized into autoclave at 121 ℃ for 20 minutes. After sterilization process in autoclave, the process was continued with sterilization with UV light for 30 minutes. Stock culture was carried out with culture of *Klebsiella pneumoniae* was added into the erlenmeyer. Then it was incubated in stated incubator for 3 days at 37 ℃ . After that, the stock culture could be used for fermentation.

2.5 Fermentation of glycerol

Glycerol 50 ml was added into fermentor, and then stock cultures of *Klebsiella pneumoniae* as much 5, 7, and 10 % from total of glycerol were added. After that, the fermentor was closed

and fermented at 37 ℃ and 25 ℃ for 1, 2, and 3 days.

2.6 Separation of 1,3-Propanediol

The results from fermentation were distilled by atmospheric distillation method for separated another compounds beside 1,3-Propanediol in order to obtained 1,3-Propanediol with high purity.

2.7 Analysis of 1,3-Propanediol

1,3-Propanediol from separation was analyzed using Gas Chromatography to obtained the purity. The analysis was performed on QP2010 Shimadzu GC equipped with automatic sampling system and DB 5 HT column. 100% dimethyl polysiloxane was used as filler material. Distilled water was used as solvent and helium was used as a carrier gas. Temperature of oven and injection were set at 60 ℃ and 370 ℃. Temperature of ion source and surface were set at 370 ℃ and 360 ℃. The flow rate and column rate were set at 125.1 ml/min and 2.42 mil/min. The pressure was set at 100 kPa.

2.5 Other analysis

The physical properties of crude glycerol and purified glycerol such colour, density, free faty acids (FFA), water content, ash content, glycerol content were analyzed in this study.

3. RESULTS

3.1 Purification of Glycerol

The results of physical properties between crude glycerol and purified glycerol is presented in Table 1.

Table 1. Characteristics of crude glycerol and purified glycerol

Physical Properties	*Crude* glycerol	Purified Glycerol
Colour	Reddish Brown	Clear
Density	1.24	1.2676
FFA	26.22	0.92
Water (%)	2.64	1.265
Ash (%)	11	6
Glycerol (%)	29.306	93.1924

3.2 Effect of fermentation time on the purity of 1,3-Propanediol

Effect of fermentation time on the purity of 1,3-Propanediol formed are presented in Figure 1. The data is the relationship of fermentation time on the purity of 1,3-Propanediol produced in fermentation temperature conditions at 37 ^{o}C and volume of inoculums was 10 % from total of glycerol that was fermented. From Figure 1, it can be seen that the compounds are not known, therefore the compounds were classified based on the retantion time from results of the gas chromatography (GC). Siregar [12] ferment glycerol into 1,3-Propanediol produced several chemical compounds such as ethanol, formic acid, acetic acid, lactic acid, butyric acid, 2,3-butanediol and succinic acid. In Figure 1 shows that the purity of 1,3-Propanediol increased with increasing fermentation time while glycerol fermentation time has run out at first day. The purity of 1,3-propanediol formed at first day was 59.4444%, at second days was 60.7145% and third day was 73.7002 %. Increased 1,3 propanediol in the second and third day due to some other compounds that are formed on the first day by the bacterium Klebsiella pneumoniae transformed into 1,3 propanediol, this can be seen with decreasing purity of compounds D, E, and G are formed. Fermentation time affect the antibacterial activity, because the longer the fermentation, the bacteria is active, the greater the number, so it has the ability to break down the larger substrate [13].

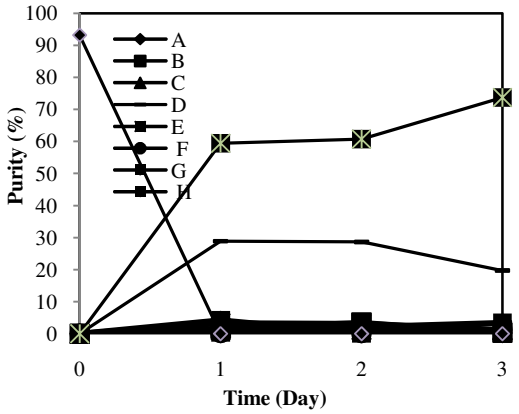

Fig. 1. Effect of Fermentation Time on The Purity of 1,3-PD (Fermentation Temperature of 37 ^{o}C and Inoculum Volume of 10%)

3.3 Effect of Inoculum Volume on the purity of 1,3-Propanediol

Effect of the addition of inoculum volume to 1,3-propanediol are presented in the figure 2. The data is the relationship of inoculum volume on fermentation temperature conditions at 37 ^{o}C and fermentation time was third days. From the figure 2, it can be seen that the purity 1,3 propanediol increases with increasing volume of

inoculum in the fermentation of glycerol. The purity of 1,3-propanediol formed in the volume of inoculum 5 % was 63.5320%, At 7 % volume, the purity of 1,3-Propanediol increased to 72.9740%, and 10 % volume of inoculum the purity of 1,3-Propanediol was 73.7002%. In addition the volume of inoculum 5, 7, and 10% experienced an increase in the purity of 1,3 propanediol. One of the factors that affect the fermentation process is food for microbes or nutrients found in the medium [14]. On the addition of 5-7% of bacteria increased their high purity on 1,3 propanediol formed. Increased purity of 1,3-propanediol on the addition of inoculum from 5-7% due to bacterial nutrients contained in the media is still sufficient for the bacteria to produce fermented products [15], while the addition of 10% volume increase in the purity of the bacteria do not produce 1,3 propanediol were large, the increase in the purity of 1,3 propanediol slightly in volume of 10 % possibly due to bacterial microbial population began to experience death. These deaths occur because the necessary nutrients and bacteria reduced bacterial excretion results had accumulated in the medium, thus disrupting breeding and subsequent bacterial growth [14].

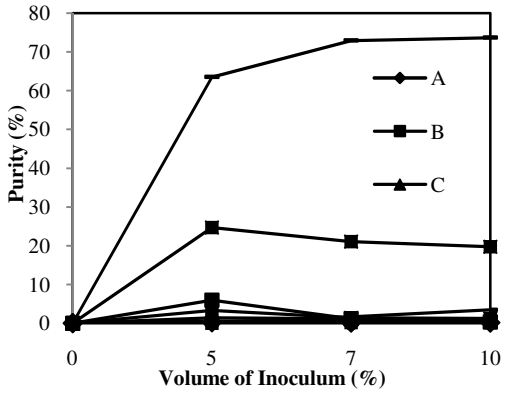

Fig. 2. Effect of Inoculum Volume on The Purity of 1,3-PD (Fermentation Temperature of 37 °C and Fermentation Time of 3 days)

3.4 Effect of fermentation temperature on the purity of 1,3-Propanediol

Fermentation was carried out at volume of inoculums was 10% from total of glycerol that was fermented and lasted for 3 days. Figure 3 shows the purity of 1,3-Propanediol was 62.4343 % when fermentation lasted at 25℃. But when fermentation lasted at 37℃, the purity of 1,3-Propanediol decreased into 73.7002 %.

The purity of 1,3-propanediol formed at high glycerol fermentation to fermentation temperature 37 °C. Temperature holds an important role, because it can directly affect the activity of microorganisms and will indirectly affect the purity of the products [16]. Temperature affects the rate of microbial growth, rate of enzyme synthesis, and rate of enzyme inactivation [17]. Each of bacteria has the optimum, maximum, and minimum temperature. If the environment temperature is less than the minimum temperature or greater than maximum temperature, the activity of enzim would be stopped even at the high temperature will be occur denaturation of enzyme [18]. Klebsiella pnemoniae grow on simple and complex medium, aerobic and can live at the optimum temperature 37 °C [19]. In this study, the high purity of 1,3-Propanediol was obtained at 37 ℃.

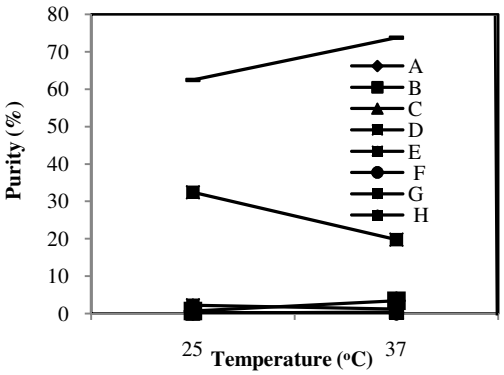

Fig. 3. Effect of Fermentation Tempertaure on The Purity of 1,3-PD (Inoculum Volume of 10% and Fermentation Time of 3 days)

4. CONCLUSION

Klebsiella pneumoniae is able to convert glycerol from biodiesel waste into 1,3-Propanediol. Fermentation time, inoculum volume, and fermentation temperature had a profound effects on the activity of *Klebsiella pneumoniae* to produced 1,3-Propanediol. The best temperature for fermentation was obtained at 37 °C, time for fermentation was obtained at 3, and inoculum volume for fermentation was obtained at 10% with the purity of 1,3-Propanediol was 73.7002 %

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