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Production Process of Hydrolysate from Steam Explosion of Oil Palm Trunk for Xylitol Fermentation

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ABSTRACT

The steam-exploded hemicellulose hydrolysate of 100 g oil palm trunk chips was acid posthydrolysed under the condition of autoclaving at 120 °C with 1%w/v H_2SO_4 for 30 min. The highest amounts of xylose and glucose obtained were 8.15 g and 2.46 g, respectively. The optimal adsorption condition to remove toxic compounds in hemicellulosic hydrolysate was achieved in two steps. Five percents (w/v) of activated carbon were used in the first step for 30 min then 2% (w/v) activated carbon were used in the second step for 60 min. The comparisons were made between two sources of fermentation media, hydrolysate and pure xylose. It was found that fermentation of the hydrolysate with *Candida magnolia* TISTR 5664 gave xylitol at 0.08 g Yp/s of xylitol/g of xylose whereas fermentation of pure xylose gave xylitol at 0.29 g Yp/s of xylitol/g**.** The fermentation with *C. guilliermondii* FTI 20037 from the hydrolysate gave xylitol at 0.12 g Yp/s of xylitol/g of xylose whereas the fermentation from pure xylose gave xylitol at 0.30 g Yp/s of xylitol/g of xylose.

Key words: xylitol, oil palm trunk, steam explosion, hydrolysate

INTRODUCTION

Oil palm trunks are agricultural by-products of lignocellulosic nature in Thailand. They are interesting biomass sources and precious substances, and can be fractionated using a sequences of chemical treatments allowing the separation of cellulose, hemicellulose and lignin in separate steam. With this approach, various fractions coming from the process can be utilized for different product applications. Oil palm trunk consists of 37.14% alpha-cellulose, 30.59% pentosan, 22.32% lignin, 8.07% ethanol/benzene extractive and 8.56% ash on dry weight (Rivas *et al*., 2002).

The hemicellulose fraction can be easily removed by dilute-acid hydrolysis (Dominguez *et al*., 1997), steam explosion (Motane *et al*., 1998) and autohydrolysis (Rivas *et al*., 2002). However, steam explosion and autohydrolysis lead to large amount of oligosaccharides which have to be converted into monosaccharides (by acidic or enzymatic catalysis) before fermentation (Rivas *et al*., 2002). The resulting hydrolysate is rich in

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fermentable sugars, mainly xylose, which can be used as a fermentation medium for xylitol production (Canettieri *et al*., 2001).

Xylitol is pentitol with an increasing interest owing to its diabetic and clinical properties. This low caloric sweetener has negative heat of solution, anticarcinogenic properties, and is suitable as sugar substitute for diabetics (Emodi, 1978). Xylitol is produced by chemical or biotechnological routes from D-xylose recovered from lignocellulosic material. An advantage of the biotechnological process over the chemical one is cost reduction associated with the non-necessity for xylose purification (Parajo *et al*., 1998).

Many yeasts, specially those of genus *Candida* (Winkelhausen and Kuzmanoua, 1998) produce high yields of xylitol from xylose. However, the xylitol production is hampered by variety of toxic compounds normally present in hemicellulose hydrolysates, which can be included in three main groups: furan derivatives, aliphatic acids and phenolic compounds. These compounds are resulted from hydrolysis of lignocellulosic structure (Converti *et al*., 2000) and their concentrations in the fermentation medium depend on the type of raw material and hydrolysis condition employed. For toxicity of the hydrolysate to be reduced, it is necessary to treat before fermentation (Winkelhausen and Kuzmanoua, 1998). Some examples of chemical treatment capable of inducing precipitation of toxic compounds are pH adjustment (Martinez *et al*., 2001), detoxification with ion-exchange resin and adsorption on activated carbon or activated charcoal (Lee *et al*., 1993). These treatments can be employed separately, but lately there has been a tendency to use them in different combinations (Alves *et al*., 1998).

This work deals with the fermentation of xylose containing hemicellulose hydrolysate obtained from steam exploded of oil palm trunk. The effect of detoxification with adsorption on activated carbon was studied before the feasibility for xylitol fermentation.

MATERIALS AND METHODS

Plant materials

Twenty-seven year-old oil palm trunks collected from Krabi Province, Thailand, were chopped into chips with particle size smaller than 5 mm ×1 inch ×1 inch, air dried to 10% moisture and stored at room condition until use.

Preparation of hydrolysates

Pretreatment by steam explosion

One hundred gram of oil palm trunk chips were steam-exploded in 2.5 l batch cooker at 214 °C for 2 min (Log Ro=3.65). Water was added to the wet pretreated material to make up the volume of 1 l and boiled at 80 °C for 30 min. Then the hemicellulose hydrolysate was recovered by filtration with cheese cloth.

Acid posthydrolysis of hemi-cellulose hydrolysate

Acid posthydrolysis of hemicellulose hydrolysate was carried out to cleave the xylooligosaccharides into monomeric sugars by autoclaving at 120 °C with the concentrations of H_2SO_4 varied from 1, 2, 3 and 4 %w/v and time varied from 15, 30, 45 and 60 min.

Treatment of hydrolysate

pH adjustment

The hydrolysate from acid post-hydrolysis was adjusted with CaO to pH 7.0-8.0 and the $CaSO_4$ precipitates were removed by filtration with filter paper (Whatman No.4, USA).

Activated carbon adsorption

Two-step detoxification of hydrolysate by powdered activated carbon at pH 7.0-8.0 was studied. First, the hydrolysate was mixed with activated carbon, varied from 3, 5, 7 and 10%w/v and agitated at 200 rpm by platform shaker (Innova 2100, New Brunswick Scientific,

USA) at room temperature for 1 h. After that, the reaction time was varied from 0.5, 1, 2, 4, 6, 8, 12, 18, 24 and 48 h. Secondly, the activated carbon was varied from 1, 2 and 3%w/v for 1 h, while the reaction time was varied from 15, 30, 45 and 60 min.

Concentration of hydrolysate

In selected experiments, the xylose concentration of hydrolysate was increased to the desired level by vacuum evaporation at temperature below 60 °C.

Xylitol fermentation (batch culture)

Microorganisms and inoculum preparation

Candida guilliermondii FTI 20037 (ATCC, USA) or *Candida magnolia* TISTR 5664 (TISTR, Thailand) was grown in 250 ml Erlenmeyer flask containing 50 ml medium with 20 g/l xylose, 10 g/l yeast extract and 1.7 g/l yeast nitrogen base. They were incubated on a rotatory shaker (170 rpm) at 30 °C for 24 h. Afterwards, the cells were collected by refrigerated centrifugation (GRX-220 High Speed, Tomy, USA) at 8,000 g, 4 °C and resuspended in sterile distilled water.

Media and fermentation condition

First, the treated hydrolysate or pure xylose was autoclaved at 110 °C for 15 min. They were used as carbon sources with supplementation of 1.7 g/l yeast nitrogen base without amino acid and ammonium sulfate, 5.0 g/l urea and 1.0 g/l yeast extract for *C. guilliermondii* FTI 20037 and with supplementation of 1.7 g/l yeast nitrogen base without amino acid and ammonium sulfate and 2.27 g/l urea for *C. magnolia* TISTR 5664. The inoculum was then seeded at an initial density of OD_{660} = 1.0 for *C. guilliermondii* FTI 20037 and of OD_{600} = 1.0 for *C. magnolia* TISTR 5664. The fermentation runs were carried out in 125 ml Erlenmeyer flasks containing 50 ml of fermentation media at 30 °C on platform shaker (Innova 2100, New Brunswick Scientific, USA) at 170 rpm for 0, 12, 24, 48 and 72 h.

Analytical method

Sugar (glucose, xylose and xylitol), acetic acid, furfural and 5-hydroxymethylfurfural (HMF) contents in hydrolysate were analysed by an LC10A High Performance Liquid Chromatography (Shimadzu Co. Ltd., Kyoto, Japan). Aminex HPX-87C column (300 × 7.8 mm, Bio-Rad, USA) was used at 80 °C with deionised water as an eluent with connection to RI detector for sugar analysis. Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad, USA) operating at 65 °C with 0.005 N H_2SO_4 as an eluent and UV-vis detector at 280 nm were used for furfural and HMF analysis, and at 210 nm for acetic acid analysis. The flow rate was set at 0.6 ml/min with a sample injection volume of 20 μl.

The phenolic compounds were detected by Folin-Ciocalteus reagent method at 735 nm whereas Platinum-Cobalt method was used for color measurement. Cell mass was measured by spectrophotometer (OD at 600 nm for *C. magnolia* TISTR 5664 and at 660 nm for *C. guilliermondii* FTI 20037) and the optical density of cell suspension was determined from a standard curve (OD versus dry cell weight).

RESULTS AND DISCUSSION

Preparation of hydrolysate

The hemicellulose hydrolysate from steam explosion pretreatment of 100 g oil palm trunk chips contained 1.68 g xylose, 0.56 g glucose, 0.55 g furfural, 0.29 g HMF, 0.05 g phenolic compounds and 1.14 g acetic acid. The xylose content was actually very low when compared with the one in oil palm trunk raw material (18.47% w/w). Therefore, acid posthydrolysis of hemicellulose hydrolysate was further performed with 1%w/v H_2SO_4 for 30 min to obtain higher xylose content of 8.15 g, 2.46 g glucose, 0.69 g furfural, 3.17 g HMF and 2.33 g

acetic acid. This result indicated that post-hydrolysis of the hemicellulose solution under acidic condition could improve %xylose recovery by 24.12% w/w.

Treatment of hydrolysate

Analysis of hemicellulose solution for toxic agents before adsorption showed that it contained color intensity, phenolic compound, furfural, and HMF at 24300 Pt-Co, 30.72 mg/l, 350.00 mg/l, and 1580.00 mg/l, respectively. These agents were proved to inhibit microorganism growth (Clark and Mackie, 1984), thus they needed to be removed before further being used as fermentation substrate. The optimal adsorption condition to remove these toxic compounds was achieved in two steps. Five percents (w/v) of activated carbon were used in the first step for 30 min then 2% (w/v) activated carbon was used in the second step for 60 min. It was found that the intensity of color, the amount of phenolic compounds, of furfural and of HMF were reduced by 99.96, 99.15, 100 and 99.94%, respectively.

Xylitol fermentation

The results of xylitol fermentation from pure xylose or hydrolysate medium by *C. magnolia* TISTR 5664 or *C. guilliermondii* FTI 20037 are presented in Figure 1. The 26.49 g/l pure xylose as medium for fermentation of *C. magnolia* TISTR 5664 produced 7.54 g/l xylitol or 0.29 g Yp/s of xylitol/g of xylose, whereas

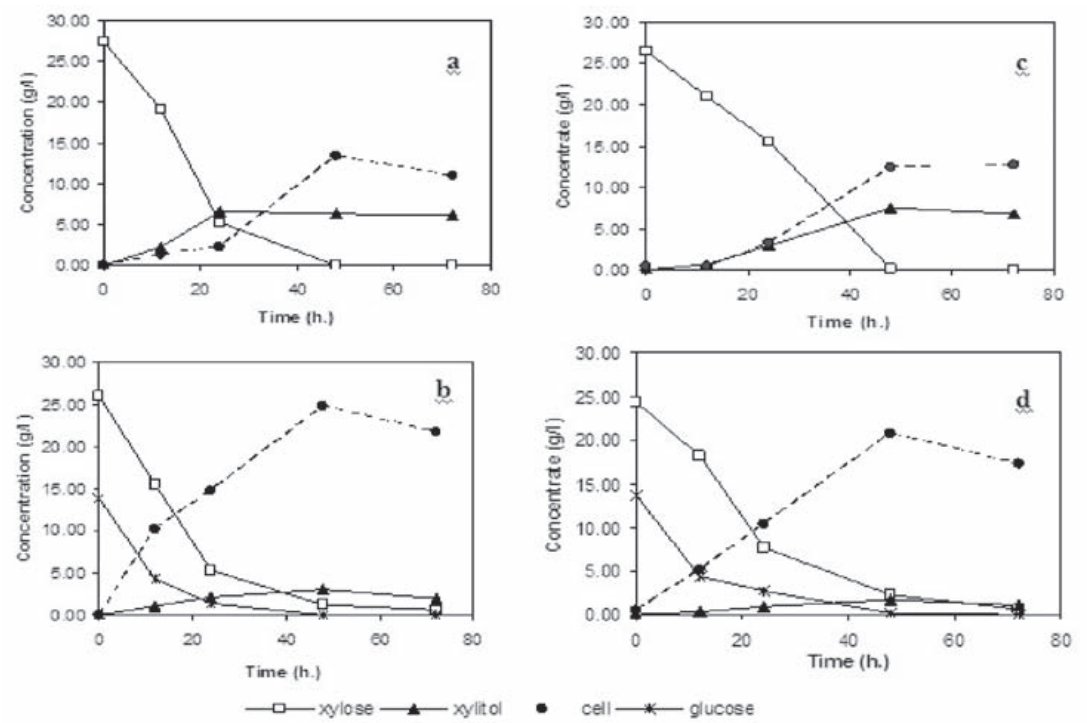

Figure 1 Batch fermentation of xylitol fermentation.

a) Xylitol production using pure xylose by *Candida guilliermondii* FTI 20037
b) Xylitol production using hydrolysate by *Candida guilliermondii* FTI 20037
c) Xylitol production using pure xylose by *Candida magnolia* TISTR 5664
d) Xylitol production using hydrolysate by *Candida magnolia* TISTR 5664

the fermentation from hydrolysate with 24.35 g/l xylose and 13.69 g/l glucose gave 1.66 g/l xylitol or 0.08 g Yp/s of xylitol/g of xylose. The fermentation with *C. guilliermondii* FTI 20037 from pure 27.48 g/l xylose gave 6.60 g/l or Yp/s 0.30 g of xylitol/g of xylose, while the fermentation from hydrolysate with 26.10 g/l xylose and 13.87 g/l glucose gave 2.98 g/l xylitol or 0.12 g Yp/s of xylitol/g of xylose. This indicated that xylitol fermentation from pure xylose was more efficient than that obtained from oil palm trunk hydrolysate. This was due to the glucose content in hydrolysate which inhibited xylitol production by *C. magnolia* TISTR 5664 or *C. guilliermondii* FTI 20037. Furthermore, hexose, particularly glucose, could affect xylose metabolism by a partial repression or inhibition of transport systems or catabolic enzymes (Webb and Lee, 1990). However, the cell growth in the hydrolysate was higher than in pure xylose as it might be observed that the hydrolysate contained glucose which is the best carbon source for microorganism growth. This could also support the results of Tochampa *et al*. (2002) that without glucose in the media, *C. mogii* consumed xylose as well as forming xylitol. When grown in a mixture of xylose and glucose, the yeast consumed glucose faster and more easily than consuming xylose. Furthermore, glucose to xylose ratios as well as the biomass yield were high.

CONCLUSIONS

After the hemicellulose hydrolysate from steam explosion of oil palm trunk chips was acid posthydrolysed under optimum condition, the higher amount of xylose and glucose were obtained. Toxic compounds obtained after posthydrolysis were harmful to microorganisms, however, they could be reduced in two steps of adsorption using activated carbon. In xylitol fermentation, the comparison was made between xylose and oil palm trunk hydrolysate. It was shown that pure xylose gave higher yield of xylitol than that obtained from hydrolysate. According to the presence of glucose in hydrolysate, xylitol production by yeast was inhibited. Following these results, there is still need to further optimise the condition for xylitol fermentation to obtain more yield.

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