Production of xylitol from corn cob hydrolysate through acid and enzymatic hydrolysis by yeast

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Abstract. The abundance of corn production in Indonesia offers the potential for its application as the raw material for biorefinery process. The hemicellulose content in corn cobs can be considered to be used as a raw material for xylitol production. The purpose of this research was to study the effect of hydrolysis methods for xylitol production and the effect of the hydrolyzed corn cobs to produce xylitol through fermentation. Hydrolysis methods that would be evaluated were acid and enzymatic hydrolysis. The result showed that the xylitol yield of fermented solution using enzymatic hydrolysates was 0.216 g-xylitol/g-xylose, which was higher than the one that used acid hydrolysates, which was 0.100 g-xylitol/g-xylose. Moreover, the specific growth rate of biomass in fermentation using enzymatic hydrolysates was also higher than the one that used acid hydrolysates, 0.039/h compared to 0.0056/h.

Introduction

Xylitol is a five-carbon polyalcohol sugar with a similar level of sweetness as that of sucrose, but contains only 2.4 cals/g, while sucrose 4 cal/g. Xylitol is very suitable to be consumed by people with diabetes because it has a low glycemic index [1]. Xylitol can be produced from xylose, either through fermentation or chemical processes. A monosaccharide xylose is produced from hydrolysis of hemicellulose with the main content in the form of xylan. Xylitol is also known as wood sugar because it is commonly contained in the woods that are rich with hemicellulose.

In the industrial scale, xylitol is produced chemically by reducing the pure xylose by Hydrogen gas at a temperature of 80°C-140°C used metal catalysts [2] . This method is costly because xylose derived from hemicellulose hydrolysis needs to be purified beforehand [3]. Nowadays, the world's production of xylitol is about 20,000 to 40,000 tons per year with a value of 40 to 80 million euros [4]. Whereas through biotechnological methods, xylitol production process is done by utilizing catalyst of microorganisms or enzymes. Microorganisms that can be used are the types of bacteria, fungi, and yeasts [2]. However, several studies have shown that xylitol production by using yeast produces xylitol yield more than the production of xylitol using bacteria and fungi [2].The advantages of producing xylitol with the bioprocess, among others, are: it does not cause environmental problems because it does not produce metal waste; it has a relatively low cost because the process takes place at room temperature and pressure of 1 atm; and there are many types of microorganisms capable of producing the xylose reductase enzyme which can turn xylose into xylitol and utilize hemicellulose hydrolysate contained in plants.

Hemicellulose is obtained from lignocellulosic waste that mainly contains 35-50% cellulose, 20-35% hemicellulose and 10-25% lignin [5,6]. Lignocellulosic wastes that are abundant in Indonesia are rice husks, tapioca pulp, empty fruit-bunches of oil-palm, and corncobs.

Corn is one of the agricultural products that are commonly produced in Indonesia. The national maize production amounted to 19.03 million tons, an increase of 2.81% compared to 2013. The plantation area in 2014 reached 3.91 million ha, while the harvesting area in 2014 amounted to 3.83 million hectares (ha) with the productivity of 4.9 tons per ha. Corn utilization is currently very diverse, ranging from food to biofuels. The fruit consists of 30% biomass waste in the form of corn cobs [7]. However, utilization of waste corn cobs in Indonesia is still limited when the content of hemicellulose in corn cobs is quite high, namely at 36%. Hydrolysis of hemicellulose will result in a constituent component which is generally a monosaccharide that is composed of the five-carbon chain. Hydrolysis is a chemical process of adding water molecules in a chemical compound that causes the termination of a chemical bond in the compound. This reaction is used to break down a polymer into its monomers. One of the results of the hydrolysis of hemicellulose is xylose which can be processed further into products of high economic value such as xylitol [8].

This study carried out two different types of hydrolysis process, that were acid hydrolysis and enzymatic hydrolysis on corn cobs to produce xylose. Hydrolysis by acid will yield a mixed product [2]. Therefore, in acid hydrolysis of corn cob, it is not only hemicellulose that will be hydrolyzed but also the cellulose whereas the monosaccharide can be further converted into another substance [5]. The resulted products are a mixture of compounds among other containing xylose, arabinose, and glucose. In addition, it will also produce byproducts in the form of oligomers, furfural, and acetic acid. Furfural compounds are toxic to some microorganisms and should be removed from the hydrolysate for it can be inhibitors in the fermentation process which may reduce the concentration of xylitol produced. The remaining acid from the hydrolysis process must also be neutralized because it can inhibit the fermentation reaction. On the contrary, in the process of the hydrolysis with enzymes, xylose will be produced more specifically because enzymatic hydrolysis does not produce mixed products or cause any product decomposition [9]. Therefore, it is important of this research to assess the effect of the acid hydrolysate and enzymatic hydrolysate in the production of xylitol by yeasts. Overall, this study was intended to turn corn cob biowaste into xylitol. Specifically, the purpose of this research was to study the effect of types of hydrolysis method on the production of xylitol via fermentation.

Materials and Methods

Corn cobs obtained from the Faculty of Agriculture, Universitas Padjadjaran, is the type of Unpad hybrid F1 originated from the crossover between Unpad A#001 x Unpad#002 that were cut, cleaned and then washed repeatedly with clean water. It was obtained from Arjasari, Bandung regency, with harvest age of 15 weeks of the growing season [10]. Afterward, the corncobs were dried in an oven at 50°C for 24 hours. Furthermore, it was crushed using a disk mill and screened with a sieve of 60 mesh in order to produce more refined corn cob powder. The composition of corn cob used was tested for its content: lignin, cellulose, and hemicellulose [11], and the morphology of the surface of the material using SEM (Scanning Electron Microscope). The tests were conducted to determine the level of hemicellulose contained in the corncobs which will then be used as a raw material in the manufacture of xylitol.

Microbe used for the fermentation was *Debaryomyces hansenii* ITBCC R85, obtained from Microbiology and Bioprocess Laboratorium, Chemical Engineering Institute of Technology Bandung. The yeast was maintained by swiping the old yeast cells in a medium of slant GYE (Glucose-Yeast Extract) agar and incubating at 30°C for 48 hours.

1.1. Hydrolysis Process

Acid hydrolysis was conducted based using sulfuric acid concentration of 6%, at the temperature of 120°C for 15 min [6]. Solid loading applied was 1 gram of corn cob fiber on the dry basis in 8 g of solution.

Enzymatic hydrolysis was performed which solid was pretreated with autohydrolysis at 121ºC for 15 minutes in an autoclave. Then, samples were hydrolysis using xylanase at 60 ℃, pH 5 and solid loading 15% [8,12].

1.2. Fermentation

The hydrolysates were further used as the substrate for the fermentation process. The fermentation process conducted using *D.hansenii* inoculum in a batch process at 30°C and at the anaerobic condition for 96 hours in Bioreactor. The solution was sampling at the beginning of the fermentation, at 24 hours fermentation, and at the end of fermentation [7]

1.3. Analysis

Samples of the fermentation liquid were analyzed by using a spectrophotometer and HPLC. From the analysis by a spectrophotometer, the cell growth curve was obtained, and from the HPLC analysis, the composition of the compound in the fermentation broth was observed.

Pulps from hydrolysis process were also analyzed using SEM (Scanning Electron Microscope) serves to determine the morphology of the surface of the material. Images that were obtained from SEM analysis were then processed using ImageJ 1.4 software to determine the average size of the particles and the pores of corn cob substrate.

The obtained data were further processed to parameterize the process performance following the growth of yeast (measured as specific growth rate), the product yield, and the utilization of xylose substrate. The specific growth rate (μ) was calculated using Equation 1 [12] :

$$\mu = \frac{1}{X}\frac{dX}{dt} \qquad (1)$$

Yield of biomass ($Y_{X/S}$) (g / g) is the biomass yield which is determined using the results of the growth curve of cells (dry cells), namely the ratio of the concentration of cells with the substrate concentration used includes xylose and glucose and expressed by Equation 2 [12]:

$$Y_{X/S} = -\frac{\Delta X}{\Delta S} = \frac{X - X_o}{S_o - S} \qquad (2)$$

Product yield ($Y_{P/S}$) (g/g) of xylitol products and other metabolites was calculated by Equation 3 [12]:

$$Y_{P/S} = -\frac{\Delta P}{\Delta S} = \frac{P - P_o}{S_o - S} \qquad (3)$$

Product yield per cell biomass (($Y_{P/X}$) (g/g) is the ratio between the number of xylitol products obtained by biomass produced at the end of fermentation (Equation 4) [12]:

$$Y_{P/X} = -\frac{\Delta P}{\Delta X} = \frac{P - P}{X - X_o} \qquad (4)$$

Xylose utilization (%) is the amount of xylose used by the yeast in the production of biomass and products, obtained by comparing the amount of xylose that was used during the fermentation with the initial xylose concentration.

Results and Discussions

1.4. Characterization of Corn Cob

Before being used as a substrate hydrolysate in the research, characterization on corn cob fiber was performed. Corn cob fiber characterization performed included the test of cellulose, hemicellulose, and lignin (Table 1).

Table 1. Corn cob fiber composition.

Fiber Components	% dry basis	
	Unpad Hybrid Corn Cobs	Reference[a]
Hemicellulose	41.170 ± 0.930	36.210
Cellulose	20.890 ± 0.220	34.400
Lignin	16.260 ± 0.210	18.800

[a]Koswara, 1991

Hemicellulose concentration of Unpad hybrid corn cobs is 41.17 (% DB) where the value is greater than the corn cobs, in general, that is 36.21 (% db) (Table 1). Hemicellulose is sugar polymer composed of xylose monomer and glucose [13]. The higher the hemicellulose composition, the more xylose available. On the contrary, Unpad hybrid corn cobs possess cellulose content of 20.89 (% DB) which is smaller in value when compared to cellulose content of corn cobs in general, i.e. 34.40 (% db). Cellulose is a polymer composed of glucose monomers [14], so that after the hydrolysis process occurs, the resulted glucose concentration was expected to be lower. The high glucose concentration in hydrolysate will inhibit the fermentation process of xylose into xylitol [5]. Unpad hybrid corn cobs have a lignin content of 16.26 (% DB) where the number is less compared to the lignin content of corn cobs in general, i.e. 18.80 (% db). Lignin is a three-dimensional polymer consisting of phenylpropane units tied by the ether linkage (C-O-C) and carbon bond (C-C). Lignin is resistant to hydrolysis because of arylalkyl bond and ether bond, and is insoluble in water, acid, and a hydrocarbon solution. Meanwhile, the other fiber components such as hemicellulose which has xylose monomer in the substrate will stick together with each other. Therefore, the lignin content that is too high on the substrate will inhibit hydrolysis process to produce xylose [9]. Based on the test results of the three components of the fiber, Unpad hybrid corn cobs have a higher potential to be used as the substrate of hydrolysis which produces xylose in xylitol when compared to corn cobs in general because it has higher hemicellulose content.

1.5. Effects of Hydrolysis Types on The Composition of Hydrolysate

As expected from the corn cob composition analysis, the hydrolysis results showed that a sample of corn cobs produced a primary product in the form of xylose and byproducts in the form of glucose and acetic acid (Table 2).

Table 2. Components of corn cob hydrolysate.

Components (g/L)	Type of Hydrolysis	
	Enzymatic Hydrolysis	Acid Hydrolysis
Xylose	2.070 ± 0,060	5.530 ± 0,140
Glucose	3.590 ± 0.120	4.070 ± 0,080
Acetic acid	1.150 ± 0,020	0.280 ± 0,000

Xylose was produced from the hydrolysis of hemicellulose, while glucose was the hydrolysis result of cellulose. Even though the solid loading applied in the acid hydrolysis was lower than enzymatic hydrolysis, which was 12.5% in the acid hydrolysis and 15% in the enzymatic hydrolysis, the concentration of xylose produced by acid hydrolysis was higher than those produced by the enzymatic hydrolysis. Besides that, both acid and enzymatic hydrolysis produced glucose, which glucose of enzymatic hydrolysis was higher than acid hydrolysis. This might be caused by the high temperature used in the hydrolysis process, 121^{o}C, that could facilitate the breaking of xylose and cellulose from the substrate [6]. The higher concentration of acetic acid in the enzymatic hydrolysate when compared to

those in acid hydrolysate was related to the addition of acetate buffer in the enzymatic hydrolysis process.

1.6. Effect of Enzymatic and Acid Hydrolysate on Microorganism's Growth During Fermentation

The fermentation using acid hydrolysate went through a phase of adaptation for 2 hours and the logarithmic phase from the 2nd until the 24th hour (Figure 1). The fermentation lasted for 96 hours. Fermentation using enzymes hydrolysate went through a phase of adaptation for 2 hours and the phase of logarithmic from the 2nd hour till the 24th hour. Based on previous studies [15] about logarithmic phase on fermentation using Oil Palm Empty Fruit Bunches (OPEFB) enzymatic hydrolysates at similar fermentation conditions, it was obtained that microbes during fermentation went through the logarithmic phase until the 46th hours. Faster logarithmic phase was thus obtained in this study.

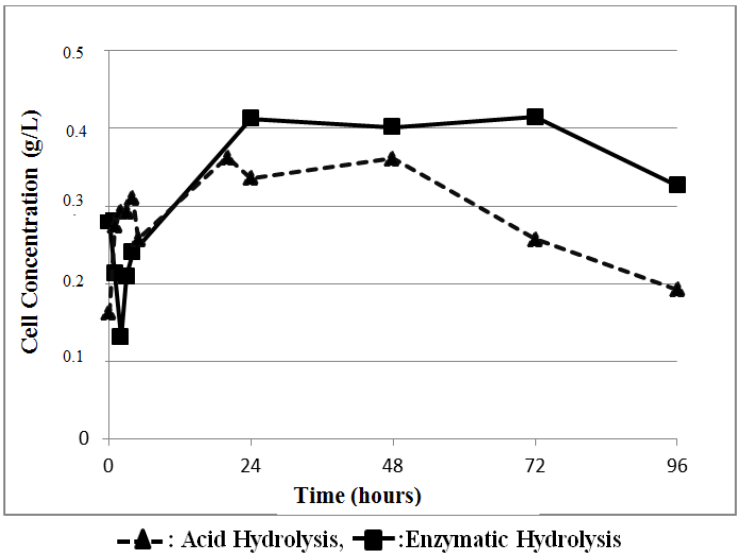

Figure 1. Profile of the growth of *D. hansenii* in different hydrolysate during fermentation.

Fermentation using acid hydrolysate went through a phase of adaptation for 2 h and the logarithmic phase from the 2 - 24 h fermentation time. The fermentation lasted for 96 hours. Fermentation using enzymes hydrolysate went through a phase of adaptation for 2 hours and the phase of logarithmic from untill the 24th hour. Based on previous studies [15] about logarithmic phase on fermentation using Oil Palm Empty Fruit Bunches (OPEFB) enzymatic hydrolysates at similar fermentation conditions, it was obtained that microbes during fermentation went through the logarithmic phase at 46 h fermentation time. Faster logarithmic phase was thus obtained in this study.

Fermentation using an enzymatic hydrolysate had the specific growth rate (μ) of 0.039 h^{-1}, whereas fermentation using acid hydrolysate had a μ of 0.0056 h^{-1}. From the data obtained, the fermentation using enzymes hydrolysate had a specific growth rate value that was greater than the fermentation using acid hydrolysate.

μ value resulted was small or nearly zero. It indicates that there is no significant growth of the cells. Slow cell growth caused by the tendency of microbial cells utilize sugars to form a metabolite product under conditions of high initial cell concentration. Limitations of available oxygen can also be one of the reasons for the absence of the cell growth in the fermentation with the condition of a high initial concentration of cells [7].

1.7. Effects of Types of Hydrolysis on The Production of Metabolic Products

Fermentation using enzymatic hydrolysate as well as acid hydrolysate showed that the utilization of glucose tended to be higher compared to the xylose utilization (Table 3).

Table 3. Fermentation product of enzyme hydrolysate and acid hydrolysate.

Products	Type of Hydrolysis	
	Enzymatic Hydrolysis	Acid Hydrolysis
Initial Xylose Concentration (g/L)	2.070 ± 0.060	5.530 ± 0.140
Final xylose concentration (g/L)	1.040 ± 0.070	3.550 ± 0.020
Xylose Utilization (%)	49.750 ± 2.290	35.800 ± 2.660
Initial Glucose Concentration (g/L)	3.590 ± 0.120	4.070 ± 0.080
Final Glucose Concentration (g/L)	0.380 ± 0.010	1.880 ± 0.040
Glucose utilization(%)	89.420 ± 0.090	53.810 ± 2.530
Initial Xylitol Concentration (g/L)	0.000 ± 0.000	0.000 ± 0.000
Final Xylitol Concentration (g/L)	0.090 ± 0.020	0.200 ± 0.010
Xylitol Yield from xylose t = 72 h (g/g) ($Y_{P/S}$)*	0.216 ± 0.000	0.000 ± 0.000
Xylitol Yield from xylose t = 96 h (g/g) ($Y_{P/S}$)*	0.100 ± 0.050	0.100 ± 0.490
Initial Cells Concentration (g cell/L)	0.281 ± 0.900	0.163 ± 0.015
Final Cells Concentration (g cell/L)	0.326 ± 0.001	0.193 ± 0.012
Biomass Yield of Substrate (g/g) ($Y_{X/S}$)*	0.031 ± 0.001	0.013 ± 0.015
Xylitol Yield of Concentration cells (g/g) ($Y_{P/X}$)*	1.500 ± 0.140	1.000 ± 0.089
Specific Growth Rate (h^{-1}) (μ)	0.039 ± 0.001	0.0056 ± 0.001

The higher glucose utilization was due to the fact that glucose was used dominantly as the food source by yeasts during the fermentation process. Something similar happened to the research Parajo (1998) [16] where the fermentation occurred using wood hydrolysate that had been concentrated as a substrate.

Based on Table 3, xylitol began produce in the fermentation using enzymatic hydrolysate at the 72 hour fermentation time, with the xylitol yield of 0.216 g xylitol/g xylose (xylitol concentration of 0.16 g/L), while for the acid hydrolysate, xylitol began to form at the 96 hour or in the late time of fermentation with yield 0.100 g xylitol/g xylose (xylitol concentration of 0.2 g/L). At the 96th hour, the xylitol produced by the enzyme hydrolysis decreased to 0,100 g of xylitol/g xylose. The decrease of xylitol yield might be caused by the consumption of xylitol by microbes as a source of food due to low levels of xylose and glucose at the end of fermentation. In another study, the yield of xylitol using the enzymatic hydrolysate with fermentation conditions similar to this study equaled to 0.098 g xylitol/g xylose [15]. Meanwhile, for the xylitol yield using acid hydrolysate for the same fermentation condition was 0.084 g of xylitol/g xylose. The yield of biomass on fermentation using enzymatic hydrolysate possessed a higher value when compared to the fermentation using acid hydrolysate [8]. The higher

value of this yield can be caused by not accounting for acetic acid content as a substrate in the fermentation of hydrolysate (indicated by the yield of acetic acid to the substrate which is negative).

Xylose and glucose from both types of hydrolysates continued to decline as it continued to be used by microbes to produce xylitol and other metabolite products (Figure 2). The concentration of glucose in the enzymatic hydrolysate decreased faster than the xylose concentration in the acid hydrolysate. This resulted in higher values of glucose utilization in the enzyme hydrolysate. The decline in xylose concentration, both acid and enzyme hydrolysate, was not as fast as the decrease in the concentration of glucose. Based on the decrease of concentration, the substrate produced greater utilization value than the xylose utilization, both for the acid hydrolysate and enzymes hydrolysate. In this case, the enzymatic hydrolysis had a higher utilization value, both glucose and xylose utilization. The high utilization of glucose indicated that the yeast that conducts the fermentation process tended to consume glucose so that the yeast will be more likely to produce other metabolite products like ethanol than xylitol.

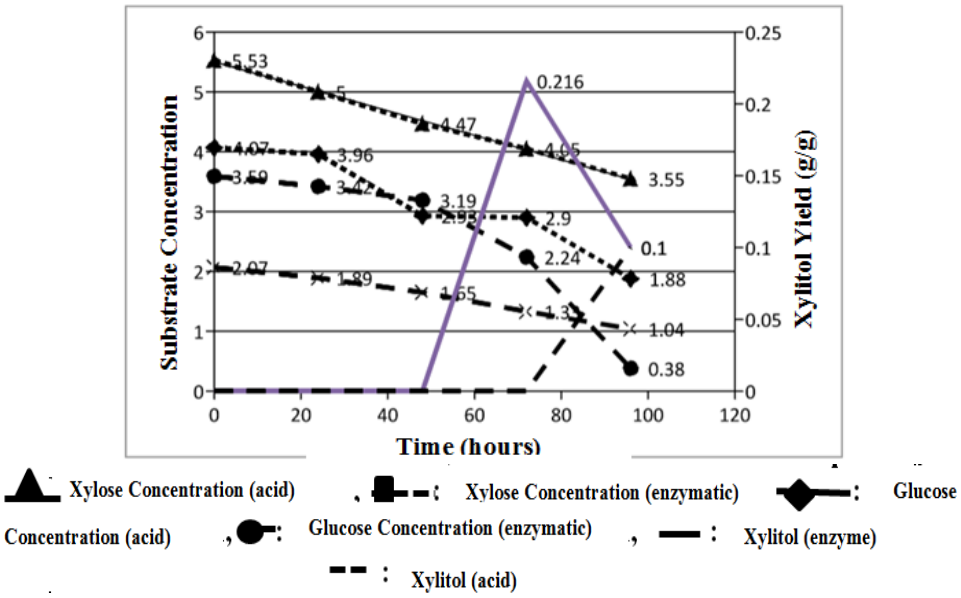

Figure 2. The concentration of xylose and glucose, and yield of xylitol per 24 hours.

Fermentation using enzymatic hydrolysate provides greater and faster xylitol yield and when compared to the acid hydrolysate. The higher yield of xylitol on fermentation using enzyme hydrolysate can be caused by the greater amount of xylose consumed compared to acid hydrolysate. In addition, the higher yield of xylitol on enzyme hydrolysate fermentation indicated the absence of inhibitor compounds that can interfere with the activity of *D. hansenii* [17]

1.8. Effectsof Enzyme and Acid Hydrolysate on other Metabolites Products

The production of metabolite products such as ethanol and acetic acid continued to increase, even the acquisition is indicated to continue to increase along with the increase of duration of fermentation (Table 4). A tendency to produce ethanol than xylitol and is indicated by the greater yield of ethanol when compared to the yield of xylitol.

Because the fermentation process takes place under anaerobic conditions, the oxygen dissolved in the broth of low fermentation. The low dissolved oxygen causes microbes to tend to convert glucose into ethanol and a small portion xylose into ethanol [18]. It also indicates that the ratio of glucose to xylose in the substrate is too big.

Table 4. Yield of other metabolites product.

Information	Fermentation Medium	
	Enzymatic Hydrolysis	Acid Hydrolysis
Ethanol Yield of Substrate t = 0 h (g/g) ($Y_{P/S}$)*	0.000 ± 0.000	0.000 ± 0.000
Ethanol Yield of Substrate t = 24 h (g/g) ($Y_{P/S}$)*	0.104 ± 0.000	0.000 ± 0.000
Ethanol Yield of Substrate t = 48 h (g/g) ($Y_{P/S}$)*	0.130 ± 0.000	0.000 ± 0.000
Ethanol Yield of Substrate t = 72 h (g/g) ($Y_{P/S}$)*	0.151 ± 0.009	0.000 ± 0.000
Ethanol Yield of Substrate t = 96 h (g/g) ($Y_{P/S}$)*	0.177 ± 0.140	0.000 ± 0.000
Acetic Acid of Substrate t = 0 h (g/g) ($Y_{P/S}$)*	0.271 ± 0.012	0.067 ± 0.001
Acetic Acid of Substrate t = 24 h (g/g) ($Y_{P/S}$)*	0.344 ± 0000	0.094 ± 0.000
Acetic Acid of Substrate t = 48 h (g/g) ($Y_{P/S}$)*	0.366 ± 0.000	0.098 ± 0.000
Acetic Acid of Substrate t = 72 h (g/g) ($Y_{P/S}$)*	0.375 ± 0.000	0.108 ± 0.000
Acetic Acid of Substrate t = 96 h (g/g) ($Y_{P/S}$)*	0.394 ± 0.007	0.137 ± 0.004
Glycerol Yield Of Substrate (g/g)($Y_{P/S}$)*	0.000 ± 0.000	0.000 ± 0.000

Acid hydrolysate fermentation has a smaller glucose conversion value than the enzymes hydrolysate fermentation. This small value of the glucose conversion causes the absence of formation of ethanol and glycerol which are metabolite products produced by microbes when consuming glucose as substrate.

1.9. Scanning Electron Microscope (SEM) Analysis

Material test by means of SEM (Scanning Electron Microscope) serves to quantitatively and qualitatively determine the morphology of the surface of the material. SEM (Scanning Electron Microscope) is one type of electron microscopes capable of producing a high-resolution picture of a sample's surface. Therefore, the image generated by SEM has qualitative characteristics in two dimensions because it uses electrons instead of light waves and is useful for determining the structure of the sample's surface. SEM characterizes the material in the form of a thin layer having a thickness of 20 μm from the surface, images of surface topography in the form of protrusions, indentations and the thickness of a thin layer of a cross-sectional.

SEM or scanning electron microscope focuses the electron beam at the surface of the object and takes a picture by detecting electrons that appear on the surface of the object. Various types of SEM allow for different usages, such as morphological study, high-speed composition analysis, surface roughness, porosity, particle size distribution, homogeneity of materials or environmental studies on the

subject of the sensitivity of the material. The test result of corn cob substrate by means of SEM can be seen in Figure 3-8.

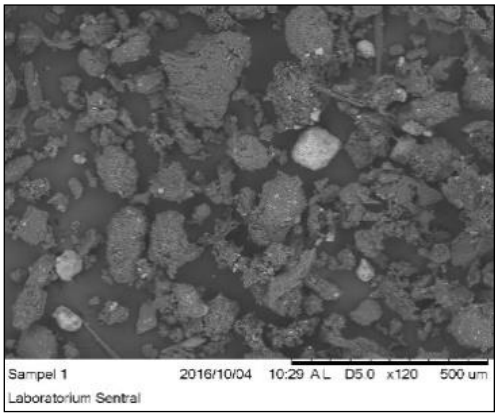

Figure 3. Particle shape (120x magnification) of corn cob substrate before hydrolysis.

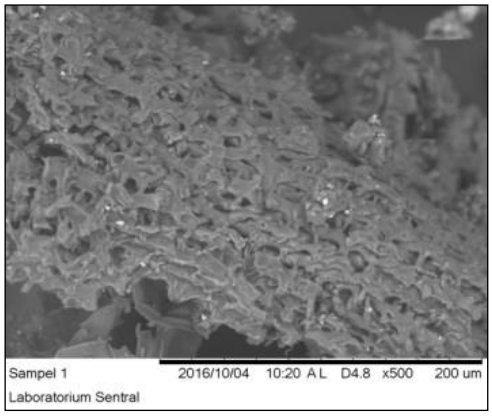

Figure 4. Particle pores (500x magnification) of corn cob substrate before hydrolysis.

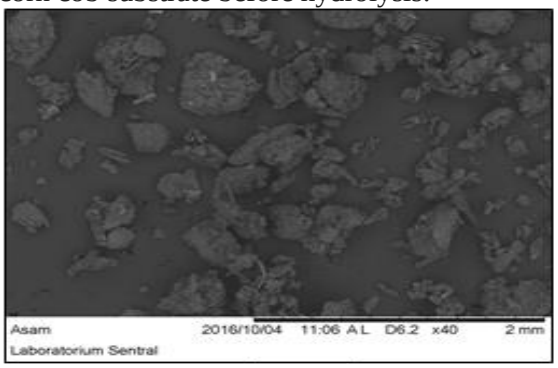

Figure 5. Particle shape (40x magnification) of corn cob substrate after acid hydrolysis.

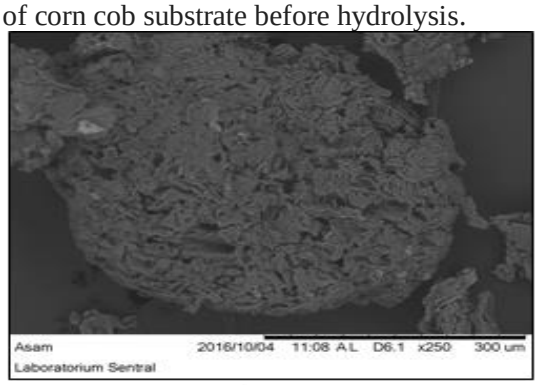

Figure 6. Particle pores (250x magnification) of corn cob substrate after acid hydrolysis.

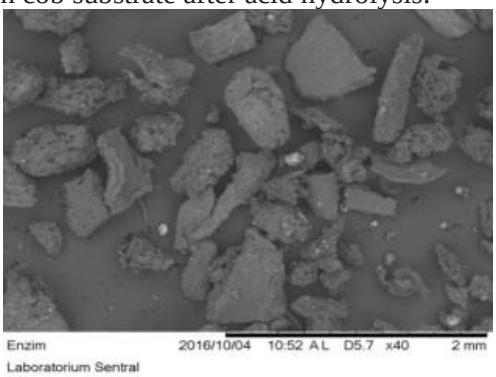

Figure 7. Particle shape (40x magnification) of corn cob substrate after enzymatic hydrolysis.

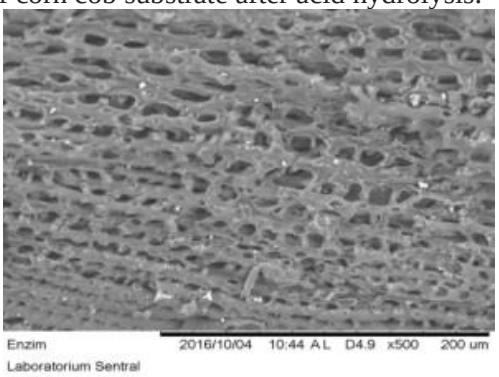

Figure 8. Particle pores (500x magnification) of corn cob substrate after enzymatic hydrolysis.

Enzymatically-hydrolyzed corncobs had a much larger average area of the particle (4.49×10^4 μm^2) when compared to corn cobs before being hydrolyzed (19.35×10^2 μm^2) and after being hydrolyzed with acid (4.20×10^4μm^2). The average size of the corncob's pores after hydrolyzed by the enzyme (33.17 μm^2) was also greater when compared to corn cobs before hydrolyzed (11.22 μm^2) and after acid hydrolysis (27.54 μm^2) (Table 5).

Table 5. Morphological test on corn cob substrate before and after hydrolysis

Components (g/L)	Average Particle Size (μm^2)	Average Pore Size (μm^2)
Before Hydrolysis	19.35×10^2	11.22
After Acid Hydrolysis	4.20×10^4	27.54
After Enzymatic Hydrolysis	4.49×10^4	33.17

The result of enzymatic hydrolysis enlarged the pore size to be more than the original corncob substrate and more than the result of acid hydrolysis. It was caused by incubation time in the hydrolysis process, namely 72 hours. Meanwhile, the particle size of enzymatic hydrolysis result was enlarged due to the clotting of the substrate by buffer acetate used during hydrolysis process. The enlarged particle size of corn cob substrate on acid hydrolysates is because acid can destruct some crude fibers by lowering the pH value. At low pH conditions, crude fiber contained in a material will clot. The clotting causes the particles to grow in size. Meanwhile, the enlargement of the pore of corn cob fiber's particle occurs due to the disconnection of fiber chains into sugar monomer that is dissolved in the hydrolysate liquid [8].

Conclusions

This study was explored the effect of the enzymatic and acid hydrolysis on xylitol fermentation product. The type of hydrolysis applied affected the growth of *D. hansenii* and correspondingly, the xylitol production by these microbes. Fermentation using enzymatic hydrolysate gave higher xylitol yield ($Y_{P/S}$) and specific growth rate (μ) than the fermentation using acid hydrolysate. These phenomena were very likely to be caused by the different concentration of glucose and fermentation inhibitors in the hydrolysate.

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