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Xylitol Obtained by Fermentation of Hydrolysate from Steam Explosion of *Vetiveria zizanioides* Nash

Sawitri Chuntranuluck[1,*], Pilanee Vaithanomsat[2] and Suwichaya Rodkamnerd[1]

ABSTRACT

Xylitol is one of the most expensive polyol sweeteners with specific health claims. Xylitol can be obtained from the hemicelullosic fraction of lignocellulosic materials containing xylose, such as vetiver grass (*Vetiveria zizanioides* Nash). Steam explosion is an efficient process for the fractionation of the main components of lignocellulosic residues. Vetiver grass was steam exploded at 17 $kg.cm^{-2}$ (208 °C) for 5 min and hydrolyzed with 1% weight per volume (w/v) sulfuric acid at 120 °C for 45 min. Hydrolysate with 6.528 $g.L^{-1}$ xylose was obtained. The inhibitory by-products in the hydrolysates affecting the bioconversion of the xylose into xylitol were acetic acid, 5-hydroxymethylfurfural, furfural and phenolic compounds. These inhibitory by-products were reduced by the addition of 10% (w/v) activated carbon under agitation (250 rpm) for 4 hr. After the detoxification treatment, acetic acid, 5-hydroxymethylfurfural, furfural and phenolic compounds were reduced by 47.5, 92.5, 98.7 and 14.3%, respectively. Then, the detoxified hydrolysate was used as the fermentation medium for xylitol production by *C. guilliermondii* FTI 20037; the xylitol yield per amount of xylose and productivity was 0.359 $g.g^{-1}$ and 0.110 $g.L^{-1}.hr^{-1}$, respectively. When comparisons were made with pure xylose as a substrate, the xylitol yield and productivity was 0.662 $g.g^{-1}$ and 0.379 $g.L^{-1}.hr^{-1}$, respectively.

Keywords: vetiver grass, steam explosion, hemicellulose, activated carbon

INTRODUCTION

Thailand is facing problems of soil degradation by water erosion and from chemicals used in agriculture (Land Development Department, 1992). Therefore to conserve soil and water, government agencies are campaigning for the planting of vetiver grass, with stimulation by cutting leaves and clumps resulting in vetiver grass sprouting and growing, which in turn leads to greater capability in conserving soil and water (Land Development Department, 1992). The biomass obtained is considered as waste that can be processed into value-added products such as xylitol from the xylose in the waste (Datar *et al.,* 2007). Pessoa *et al*. (1997) has described how xylose can be derived from the chemical decomposition technique of steam explosion at high temperature and pressure. The steam dissolves the hemicellulose from the fiber without the use of chemicals that destroy the environment. After the degradation of vetiver grass by steam explosion, hemicellulose hydrolysate is obtained, which contains xylose, xylose oligomers

1 Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand.

2 Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI), Kasetsart University, Bangkok 10900, Thailand.

* Corresponding author, e-mail: fagisvtc@ku.ac.th

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and derivatives that are inhibitory compounds. The decomposition of lignocellulosic materials emits inhibitory compounds such as acetic acid, 5-hydroxymethylfurfural, furfural and phenolic compounds that affect the growth of microorganisms in the xylitol production process. Therefore, the hydrolysate has to be treated prior to microbial utilization. The inhibitory compounds can be reduced by several ways such as adsorption by activated carbon, by ion exchange using ion exchange resin or by using enzymes (Clark and Mackie, 1984).

At present, xylitol production by chemical methods is expensive, especially the purification process. Therefore, biotechnological production of xylitol from fermented hemicellulosic hydrolysate using microorganisms such as fungi, bacteria and yeast has become more attractive since the downstream process is expected to be cheaper (Pessoa et al.,1997). A large amount of xylitol is obtained using *Candida* yeasts like *C. mogii*, *C. tropicalis* and *C. guilliermondii*. (Granström *et al.,* 2001).

The purpose of the current research was to study the xylitol production from hemicellulosic hydrolysate obtained by the steam explosion of vetiver grass to create added value from vetiver grass. The xylitol production from fermented hydrolysate was also compared with the production from pure xylose.

MATERIALS AND METHODS

Preparation of material and hydrolysate

The vetiver grass (*Vetiveria zizanioides* Nash) used was a strain originally from Sri Lanka and was obtained from the Land Development Department, Muang district, Phichit province, Thailand. Samples were dried and chopped into chips with a length of 2.5 cm and then analyzed for chemical composition. A sample of 200 g of dry vetiver grass was steam-exploded in a 2.5 L batch cooker (Kitto Koatsu; Japan) at 17 $kg.cm^{-2}$ (208 °C) for 5 min. Water was added to the wet, pretreated material to make up the volume to 1.2 L and the mixture was then boiled at 80 °C for 30 min after which the hemicellulose hydrolysate was recovered by filtration. Acid hydrolysis of hemicellulose hydrolysate was carried out to cleave the xylo-oligosaccharides into monomeric sugars by autoclaving at 120 °C with 1% weight per volume (w/v) H_2SO_4 for 45 min. Sugars (glucose and xylose) and their derivatives were analyzed.

Removal of inhibitory compounds

The hydrolysate from acid hydrolysis was detoxified by mixing with 10% w/v powdered activated carbon size 8—20 mesh and agitated at 250 rpm at room temperature for 4 hr. The sugars (glucose and xylose) and their derivatives were analyzed. The xylose concentration of the hydrolysate was increased to the desired level by vacuum evaporation at a temperature of 55 °C.

Xylitol fermentation (batch culture)

Preparation of microorganisms and inoculum

Candida guilliermondii FTI 20037 (ATCC; Manassas VA, USA) used in the study was grown on yeast potato dextrose (YPD) agar slant at room temperature for 24 hr. The 1-2 loop inoculum was seeded into 250 mL Erlenmeyer flasks containing 50 mL yeast extract peptone xylose (YPX) broth. The flasks were incubated on a rotatory shaker (250 rpm) at 30 °C for 24 hr. Afterward, the cells were centrifuged at 5,000 rpm for 10 min (Pumipat *et al.,* 2008).

Media and fermentation condition

Comparison studies were made between xylitol fermentation from pure xylose and fermented hydrolysate media with an initial xylose concentration of 25 $g.L^{-1}$. The *C. guilliermondii* FTI 20037 was grown in 500 mL Erlenmeyer flasks containing a basal medium with 1.7 $g.L^{-1}$ yeast nitrogen base (without any amino acid and ammonium sulfate), 5.0 $g.L^{-1}$ urea and 1.0 $g.L^{-1}$

yeast extract. The inoculum was seeded into 500 mL Erlenmeyer flasks containing 300 mL fermentation medium at an initial density optical density at 600 nm of 1.0 with a dry cell weight of 1.85 $g.L^{-1}$. The fermentation runs were carried out at 30 °C and 170 rpm (Pumipat *et al.,* 2008).

Analytical method

The phenolic compounds were detected by the Folin-Ciocalteu reagent method using a spectrophotometer (Thermo Spectronic Helios, Thermo SCIENTIFIC; West Palm Beach, FL, USA) at 765 nm with gallic acid as the standard (Gao *et al.,* 2000). The contents of the sugars (glucose, xylose, arabinose and xylitol), acetic acid, furfural and 5-hydroxymethyfurfural (HMF) in the hydrolysate were analyzed by high performance liquid chromatography (LC10A, Shimadzu Co. Ltd.; Kyoto, Japan) with an Aminex HPX-87C column (Bio-Rad; Hercules, CA, USA) with a size of 300 × 7.8 mm. The flow rate of the mobile phase (5 mM H_2SO_4) was set at 0.6 $mL.min^{-1}$ at 60 °C with connection to a refractive index detector for sugar and acetic acid analysis and connection to an UV-vis detector at 277 nm for furfural and HMF analysis.

RESULTS AND DISCUSSION

Chemical composition of vetiver grass and hydrolysate

The chemical analysis of the dry vetiver grass indicated that the amounts of extractive, lignin, holocellulose, cellulose, pentosan and ash were 9.12, 9.97, 59.85, 30.71, 29.13 and 2.89%, respectively. After hydrolysis by steam explosion and sulfuric acid, the chemical composition of the hydrolysate obtained was glucose, xylose, acetic acid, furfural, 5-HMF and phenolic compounds with amounts of 0.749, 6.528, 3.397, 1.117, 0.120 and 0.077 $g.L^{-1}$, respectively.

Removal of inhibitory compounds

After adsorption, analysis of the inhibitory compounds in the hydrolysate showed that 10% (w/v) activated carbon at 250 rpm for 4 hr at room temperature could reduce the amounts of acetic acid, furfural, 5-HMF and phenolic compounds to 1.783, 0.015, 0.009 and 0.066 $g.L^{-1}$, which were equivalent to percentage removals of 47.5, 98.7, 92.5 and 14.3%, respectively. These figures were acceptable levels for application in industrial systems in a reasonable time according to the research of Villarreal *et al.* (2006) who studied the removal of toxins in hydrolysate from *Eucalyptus grandis*. When comparisons were made between using activated carbon and ion exchange resins for the removal of toxic substances, activated carbon gave percentage removals of 10–33, 100, 100 and 80–90% for acetic acid, furfural, 5-HMF and phenolic compounds, respectively, while the use of ion exchange resins gave percentage removals of 100, 95, 95 and 85–93% for acetic acid, furfural, 5-HMF and phenolic compounds, respectively. Carvalheiro *et al.* (2004) studied toxic removal in Brewery's spent grain hydrolysate using shaking with 10% (w/v) activated carbon for 1 hr at room temperature and reported a reduction of 95% in furfural and 5-HMF.

Xylitol fermentation (batch culture)

The results of xylitol fermentation from pure xylose with an initial concentration of 25 $g.L^{-1}$ are presented in Figures 1a and 2c. It was found that the maximum xylitol yield of 18.23 $g.L^{-1}$ was obtained at 48 hr while the xylose remaining in the culture medium was 0.13 $g.L^{-1}$. The dry cell weight, rate of xylitol production (Q_p) and pH were 9.72 $g.L^{-1}$, 0.379 $g.L^{-1}.hr^{-1}$, and 5.70, respectively. During the period 0–48 hr, the average cell yield per amount of xylose ($Y_{x/s}$), the average xylitol yield per amount of xylose ($Y_{p/s}$) and the specific growth rate were 0.262, 0.662 $g.g^{-1}$ and 0.020 μ, respectively.

From the study of xylitol fermentation by *C. guilliermondii* FTI 20037 with a pure xylose medium, Pumipat *et al.* (2008) found that with a starting concentration of 27.48 $g.L^{-1}$, xylose

at 30 °C and 170 rpm, the maximum xylitol production of 6.60 $g.L^{-1}$ was obtained at 48 hr, and the maximum xylitol yield per amount of xylose was 0.30 $g.g^{-1}$, while Barbosa *et al.* (1988) found that with a starting concentration of 40 $g.L^{-1}$ xylose at 30 °C and 200 rpm, the maximum xylitol production and the xylitol yield per amount of xylose were 23 $g.L^{-1}$ and 0.30 $g.g^{-1}$, respectively.

The results of the xylitol fermentation from detoxified hydrolysate with an initial concentration of 25 $g.L^{-1}$ xylose are presented in Figures 1b, 1c, 1d and 2c. It was found that the maximum xylitol yield of 7.94 $g.L^{-1}$ was obtained at 72 hr while the xylose remaining in the culture medium was 0.99 $g.L^{-1}$. The dry cell weight, Q_p and pH were 9.69 $g.L^{-1}$, 0.110 $g.L^{-1}.hr^{-1}$, and 6.45, respectively. The $Y_{x/s}$ value during the period 4–96 hr was 0.229 $g.g^{-1}$. The $Y_{p/s}$ value during the period 8–72 hr was 0.359 $g.g^{-1}$ and the specific growth rate was 0.014 μ, consistent with other research, such as Canilha *et al.* (2008), who studied xylitol production from wheat straw hydrolysate that was detoxified by 10% (w/v) activated carbon shaking at 200 rpm for 1 hr. A sample of 50 mL hydrolysate with a starting xylose concentration of 30 $g.L^{-1}$ in a 250 mL Erlenmeyer flask was fermented by *C. guilliermondii* FTI 20037 at 30 °C and 200 rpm. It was found that a maximum xylitol yield of 24 $g.L^{-1}$ was obtained, with the xylitol production rate being 0.34 $g.L^{-1}.hr^{-1}$ and the xylitol yield per amount of xylose was 0.48 $g.g^{-1}$.

Table 1 shows that pure xylose was a more appropriate nutrient than detoxified hydrolysate according to the presence of other substances such as glucose, arabinose, acetic acid, furfural, 5-HMF, and phenolic compounds. Felipe *et al.* (1995) found that 1 $g.L^{-1}$ acetic acid caused lower xylitol production while 3 $g.L^{-1}$ of acetic acid inhibited xylitol production by *C. guilliermondii* FTI 20037. Villa *et al.* (1998) found that 0.1–4.0 $g.L^{-1}$ of phenolic compound inhibited xylitol production. Alves *et al.* (1998) found that 1 $g.L^{-1}$ of 5-HMF inhibited the growth rate and fermentation of *Saccharomyces cerevisiae*. Amounts of 0.5, 1.0 and 2.0 $g.L^{-1}$ of 5-HMF inhibited the growth rate of *Pichia stipitis* to 43, 70 and 100%, respectively (Delgenes *et al.,* 1996). Wanhlbom and Hägerdal (2002) found that *S. cerevisiae* could reduce furfural and 5-HMF into furfuryl alcohol and 5-hydroxymethylfurfuryl alcohol, respectively, which resulted in the conversion of xylitol to xylose.

Pure xylose was used at a faster rate than xylose in detoxified hydrolysate by C. *guilliermondii* FTI 20037 (Figures 1a and 1b). Glucose, furfural, and 5-HMF were used up within the first 20 hr (Figures 1c and 1d). During this period, glucose in the hydrolysate was used instead of xylose (Figure 1c), Furfuryl alcohol and 5-hydroxymethylfurfuryl alcohol converted xylitol to xylose (Wanhlbom and Hägerdal, 2002). As a result, the cell yields from both substrates were equal (Figure 2a) but the cell yield in hydrolysate was higher than the xylitol yield (Figure 2b). The presence of acetic acid, total phenolic compounds, furfural and 5-HMF in the hydrolysate-inhibited cells affected the growth rate and considerably decreased the yield of xylitol (Figures 2b and 2c). However furfural and 5-HMF were used up within the first 20 hr (Figure 1d). The amount

Table 1 Xylitol production, xylitol yield per amount of xylose ($Y_{p/s}$), cell yield per amount of xylose ($Y_{x/s}$) and the rate of xylitol production (Q_p) from various materials.

Media	Xylitol production ($g.L^{-1}$)	$Y_{p/s}$ ($g.g^{-1}$)	$Y_{x/s}$ ($g.g^{-1}$)	Q_p ($g.L^{-1}.hr^{-1}$)
Pure xylose	18.23±0.52	0.662±0.02	0.262±0.004	0.379±0.010
Hydrolysate (detoxification)	7.94±0.52	0.359±0.07	0.229±0.010	0.110±0.001

Results are the average of two independent experiments ± SD, each conducted with three replicates.

of total phenolic compounds was quite constant throughout the fermentation.

Glucose and arabinose (Figure 1c) inhibited the enzymes involved in xylose metabolism as reported by Nigam and Singh (1995), with the rates of glucose utilization being faster than the rates of xylose utilization. In the fermention of xylose for xylitol production in the presence of glucose, arabinose, ethanol, methanol and organic acids, Saha and Bothast (1999) found that *Candida peltata* NRRL Y-6888 preferentially utilized glucose > xylose > arabinose from mixed substrates, while arabinose had no inhibitory effect on xylitol production. Thus, glucose was used as a basic growth nutrient prior to the use of xylose in xylitol production. Glucose and arabinose cause longer durations for the log phase (Ooi *et al.*, 2002); therefore, the use of xylose in hydrolysate as a substrate ended within 72 hr, while the use of pure xylose ended within 48 hr. Derivatives in the hydrolysate considerably blocked the functioning of the enzyme and the selective membrane of the cell. As a result, the growth and xylitol production decreased compared with pure xylose. In the xylitol production, a pH change occurred during the fermentation process, increased during the log phase and then remained constant during the stationary phase (Figure 1b).

CONCLUSION

Steam explosion and acid hydrolysis of vetiver grass samples produced hydrolysate with a yield of 6.528 $g.L^{-1}$ of xylose. However,

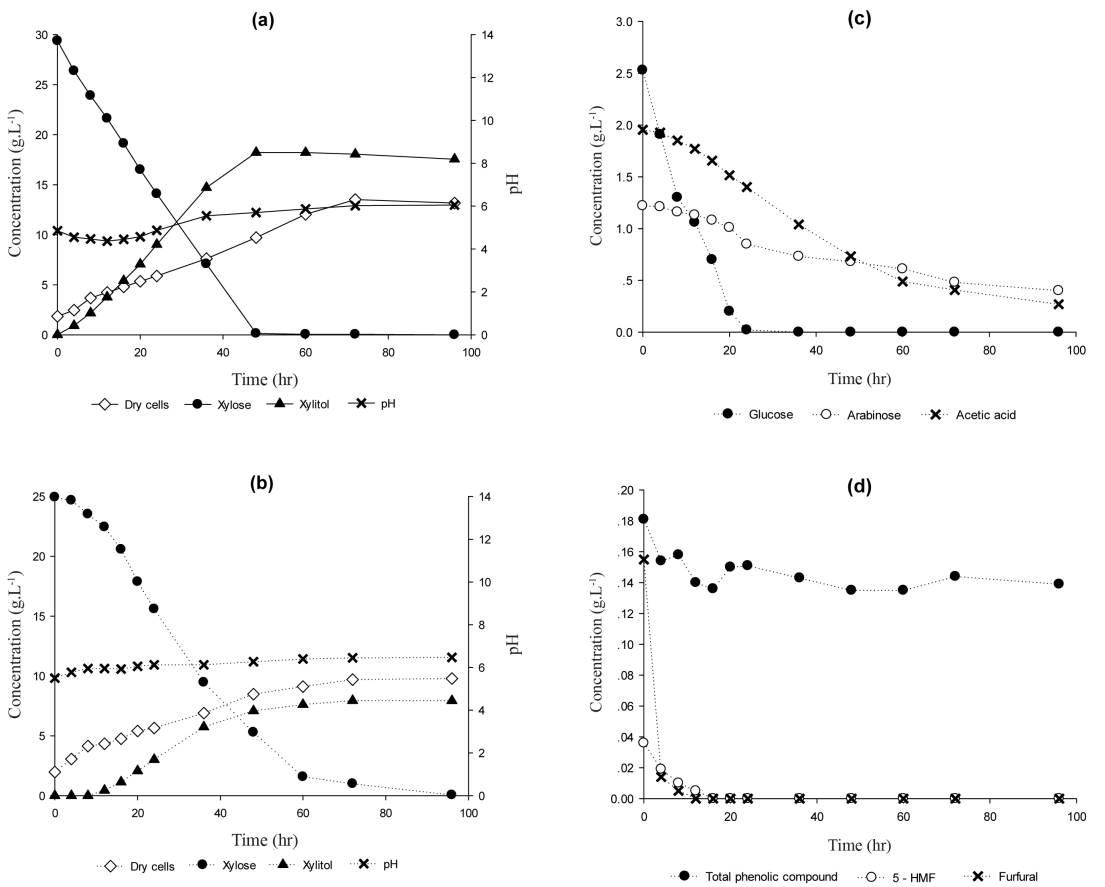

Figure 1 Results of xylitol fermentation from pure xylose (a) and from hydrolysate (b) with the presence of inhibitory compounds (c and d).

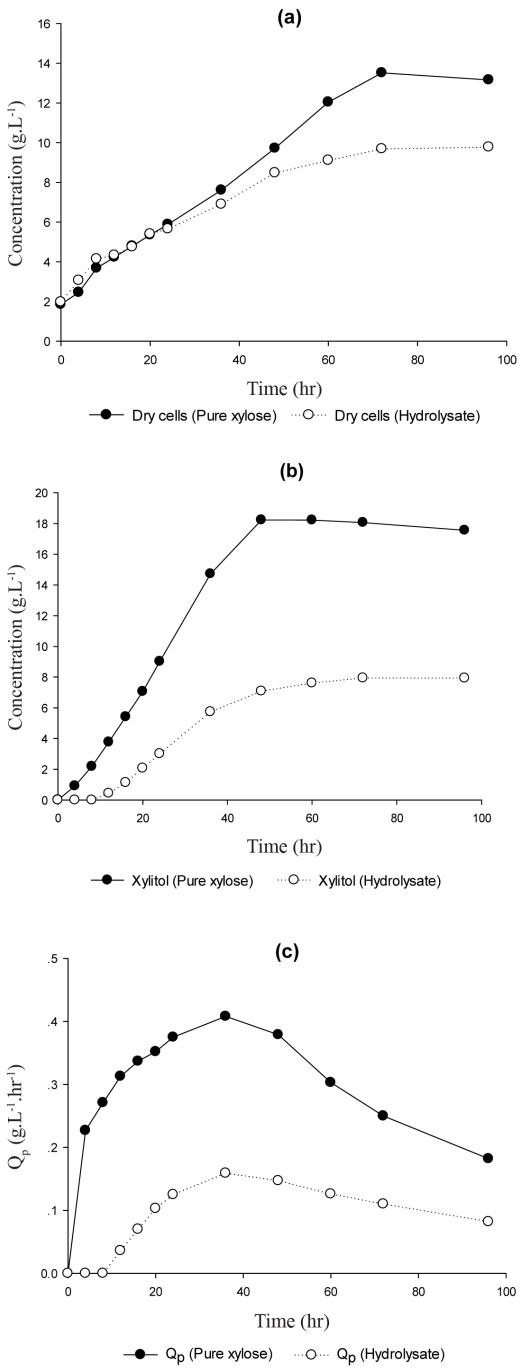

Figure 2 Fermentation with pure xylose and detoxified hydrolysate: (a) Comparison of dry cell yield, (b) Xylitol production (c) Rate of xylitol production (Q_p).

the hydrolysate contained inhibitory compounds that reduced the growth and xylitol production of yeast. Prior to fermentation, it was necessary to reduce the amount of inhibitory compounds such as acetic acid, furfural, 5-HMF and phenolic compounds. After adsorption with activated carbon, analysis of the inhibitory compounds in the hydrolysate showed that 10% (w/v) activated carbon at 250 rpm for 4 hr at room temperature could reduce the amounts of acetic acid, furfural, 5-HMF and phenolic compounds with percentage removal amounts of 47.5, 98.7, 92.5 and 14.3%, respectively. After fermentation of the detoxified hydrolysate and pure xylose, it was found that the use of detoxified hydrolysate as a medium gave values for $Y_{p/s}$ and Q_p of 0.359 $g.g^{-1}$ and 0.110 $g.L^{-1}.hr^{-1}$, respectively, which were less than those from using pure xylose as the medium. Contamination by some inhibitory compounds left in the hydrolysate resulted in inhibition of the growth and xylitol production of *C. guilliermondii* FTI 20037. Therefore, future study is required to develop a xylitol production process that can totally remove the inhibitory compounds through other methods such as ion exchange resin.

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