Fermentation Process of Glycerol to Arabitol from Byproducts of *Reutalis trisperma* Biodiesel using Yeast of *Debaryomyces hansenii*

Efri Mardawati¹(a), Robi Andoyo¹, Mimin Muhaemin², Sarifah Nurjanah², Darajat Natawigena², Totok Herwanto³, Handarto², Gemilang Lara Utama¹, Rosalinda, Poppy L, Ade M Kramadibrata²

¹Department of Agro-Industrial Technology, Faculty of Agro-industrial Technology, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km 21. Jatinangor, Sumedang, 40600, Indonesia
²Department of Agricultural Engineering, Faculty of Agro-industrial Technology, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km 21. Jatinangor, Sumedang, 40600, Indonesia. (a)Corresponding author: efri.mardawati@unpad.ac.id

Abstract. Arabitol byproduct on the production of biodiesel *Reutalis trisperma* has calories of 0.2 cals / g equivalent to the level of sweetness of sucrose can be produced by using the yeast of *Debaryomyces hansenii*. The research was done by experimental method and descriptive analysis with two replications. The variation in this study was the ratio between the concentration of glycerol substrate and the fermentation medium added to 250 m / L of work volume in five treatments, namely: A (1: 16) crude glycerol, B (1: 16) pure glycerol, C (1: 8) crude glycerol, D (1:5) crude glycerol, and E (1:4) crude glycerol. The experimental results that the *D. hansenii ITB CC R85* yeast may accumulate the formation of arabitol during the stationary phase at 120 h. The best fermentation condition was obtained at treatment C with a specific growth rate (μ) of 0.0608/h, arabitol concentration of 0.2373 g / L, the yield of arabitol to glycerol (Yp / s) of 0.0041 g / g, the yield of biomass to the substrate (Yx / s) of 0.3214 g / g.

1. Introduction

Indonesia's energy needs are increasing along with the development of the industry and population growth. This is observed from the demand for fuel oil in Indonesia currently reaching 1.6 million barrels per day. However, this demand can not supply by the production capacity of domestic refineries which is only 850 thousand barrels per day, so the rest must be met from imports [1]. Biodiesel is an alternative fuel that can be obtained from used oil, animal fat, or plant oil that has been converted into Methyl Esters through the process of transesterification with alcohol. Glycerol is the main by-product about 1 kg of glycerol for every 9 kg biodiesel produced [2].
Sunan candlenut (*Reutalis trisperma*) or kemiri sunan in Indonesia is one of the plants that has the potential as a biodiesel feedstock because it has physical-chemical characteristics close to diesel oil. This plant is found in Indonesia. The yield of crude oil produced from the kernel (meat) sunan candlenut can reach 45-50% [3] with the yield of biodiesel reaching 88% crude oil and by-products in the form of glycerol [4]. Sunan candlenut oil as a raw material for biodiesel is toxic and makes it non-edible as food, so its use as a biodiesel feedstock does not interfere with people's needs for food ingredients. Sunan candlenut is one of the plants that has potential as a raw material of biodiesel. Besides it many found in Indonesia, the yield of crude oil produced from kernel (meat) Sunan candlenut can reach 45-50% [3] and the yield of biodiesel can reach 88% of crude oil with glycerol byproducts [4].

One of the methods to utilize the excess of by-product glycerol from biodiesel is by using glycerol as a substrate for producing arabitol. Yeast strains play an important role in the production of arabitol by bioconversion [5]. This research presents *Debaryomyces Hansenii* fermentation using biodiesel waste in the form of glycerol as substrate in arabitol production. The glycerol used is the by-product of biodiesel production from Sunan candlenut, then the arabitol result compared with arabitol using pure glycerol commercial. The purpose of this study is to determine the best addition of crude glycerol as biodiesel byproduct in producing arabitol and to evaluate the yield of arabitol produced from yeast *Debaryomyces Hansenii*.

2. Methodology

2.1. Microbial Preservation

The yeast strain of *D. Hansenii* ITB CCR85 was used in this study and obtained from Microbiology and Bioprocess Technology Laboratory of Chemical Engineering ITB. The yeast was grown in glucose yeast extract agar (GYE) for 3 days at 30°C before further use. Cultivation of yeast *D. Hansenii* using Glucose Yeast Extract (GYE) medium (10 g of Pepton, 5 g of Yeast Extract, 20 g of Glucose, 20 g of Bako agar). The inoculum and medium fermentation were made using the same composition of nutrient in 10% volume of the total fermentation volume. The composition of medium fermentation for inoculum is refer to [5]. The nutrient components and glycerol were autoclaved separately at 121 °C for 15 minutes. Determination of concentration of cells was done by measuring the absorbance of samples which taken at each particular concentration using a spectrophotometer with a wavelength of 580 and each cell in the inoculum solution was calculated using TPC (Total Plate Count) method [6].

2.2. Arabinol Fermentation

The fermentation process was conducted into a 250 mL flask shaker which is an inoculum solution of 10% of the working volume that has been incubated for 48 hours. Fermentation medium was varied in the ratio of addition of glycerol and fermentation medium with 5 different treatments ie Treatment A (1:16) glycerol, Treatment B (1:16) pure glycerol, Treatment C (1:8) crude glycerol, Treatment D (1:5) crude glycerol, and Treatment E (1:4) crude glycerol. The temperature was setted at 30°C, 150 rpm mixing speed at pH 5 and temperature 30°C in 52 h the fermentation time. The sampling was done during the fermentation time. Fermentation is terminated when the culture reached the stationary phase.

2.3. Analysis

Sample was analysed for biomass as cell concentration by the turbidimetric-gravimetric method using UV-Vis spectrophotometer [6,14]. Glycerol, glucose, arabitol, ethanol and xylitol concentrations were determined using high performance liquid chromatography (HPLC) type BioRad Aminex HPX-87H column and 5 mM H₂SO₄ as eluent as was described in [6]. The standard cell curve that relate the cell concentration with absorbance has been determined first. The concentration of cells then used to determine the rate of cell growth in the fermentation solution. The standard curves of glycerol, arabitol, ethanol, and xylitol at various concentrations also used to determine the concentration of substrate consumed and the
product arabitol and others metabolites produced. These curves correlate the concentration of the compound to the HPLC peak area.

2.4. Data Interpretation

Growth of microorganisms were parameterised as the specific growth rate that was calculated from biomass concentration data during the logarithmic phase, following equation 1.

\[
\frac{dX}{dt} = \mu X
\]

\(\mu\) is referred as specific growth rate (1/hours), the \(\mu\) value depends on substrate concentration.

In order to measure the performance of a fermentation system, the yield values are used. The general statement of yield can be described as ratio of formed product (product or cell) to used substrate, as stated on Equation 2 - 3.

\[
Y_{P/S} = \frac{(P-P_0)}{(S-S_0)}
\]

(2)

\[
Y_{P/X} = \frac{(P-P_0)}{(X-X_0)}
\]

(3)

where:

- \(P\) = arabitol product concentration (g/L)
- \(T\) = Total fermentation time (hours)
- \(Y_{P/S}\) = product-from-substrate yield (g arabitol/g xylose) or other metabolites products ie xylitol and ethanol
- \(Y_{P/X}\) = product-from-cell yield (g arabitol/g cell)
- \(X\) = Cell concentration (g/L)

3. Results and Discussion

The fermentation of the parameters of success were determined in three groups: yeast cell growth, substrate concentration especially glycerol consumed and product concentration of arabitol and xylitol.

3.1. The Ratio of Substrate Glycerol Concentration with Medium Fermentation on Growth Microorganisms.

Arabitol is produced from glycerol substrate by yeast, the yeast needs arabitol as carbon source for their growth. Glycerol-to-arabitol influenced by the concentration glycerol as substrate. In order to study the influence of each substrates on the cell growth, fermentation were conducted using either substrate concentration. Specific growth rates (\(\mu\)) of each runs were determined using Eq.1. Following, Figure 1 and Table show the cell concentration and the calculated specific growth rates for each substrate.

![Figure 1. Profile of growth D.hansenii on different substrate concentration during fermentation](image-url)
Figure 1 shows the increase in cell concentration as increasing as the ratio addition of initial substrate concentration. Cell concentrations of \textit{D. hansenii} ITB CC R85 for 152 h with crude glycerol in ratio glycerol and medium fermentation A, C, D dan E showed almost has the same cell growth at hour 0 to 32 hours. This result is in contrast to the ratio Pure glycerol and medium fermentation B which exhibit a growth rate in the lag phase that runs lower at the hour 0 to the 32 hours. Pure glycerol does not contain impurities that can support the growth of \textit{D. hansenii} as well as on the crude content of glycerol from the separation of biodiesel from Sunan candlenut. According to [7], crude Glycerol obtained a composition (based on a wt% basis): glycerol 40.0; non-glycerol organic substance (NGOs) 55.0; and moisture 5.0. NGOS consists of free fatty acid (FFA) as a 15% saponification fatty acid (SFA); fatty acid methyl ester (FAME) of 10%; and 30% methanol.

The lag phase has brief time to the increase of biomass is in the first 4 h. The average logarithmic phase ends after 72 h which is followed by the stationary phase up to 122 h. Koganti et al (2010), cell growth will usually reach a static phase at the 80 h. Furthermore, at 122 to 144 h, some of the samples had decreased (death phase) such as the ratio of glycerol concentration and medium fermentation A and D. This is due to nutrients and substrates in the cells has been exhausted and no more nutrients are used for growth. Crude glycerol indicates an inhibitory effect on cell concentration production. Then the presence of soap and methanol has been shown to negatively affect the production of DHA from crude glycerol, otherwise, high salinity (Na or K) of crude glycerol may inhibit microbial activity in anaerobic fermentation [1,8]. The Cell growth follows of sigmoid curve. The higher the substrate concentration affected to the higher the cell concentration. The effect of the substrate on the specific growth rate (\(\mu\)) of the microbes from each treatment give different results. The specific rate of growth was taken at the logarithmic phase at 4 to 32 h, calculated by plotting the ln data of cell concentration over time.

| Treatments of Ratio (Substrate: Medium) | Specific Growth Rate (\(\mu\)) (hour\(^{-1}\)) |
|----------------------------------------|-----------------------------------------------|
| Treatment A                            | 0.0505 ± 0.013                                |
| Treatment B                            | 0.0395 ± 0.025                                |
| Treatment C                            | 0.0608± 0.010                                 |
| Treatment D                            | 0.0333 ± 0.016                                |
| Treatment E                            | 0.0415± 0.027                                 |

The highest specific growth rate is by the treatment B of 0.0608 hr\(^{-1}\). The decrease and the increase of fermentation substrate concentration can causes the decrease of \(\mu\) value. Mardawati (2017), the low cell can growth is caused by the tendency of microbial cells to consume substrates or to sequester the sugars to form metabolite products under conditions of high initial cell concentration.

The growth rate using crude glycerol has greater growth compared to growth rate with pure glycerol, this is probably due to crude glycerols are having an additional nutrient impurities in the form of FFA that is not found in pure glycerol. In addition, the environmental factors also affect the specific growth rates such as pH conditions, dissolved oxygen, inoculum, temperature, and substrate concentration.

### 3.2. Effect of Glycerol Substrate Concentration on Arabitol Production

During the fermentation process, The lag phase and then log phase was measured at 28 h, it followed by the stationary phase. Arabitol starts produced at 32 h of 0.0184 g/L on the treatment phase until it ends at 152 h which is the phase of death. The yeast \textit{D. hansenii} accumulates the formation of arabitol during the stationary phase.

Arabitol began to form at 32 h of 0.0184 g/L on the treatment D and the treatment B of 0.0858 g/L, at 120 h in all treatment arabitol have been formed. The treatment B produce arabitol of 0.1881 g/g, at the treatment A, C and D arabitol produce at the 120 h of 0.0465 g/L, 0.2347 g/L, and 0.0083 g/L while at 152 h, there are no arabitol is formed again at all treatments. It is possible at that time, arabitol has been
consumed by yeast as a nutrient source due to the low or exhausted levels of glycerol at the end of the fermentation. In Table 2, the yield of arabitol from each experiments are presented.

Table 2. Glycerol Substrate on Yield of Arabitol

| Description                              | Ratio (Concentration Substrate : Medium Fermentation) |
|------------------------------------------|-----------------------------------------------------|
| **Concentration of Arabitol**            | A   | B   | C   | D   | E   |
| t = 0 h (g / L)                          | 0.000 ± 0 | 0.0289 | 0.000 ± 0 | 0.0184 | 0.000 ± 0 |
| t = 120 h (g / L)                        | 0.0449 | 0.1937 | 0.2373 | 0.000 ± 0 | 0.000 ± 0 |
| Yield value arabitol to glycerol         | ± 0.00015 | ± 0.00046 | ± 0.0002 | 0.000 ± 0 | 0.000 ± 0 |
| t = 32 h (g / g) (YP/S)*                 | ± 0.0001 | ± 0.0001 | ± 0.0001 | 0.000 ± 0 | 0.000 ± 0 |
| Yield value arabitol to glycerol         | ± 0.0003 | ± 0.0001 | ± 0.0009 | ± 0.0001 | 0.000 ± 0 |
| Yield of biomass to substrate            | 0.2453 | 0.2996 | 0.3214 | 0.1343 | 0.2016 |
| (g / g) (YXS)*                           | ± 0.0069 | ± 0.0014 | ± 0.0221 | ± 0.0046 | ± 0.0052 |
| Yield of arabitol to cell concentration  | 0.000 ± 0 | 0.0072 | 0.000 ± 0 | 0.0019 | 0.000 ± 0 |
| t = 32 h (g / g) (YP/X)*                 | ± 0.00011 | ± 0.0001 | ± 0.0001 | ± 0.0001 | ± 0.0001 |
| Yield of arabitol to cell concentration  | 0.0045 | 0.0126 | 0.0129 | 0.000 ± 0 | 0.0007 |
| t = 120 h (g / g) (YP/X)*                | ± 0.0004 | ± 0.0051 | ± 0.0004 | ± 0.0001 | ± 0.0001 |

The average arabitol was formed at 120 h, this is similar with Koganti et al (2010), arabitol produced from consumed glycerol at 120 h. Arabitol increases along with the increasing of initial glycerol concentration, especially at concentrations of 50 g / L to 90 g / L. The higher of glycerol added, the higher the concentration of glycerol. The longer the fermentation time, the less glycerol is produced and the increased arabitol formation is formed. The result of the glycerol concentration determines the value of the biomass yield on the substrate and the yield value of the product on the substrate.

Glycerol has decreased because it continues to be used by microbes to produce arabitol and other metabolite products. Based on the decrease of the substrate concentration resulted in the conversion value of utilization.

The highest value of arabitol is at the treatment C 120 h that is 0.2373 g/L, with yield value arabitol to glycerol (YP/s) equal to 0.0041 g/g, yield of biomass to substrate (Yx/s) of 0.3214 g/g. This treatment gives the highest of arabitol yield. This result is consistent with the statement of [5] suggesting that the yield of arabitol increases with increasing concentrations of glycerol, especially from 50 to 90 g/L.

The lowest yield of arabitol is at the treatment D. Arabitol formed at 120 h with the yield of arabitol to glycerol (YP/s) of 0.00014 g/g, the value of the biomass yield on the substrate formed (Yx/s) of 0.2016 g/g. The low arabitol in the high crude glycerol addition can be affected by several factors, such as the presence of impurities in the form of saponification fatty acids and methanol and also be caused by the formation of other metabolites such as ethanol which can be inhibit of the desired product formation of arabitol.

The highest utilization 100% occurs in the treatment E arabitol was formed at 32 h only at 0.0192 g/L with yield value arabitol to glycerol (YP/s) of 0.00026 g/g, yield of biomass to substrate (Yx/s) of 0.1343 g/g. While in the 120 h arabitol is not produced anymore. The non-formation of arabitol may be due to the occurrence of arabitol consumption by microbes due to low levels of glycerol at the end of fermentation. Low concentration of substrate will decrease yield because there is no excess substrate that can be used to produce arabitol [9].
The concentration of treatment A was 42.6645 g/L. The yield of arabitol started to form at hour 120 of 0.0465 g/L, with the yield of arabitol on glycerol (Yp/s) 0.0011 g/g, the yield of biomass to the substrate (Yx/s) of 0.2453 g/g, whereas in the treatment B (pure glycerol) with the same concentration occurs the formation of arabitol at 32 h and 120 h along with the decrease of glycerol. The highest concentration of glycerol was 49.5914 g/L. The yield of arabitol formed at the 32 h of 0.0858 g/L, with the yield value of arabitol to glycerol (Yp/s) 0.0015 g/g, the yield value of biomass to the substrate (Yx/s) of 0.2534 g/g, while in the 120 h arabitol is still formed at 0.1881 g/L, with the yield value of arabitol to glycerol (Yp/s) 0.0038 g/g, the yield of biomass to the substrate (Yx/s) 0.2996 g/g. This treatment has formation of arabitol twice. Thus are due to the concentration of pure glycerol has no inhibitor or impurities. This is supported by HPLC data that the metabolites producing the lowest ethanol. Seen on the comparison between the treatment A and B did not experience enough different, whereas the best arabitol yield occurred in the treatment C.

The occurrence of fluctuating data with the addition of substrate given in this study is according with the Monod’s Model which introduced the concept of limiting substrate growth. Substrate that is too low will decrease the yield value because there is no excess substrate that can be used to produce arabitol, while the substrate that is too high by the yeast is diutilized to become other metabolites that will impact on the formation of inhibitors, causing inhibition of the formation of arabitol products[6,12].

It was seen that in the treatment of A, B, and C there was an increase of arabitol product, but in the treatment of D and E there was a decrease in the formation of arabitol products. This is supported by the opinion of [5], which states that the Monod Model expressing the rate of formation will increase if the substrate concentration increases[10]. However the rate of formation will decrease if the substrate concentration too high.

The yield of arabitol yields in this study is still low. This is supported by [12], which says osmophilic yeast such as D. hansenii can produce D-arabitol, but it usually takes too long incubation time and the yield is too small to be accepted industrially.

**Table 3. Other Metabolite Products**

| Description                        | Treatment (Substrate Concentration : Medium Fermentation) |
|------------------------------------|----------------------------------------------------------|
| Yield value of ethanol to substrate t = 32 (g/g) (Yp/s)* | A   | B   | C   | D   | E   |
|                                    | 0.0291 | ± 0.0012 | 0.0191 | 0.0104 | 0.0137 | ± 0.0003 |
| Yield value of ethanol to substrate t = 120 (g/g) (Yp/s)* | 0.02954 | ± 0.0019 | 0.0164 | 0 ± 0 | 0.0022 | 0.0031 | ± 0.0015 |
| Yield of ethanol to biomass t = 32 (g/g) (Yp/x)* | 0.1998 | ± 0.00374 | 0.4674 | 0.1741 | 0.1259 | 0.2284 | ± 0.00751 |
| Yield of ethanol to biomass t = 120 (g/g) (Yp/x)* | 0.1205 | ± 0.0047 | 0 ± 0 | 0 ± 0 | 0.0110 | 0.0229 | ± 0.0023 |
| Concentration of Xylitol (g/L)     | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
3.3. Effect of Glycerol Substrate Concentration on Other Metabolite Products

Glycerol under anaerobic conditions are assimilated by simple diffusion or active transport is phosphorylated by glycerol kinase to glycerol-3-phosphatase and then to dihydroxyacetone phosphate (DHAP) by glycerol-3-phosphatase dehydrogenase. In alternative pathways in yeasts lacking glycerol kinase involve NAD-dependent on glycerol dehydrogenase and dihydroxyacetone kinase. Dihydroxyacetone phosphate, formed in the above routes is converted to glyceraldehyde-3-phosphate and further through the gluconeogenesis pathway to glucose-6-phosphate. This compound is then converted in PPP to arabitol via ribulose-5-phosphate, then it will be phosphorylated so that D-ribulose will be reduced to D-arabitol by NADP depending on D-arabitol dehydrogenase (D-ribulose forming pathways). Other pathways convert glucose-6-phosphate to D-xylose-5-phosphate and after the diphosphorylation D-xylulose is reduced to D-arabitol by NAD depending on D-arabitol dehydrogenase (D-xylulose forms pathway) [11, 12]. In Table 3, the yield of other metabolite product from each experiments are presented.

According to Chaudhary [15], ethanol is formed at the 32 h, In accordance with a study conducted that converted glycerol to form ethanol products with the most ethanol production value is at 32 h as seen in the data (table 4). Then at the 120 h it appears that ethanol production is starting to decline. This is because the ethanol are easy to volatile and the substrate glycerol are reducing along with conversion to form arabitol. The low yield of arabitol products the high ethanol formed due to D. hansenii has a tendency to convert into ethanol in anaerobic fermentation conditions.

The Treatment using pure glycerol commercial indicates that ethanol formation is smaller variation with the addition of crude glycerol. It is possible in pure glycerol has no inhibitor compound which can accumulate ethanol formation and the yeasts tend to convert glycerol to arabitol. Many factors may inhibit the fermentation process in arabitol products, such as the presence of other substrates such as FFA, low dissolved oxygen levels can convert the substrate into ethanol, inhibitor compounds such as acetic compounds can alter cell morphology that causes cell death.

According to [5] the glycerol pathway is converted to ethanol in which glycerol is converted to dihydroxyacetone phosphate by forming ATP and oxidized to form Dihydroxy-acetone phosphate [13], then into glyceraldehyde 3-phosphate into glycolysis and then to pyruvate decarboxylation until to ethanol by alcohol dehydrogenase.

The presence of xylitol is not found in the result of fermentation, it viewed from the conversion pathway of arabitol formation, according to [5], xylitol is form when glycerol is converted into ribulose 5-phosphate then split into two stripes ribulose by ribulokinase or xylulose-5-phosphate by ribulose-5-phosphate epimerase, then forming xylulose and into xylitol by xylitol dehydrogenase. The non-existence of xylitol may due to the diminishing availability of the substrate so that it does not have enough reserves to continue the conversion pathway into xylitol. Moreover, there is no additional co-substrate of xylose support which is capable of forming xylitol and increasing the production of arabitol.

4. Conclusions
Strain D. hansenii ITB CCR85 accumulates the formation of arabitol during the stationary phase at 120 h. The optimum conditions in the maximum yield on arabitol was obtained, that is a ratio of addition glycerol substrate and medium fermentation treatment C at a temperature of 30°C, 150 rpm, pH range 4-5 with the concentration of arabitol 0.2373 g/L and the yield of arabitol to glycerol substrate (Yp/s) of 0.0041 g/g.

Reference
[1] Ardi M.S, M.K. Aroua, N. Awanis Hashim. 2014. Progress, prospect and challenges in glycerol purification process: A review. Renewable and Sustainable Energy Reviews 42 (2015) 1164–1173
[2] Dasari MA, Kiatstikul PP, Sutterlin WR, Suppes GJ. 2005. Low pressure hydrogenolysis of glycerol to propylene glycol. Appl Catal A Gen 281(1–2):225–231.
[3] Herman, M., H. T. Luntungan, dan A. Wahyudi. 2009. Kemiri Sunan (Aleurites triesperma BLANCO) Tanaman Harapan Sumber Bahan Bakar Nabati di dalam Majalah Pusat Penelitian dan Pengembangan Perkebunan hal 6.

[4] Ministry of Energy and Mineral Resources of the Republic of Indonesia. 2013. Development of Kemiri Sunan in Mining Areas. (Press conference No. 57/PUSKOM KESDM/2013).

[5] Koganti S, Kuo TM, Kurtzman CP, Smith N and Ju LK. 2010. Production of arabitol from glycerol: strain screening and study of factors affecting production yield. Appl Microbiol Biotechnol: 257–267

[6] Mardawati, E., D.W. Wira dan M.T.A.P. Kresnowati. 2015. Mirobial Production of Xylitol from Oil Palm Empty Fruit Bunches Hydrolysate: The Effect of Glucose Concentration. Journal of the Japan Institute of Energy. 2015, 94. 769-771.

[7] Dhabhai R, Elahe A, Ajay K. Dalai, Martin R. 2016. Purification of crude glycerol using a sequential physico-chemical treatment, membrane filtration, and activated charcoal adsorption. Separation and Purification Technology 168 (2016) 101–106

[8] Mardawati, E, Parlan, Rialita, T, and Nurhadi, B. 2018. Optimization of moistening solution concentration on xylanase activity in solid state fermentation from oil palm empty fruit bunches, 141, 012018, IOP Conference Series: Earth and Environmental Science

[9] Kordowska-Wiater M. 2015. Review Article Production of arabitol by yeasts: current status and future Prospects. Department of Biotechnology, Human Nutrition and Science of Food Commodities, University of Life Sciences in Lublin, Lublin, Poland

[10] Mardawati E, Purwadi R, Kresnowati MTAP, Setiadi T, 2017. Evaluation of The Enzymatic Hydrolysis Process of Oil Palm Empty Fruit Bunch Using Crude Fungal Xylanase. ARPN Engineering and Applied Sciences. 12(18):5286–5292.

[11] Zhang, G., Lin, Y. P., Li, L., Wang, Q and Ma, Y. 2014. Character of the Sugar Alcohol-Producing Yeast Pichia anomala. J Ind Microbiol Biotechnol 141,41-48

[12] Saha BC, Sakakibara Y, Cotta MA. 2007. Production of D-arabitol by a newly isolated Zygosaccharomyces rouxii. J Ind Microbiol Biotechnol 34(7):519–523

[13] Mardawati E, Andoyo R, Sukra KA, Kresnowati MTAP, Bindar Y, 2018. Production of Xylitol from Corn Cob Hydrolysate Through Acid and Enzymatic Hydrolysis by Yeast, pp. 1-11. IOP Conf. Series: Earth and Enviromental Science, IOP Publishing, UK.

[12] Saha BC, Sakakibara Y, Cotta MA. 2007. Production of D-arabitol by a newly isolated Zygosaccharomyces rouxii. J Ind Microbiol Biotechnol 34(7):519–523

[13] Mardawati E, Andoyo R, Sukra KA, Kresnowati MTAP, Bindar Y, 2018. Production of Xylitol from Corn Cob Hydrolysate Through Acid and Enzymatic Hydrolysis by Yeast, pp. 1-11. IOP Conf. Series: Earth and Enviromental Science, IOP Publishing, UK.

[14] Mardawati E, Kresnowati MTAP, purwadi, R, Bindar Y, Setiadi, T. 2018. Fungal Production of Xylanase from Oil Palm Empty Fruit Bunch via Solid State Fermentation, International Journal on Advance Science, Engineering and Information Technology, 8(6) pp. 2539-2546.

[15] Chaudhary N, 2010. Anaerobic Fermentation of Glycerol by Escherichia coli K12 for The Production of Ethanol. Thesis Department of Bioresource Engineering. McGill University, Canada