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# The Xylitol Production Efficiency From Corn Cob Waste By Using Stirred Tank Bioreactor-Tubular Loop Liquid Emulsion Membrane (LEM)

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**Abstract.** The alternative sweetener used to beverage and food with a calorie-controlled, to control diet program or to prevent tooth decay. The alternative processes that are currently being developed are producing xylitol from xylan of the cellulose biomass. The goal of this study is to determine the most influential process variable and optimum operating condition of xylitol production through enzymatic hydrolysis reaction of corn cob waste. The parameters are the time (30-120 minutes substrate ratio: starter (1:5-1:15 w/w). The result showed a high number of a cell on a substrate concentration of 10% led to increase in xylose consumption while the xylitol production is not too large because the substrate is used for yeast cell growth so that production of xylitol to be reduced and the efficiency is low. The high efficiency (24.21%) occurred in 20% of substrate concentration because the xylose consumption is used for growth and xylitol production. While the concentration of 30% decline in the consumption of xylose by yeast compared with others and the efficiency is not too high. This is because the high concentration of xylose, which led to substances inhibitor in the media and the production of xylitol becomes low.

## 1. Introduction

Xylitol is a potential alternative of sweeteners sucrose, and xylitol is a natural carbohydrate-like substance which is classified in the systematics of organic chemistry as sugar alcohol of the pentitol type with the general formula  $C_5H_{12}O_3$ . Xylitol occurs in fruits and vegetable such as strawberries, raspberries, carrot, spinach, lettuce and cauliflower for small amount. Xylitol has almost the same properties as sucrose (table sugar), makes it suitable to be used as a sucrose substitute in the food industry. Xylitol has a negative dissolution heat value, the cooling sensation it gives in the mouth. This property makes a suitable for use in the chewing gum industry. Another critical property of xylitol which is able to prevent cavitation in the teeth [9] and its non-insulin nature for regulating the metabolism makes it suitable as sucrose sweetener alternative for use as a sweetener in vitamin tablets, medicine, toothpaste and mouthwash. Besides being used in various industries, xylitol is also used in the treatment of diabetes patients [2], osteoporosis [10] and ear infection patients [17]. Xylitol has been used in 35 countries to the needs of 40,000 tons in 2006 valued at 250 million euro. Xylitol world demand as increase as the growth rate reaching 10% per year. Indonesia is still importing xylitol for



several industrial from a few countries in Europe, especially Finland, the United States (U.S.), China, India, and Japan. Xylitol can be produced from the hydrogenation process of D-xylose or xylan-rich hemicellulose hydrolysates. Currently, xylitol produced from birch wood hydrolyzate. Basically, the raw material of xylitol is biomass containing hemicellulose which one monomer of the hemicellulose is xylan. Xylans are considered to be the primary interface between the lignin and other carbohydrates in the cell wall of plants. Chemical methods that are used in the producing of xylitol is based on hydrogenation reaction of D-Xylose or hemicellulose hydrolyzate has xylan-rich using Raney nickel catalyst in the autoclave at a temperature of 135°C and pressure of 40 atm for 2.5 hours [11]. The low conversion (60%), the expensive catalyst and equipment are the disadvantages of a chemical process. Another method of producing xylitol is produced from xylonic acid [6]. Xylonic acid crystal produced and hydrogenated for 3 hours in an autoclave at a temperature of 110°C pressure of 13,000 kpa uses Ruthenium on carbon catalyst. Reaction conversion of this process reached 75.9% [6]. Xylitol can also be produced from D-glucose, D-fructose and D-galactose. D-glucose is oxidised in the autoclave using Ruthenium in carbon catalyst with 90% yield. However, the processes proved too costly for large scale production [1].

The alternative processes that are currently being developed are producing xylitol from xylan of the cellulose biomass. The research of cellulose biomass production from xylitol is being developed, and both in term of raw material, as well as in term of sources of enzyme used. The research of xylitol production from various biomass has been studied by several authors, including corn fibre raw material and wheat straw. On the other hand, the yield of biomass fermentation is mainly influenced by the type of raw material [12]. Enzymatic conversion of cellulose biomass into xylitol involved three necessary steps are the pre-treatment, hydrolysis and fermentation processes. Pre-treatment process is to facilitate access xylanase enzyme to hydrolyses xylan into xylose. Hydrolysis process to produce xylose from xylan through a process of acid hydrolysis or by enzymatic hydrolysis. Acid hydrolysis can be divided into two processes, namely, dilute Concentrated Acid Hydrolysis and Acid Hydrolysis. Dilute Acid Hydrolysis (DAH) is the oldest technology used to hydrolyse xylan. DAH process involved a 1% solution of sulfuric acid in a continuous reactor operating at high temperature, 250°C. Conversion of the process is only 50%. Concentrated of Acid Hydrolysis using concentrated sulfuric acid, followed by dissolution in water to dissolve and hydrolyse xylan into xylose. Xylan enzymatic hydrolysis has the potential to increase efficiency, conversion and productivity. Xylan enzymatic hydrolysis is involving several different enzymes. The secreted enzyme of filamentous fungi *Trichoderma reesei* can convert biomass into sugar. The higher conversion produced minimal side product, lower energy requirement and operating conditions are relatively lower are the advantages of xylan enzymatic hydrolysis. The enzymatic process is an environmentally friendly process, by using renewable raw material for the economy of waste corn cobs for the xylitol production process can increase of value for the farmer and the corn industry. Nowadays, enzymatic hydrolysis using a bioreactor microwave is an up-and-coming technology for converting biomass into xylose for conversion into xylitol by *Candida guilliermondii*.

## 2. Method

### 2.1. Preparation of Microorganisms and inoculum

Enzymes were used in the bioconversion of D-xylose into xylitol is *Candida guilliermondii*. *Candida guilliermondii* yeast cultivation maintained at a temperature of 4°C in agar medium. Then, the cultivation yeast is transferred into 125 ml Erlenmeyer which is containing 50 ml of liquid media of 30.0g / L D-xylose, 2.0 g/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1 g/L CaCl<sub>2</sub>·2H<sub>2</sub>O and 20.0 g/L solution extracts rice bran. The liquid yeast cultivation media incubated and stirred at 200 rpm and at 30°C for 24 hours. Yeast cells are centrifuged at 2000xg for 15 minutes and washed using sterile distilled water. Making the suspension is done by adding distilled water into yeast cells and used as an inoculum.

## 2.2. Preparation of Corn cob hydrolyzate

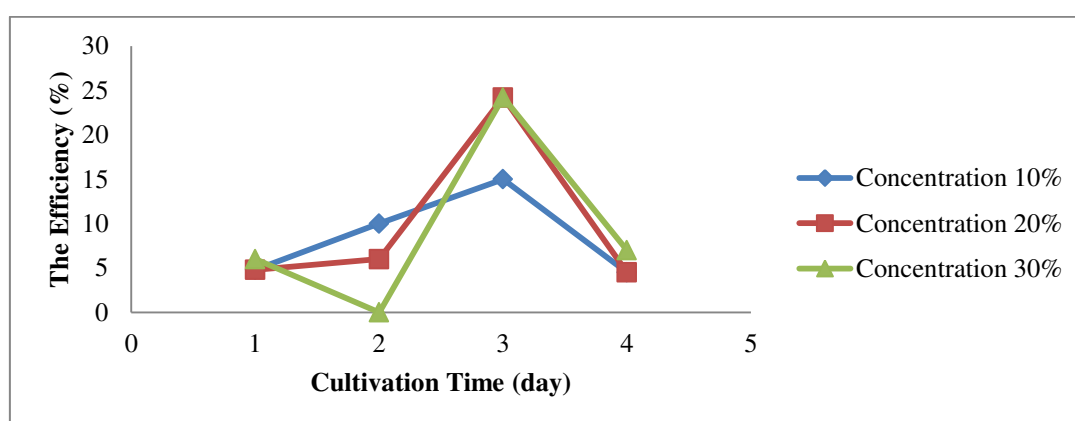
Corn cob mixed with 1% (v/v)  $\text{H}_2\text{SO}_4$  solution with a ratio of 1:10, into the reactor. Hydrolysis was carried out at  $121^\circ\text{C}$  for 10 minutes. Then, corn cob hydrolyzate was filtered and concentrated in a vacuum at a temperature of  $70^\circ\text{C}$ . The pH of hydrolyzate is neutralised using CaO and then acidified to reach a pH of 5.5 by using  $\text{H}_3\text{PO}_4$  as well as the added activated carbon. After the adsorption process completed, active carbon separated by using vacuum filtration. Corn cob hydrolyzate adsorption result of autoclave at  $111^\circ\text{C}$  for 15 minutes before used as a fermentation medium.

## 2.3. Fermentation Process

The corn cob hydrolyzate mixture with a couple of type of nutrients to the nutrient added to the inoculum preparation except D-xylose. Fermentation was done in 500 ml fermentor of cultivation medium. The stirring was carried out using a rotary shaker at 200 rpm, temperature of  $30^\circ\text{C}$  for 64 hours. Sampling has done for 6 hours to analyse the concentration of D-glucose, L-arabinose, D-xylose, xylitol, acetic acid and furfural using HPLC. The concentration of the phenolic compound has done by using the colourimetric method  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  and  $\text{K}_3\text{Fe}(\text{CN})_6$  at 700nm. The number of cells is determined directly by counting use a Neubauer chamber (area =  $1 / 400\text{mm}^2$ ; height = 0.100 mm).

## 3. Results And Discussion

### 3.1. The efficiency of Xylitol production



**Fig 1.** The Efficiency of Xylitol Production on Various Substrate Concentration of hydrolyzate Hemiselulose Corn Cob For four days cultivation

The high number of cells on a substrate concentration of 10% led to increasing in the consumption of xylitol production of xylose while not too large due to a little-used substrate for the growth of yeast cells so that production of xylitol to be reduced and the efficiency is low. The highest efficiency (24.21%) occurred in 20% of substrate concentration because the balance xylose consumption is used for growth and xylitol production. While at a concentration of 30%, the consumption of xylose by yeasts compared with other concentrations decreased so that efficiency was not too high. That is because the high concentration of xylose led to many inhibitors in the media and the production of xylitol was low. The efficiency of bioconversion process usually observed from the value of the yield. If the yield of the bioconversion process close to 1 means that almost the entire substrate consumed was converted into product. According to Barbosa et al. (1988) in Roberto et al. (1996a), the value of yield for the production of xylitol theoretically amounted to 0.917 g/g. The highest yield from the study is 0.22 g/g, lower than the theoretical value. The yield value indicated that not all of the substrate is consumed by yeast transformed into a product for substrate consumption is closely related to the growth of yeast cell is influenced by external and internal factors of the cell. The related factor

of the value of the xylitol production yield is the ambient temperature for the production of xylitol. In this research, the xylitol production temperature is actually less optimum for the growth of *C. tropicalis* the optimum growth temperature of *C. tropicalis* is 30 ° C, the less optimum growth of *C. tropicalis* caused xylitol production activity was less than optimal.

#### 4. Conclusion

The results showed that the growth of *C. tropicalis* for four days cultivation increased in the various concentrations of hemicellulose hydrolyzate bagasse. Xylitol production obtained at the optimum production media containing 20% hemicellulose hydrolyzate substrate bagasse on the third day of cultivation with the production of xylitol 10.258 g/l, yielding 0.22 g/g and the cell number of  $2.9 \times 10^8$  cells/ml. The optimum (highest) efficiency of xylitol production of hydrolyzate by *C. tropicalis* hemiselolosa is 24.21% at a concentration of 20% in the third day of substrate cultivation. Based on these results, it needs to study the variation of substrate concentration between 20% and 30% to optimise the production of xylitol by various cultivation time in hours. The research of other substances than xylose and xylitol contained in hemicellulose hydrolyzate bagasse and its influence on the production of xylitol by *Candida tropicalis*, optimisation of production temperature to optimise the growth of *C. tropicalis* and research using other yeast is also very necessary.

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