Overliming Effects on Xylitol Production from Sago Trunk Hydrolysate

Mohamad, N.L.[1], Mustapa Kamal, S.M.[1*] and Abdullah, A.G.L.[2]
[1]Department of Process and Food Engineering,
[2]Department of Chemical and Environment Engineering,
Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM,
Serdang, Selangor, Malaysia
[*]E-mail: siti@eng.upm.edu.my

ABSTRACT

Xylitol can be obtained from lignocellulosic materials containing xylose. However, the fraction of lignocellulose converted through dilute acid hydrolysis contains compounds that inhibit the fermenting micro-organisms. These inhibitors can be removed from the hydrolysate by detoxification method, prior to fermentation. This study describes effectiveness of overliming process to reduce the toxicity of hydrolysates generated from pre-treatment of sago trunk for xylitol production. The overliming pH 9 and 10 was studied and the results showed that pH 9 was showed 20% of sugar loss, which is low compared to pH 10. *Candida tropicalis* strain was used to evaluate the fermentability of overlimed sago trunk hydrolysate at pH 9 and non-overlimed hydrolysate medium. Meanwhile, Xylitol accumulation and productivity in the overlimed medium was found to be higher than the non-treated medium. The maximum production of xylitol was increased up to 74% and converted within 76 h. The results obtained improved the fermentation process when compared with the non-treated medium.

Keywords: Overliming, sago trunk, dilute acid hydrolysis, xylose, xylitol

INTRODUCTION

Sago trunk is an agricultural waste produced from the production of sago starch. The sago palm waste is the cheapest biodegradable and most readily available of all renewable natural sources existing in Malaysia (Pushpamalar *et al*., 2006). The bark of the trunk is normally used as walls, ceiling and fences; however, it was not fully utilize for higher value products as fuels and chemicals (Rahman, 2009).

The presence of hemicellulose of sago trunk waste (20-30%) can be used as a potential renewable carbon source which contains xylose as the intermediate for the production of xylitol, a five carbon sugar alcohol with high sweetening power and valuable anti-cariogenic properties (Parajo *et al*., 1998).

Xylitol production through dilute acid hydrolysis was hindered by toxic compounds normally presents in hemicellulose hydrolysates (Mussatto *et al*., 2005). These compounds will inhibit the microbial growth and lead to the decreasing of product formation in the fermentation medium. In his paper, Mussatto discussed on types of inhibitors derived from dilute acid hydrolysates. Four groups of toxic compounds derived; these were (1) sugar degradation products, (2) lignin degradation products, (3) compounds derived from lignocellulose structure, and (4) heavy metal ions (Mussatto & Roberto, 2004). Furfural and hydroxymetylfurfural were toxic compounds derived from sugar degradation products. Mussatto *et al*. (2005) observed that furfural concentrations lower than 0.5 g

L^{-1} had a positive effect on cell growth but if the concentrations were increased to 2 g L^{-1}, it would inhibit the cell growth almost completely.

Meanwhile, a variety of procedures have been employed for removing inhibitory compounds from hydrolysates for their inhibitory action in the fermentation (Cruz *et al*., 1999). Physico-chemical procedures, neutralization and overliming (Roberto *et al*., 1991), adsorption in activated charcoal (Dominguez *et al*. 1997), extraction with organic solvents (Parajo *et al*. 1996) have been used for this purpose.

According to Roberto *et al*. (1991), overliming methods was found to be low cost treatment and gives good results. This author obtained a partial removal of phenolic compound by adjusting pH to 10 with $Ca(OH)_2$ and then to 6.5 with H_2SO_4. The detoxifying effect of overliming is due to both the precipitation of toxic components and to the instability of some inhibitors at high pH (Palmqist & Hagerdal, 2000). Palmqvist *et al*. (2000) observed the pre-adjustment to pH 10 with NaOH and CaOH, which were reported to decrease the concentration of Hibbert's ketones in a dilute acid hydrolysate of spruce from 203 to 158 (22%) decrease and to the concentration of both furfural and HMF by 20%.

The main purpose of this work was to use sago trunk hemicellulose hydrolysate as a feedstock for microbial production of xylitol. The overliming method was used to monitor the effects of growth, xylitol accumulations and to confirm the suitability of overliming hydrolysates for making culture media that could easily be fermentable with *Candida tropicalis*.

MATERIALS AND METHODS

Raw Materials

Sago trunks were obtained from local plantations, dried to 7% moisture content on oven dry basis, and ground to the particle size less than 1 mm, homogenized, air-dried, stored, and used for the experiments.

Dilute-acid Hydrolysates

Sago trunk ground chips were hydrolyzed by 6% H_2SO_4 at 121°C for 45 minutes. The slurry was filtered through vacuum filtration to separate the solid containing cellulose and lignin with the liquid portion which primarily contained hemicellulose. The hydrolyzate contains 27.63 g L^{-1} xylose, 5.399 g L^{-1} glucose, 0.5 g L^{-1} furfural, 2.75 g L^{-1} acetic acid, and 3.2 g L^{-1} total phenolic compound. The pH of the initial hydrolyzate was 0.956.

Overliming Treatment

The overliming treatment was chosen to be a detoxification method by increasing the pH of hydrolysates to 9 and 10 by addition of $Ca(OH)_2$. The hydrolysate was placed on the stirrer plate and heated to 50°C. $Ca(OH)_2$ was added gradually and mixed by using stirring bar until reach the target pH. The hydrolysate was then maintained at 50°C for 30 minutes (Mohagheghi *et al*., 2006). The mixture of hydrolysate and $Ca(OH)_2$ was vacuum filtered and the recovered solids ($CaSO_4$) were removed. The overlimed filtrate pH was adjusted to 6.5 for further analysis.

Microorganism

The yeast *Candida tropicalis* was obtained from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, stored at 4°C on sabourod dextrose agar plates and sub-cultured twice a month.

Inoculum Preparation

The pre-culture medium consisted of (gL^{-1}) D-xylose, 10; yeast extract, 60; KH_2PO_4, 15; $(NH_4)_2HPO_4$, 3, and $MgSO_4 \cdot 7H_2 0$ (Yahashi *et al*. 1996). The pH was adjusted to 5 with 1 M HCl. The preculture was incubated in 250 ml Erlenmeyer flask containing 100 ml of medium, agitated at 250 rpm on a rotary platform shaker for 24 h at 30°C (Rao *et al*. 2006). The cells recovered by centrifugation at 4000 rpm for 15 minutes, then washed twice with sterile water and used for inoculum.

Fermentation

Fermentations were performed to compare the performance of overliming treated medium at suggested pH with the non-treated medium. Fermentations were carried out in 250 ml cotton-plug Erlenmeyer flask containing 155 ml of production medium provided by both medium which include 135 ml hydrolyzates, 5 ml of inoculums, and 15 ml solution of nutrients similar to inoculums (Millati *et al*., 2002). All fermentations were done in triplicates and placed in a platform shaker at 200 rpm and at a temperature of 30°C for 76 h without controlling pH.

Analysis

Xylose, glucose, and xylitol were analyzed by High Performance Liquid Chromatography (HPLC) Class VP Shimadzu, Japan, using Refractive Index Detector and Inertsil NH_2 5µm column. The mobile phases used were 75% acetonitrile and 25% deionized water with flowrate of 0.5 ml/min. Furfural compound was detected by HPLC using an ultraviolet (UV-VIS) detector and C18 column under the same conditions for sugar detection.

A phenolics compound was detected by spectral analysis using UV-vis spectrophotometer with absorbance at 280 nm (Wrolstad 2009). Cell concentration was estimated by measuring absorbance at 600 nm. The relationship between absorbance and dry weight (gL^{-1}) was given by a standard curve.

RESULTS AND DISCUSSION

Table 1 shows the composition of hydrolysates of non-treated medium and overliming detoxification method. The reduction of 85% and 65% of furfural and phenol composition in the overliming detoxified method had improved the recovered hydrolysate composition, specifically for further fermentation process. Furfural and phenol compounds, which are normally derived from sugar degradation and compounds from lignocellulosic structure, are effectively removed from the hydrolysates; however, acetic acid compound could remove only 29.3% of the hydrolysate (Mussatto & Roberto 2004). According to Felipe *et al*. (1997), the concentration of acetic acid up to 1.0 g/l improved xylose-to-xylitol bioconversion, but concentrations higher than 3 g/l were harmful to the fermentation process.

The overliming detoxification method had extensively affected the total reducing sugar. After the pH adjusted to 10, the yields of xylose and glucose were reduced by 30% and 19.5%, respectively *(Fig. 1)*. The loss of fermentable sugars is the major drawback of these methods (Villareal *et al*., 2006).

TABLE 1
Composition of hydrolysate

Composition	Non-treated medium ($g\,L^{-1}$)	Detoxified medium ($g\,L^{-1}$)
Xylose	23.145	27.066
Glucose	5.399	9.576
Furfural	2.641	0.372
Acetic acid	1.674	1.185
Phenol	0.482	0.164

TABLE 2
Volumetric productivities and yields of fermentation experiments

Medium	Time (h)	P_m ($g\,l^{-1}$)	$Y_{p/s}$ ($g\,g^{-1}$)	Q_p ($g\,l^{-1}h^{-1}$)	q_p ($g\,g^{-1}\,h^{-1}$)	$Y_{x/s}$ ($g\,g^{-1}$)	U (h^{-1})
Non-treated	76	7.523	0.325	0.098	0.041	0.25	0.039
Overliming	76	19.739	0.729	0.25	0.03	0.69	0.047

Note:

P_m, maximum production of xylitol; $Y_{p/s}$, xylitol yield on consumed xylose; Q_p, volumetric productivities; q_p, specific productivity; $Y_{x/s}$, yield of biomass on consume xylose; U, specific growth rate

In order to check the effectiveness of the detoxification*, C. tropicalis* was grown in the media made from raw non-treated and detoxified sago trunk hydrolysate. The non-treated medium was referred to medium that was subjected to only neutralize to the pH7. *Figs. 2* and *3* showed the time courses of cell growth, xylose consumption and xylitol production in the media made from the detoxified hydrolysate and non-treated hydrolysate.

The amount of glucose concentration was consumed by the micro-organism within 24 h before the depletion of xylose, and it was assumed that glucose had been converted to ethanol. The same result was also observed by Villarreal *et al*. (2006) using *C. guilliermondii* on *Eucalyptus hydrolysates*. As shown in *Fig. 2*, the production of xylitol was slowed by the inhibitors contained in the non-treated medium compared to the overliming medium. Table 3 indicates the volumetric productivity of xylitol for the non-treated medium was only 0.098 $gL^{-1}h^{-1}$ and the maximum production was 7.523 g L^{-1}. However, the fermentation process on the overliming medium improved the maximum production of xylitol to 2 folds compared to the non-treated medium with increased up to 19.739 g L^{-1}. The result of the detoxified hydrolysates of overliming methods indicates the capability of improving the yeast performance.

Fig. 3 shows the trend in xylitol accumulation and xylose consumption of the non-treated and overliming treated medium. A faster xylose consumption was reached with hydrolysates subjected to overliming process. Meanwhile, xylitol accumulation was observed to increase from the beginning of fermentation. In the non-treated medium, however, no accumulation of xylitol was observed within the 24 hours of fermentation time.

The glucose was completely consumed by yeast after 24 h and was followed by xylose fermentation. Xylose was completely consumed by 72 h with the xylitol concentration and yield of 19.832 g L^{-1} and 0.73 g xylitol/g xylose. In the non-treated medium, however, the xylose consumption was faster than the overliming medium, which is 52 h with the xylitol accumulation
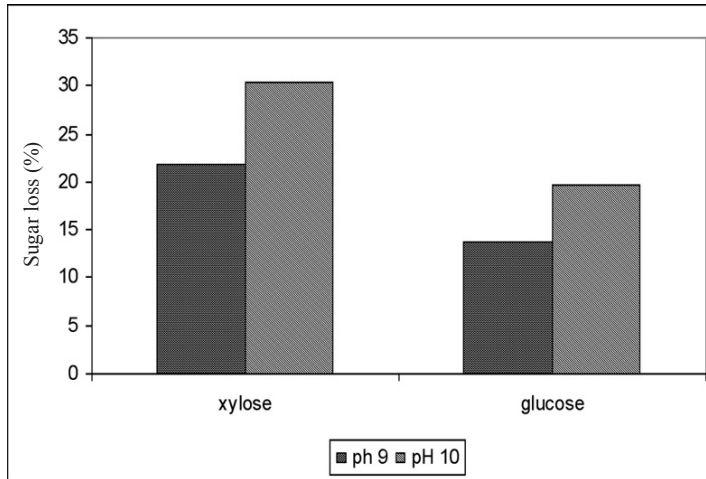
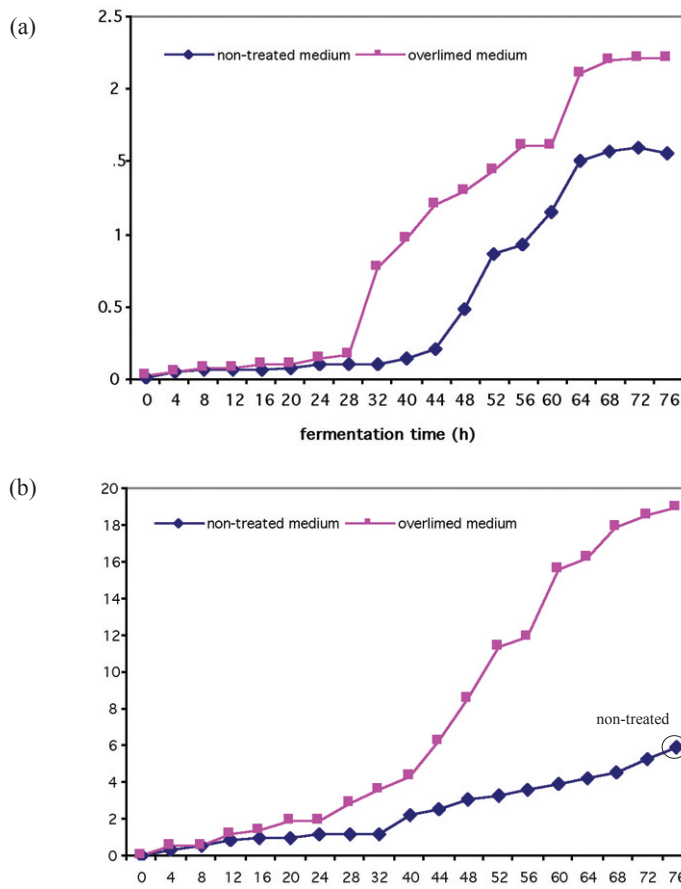*Fig. 1: Amount of sugar losses after the overliming treatment*

Fig. 2: Cell growth of C. tropicalis *on the non-treated medium (□) and overliming medium (■);
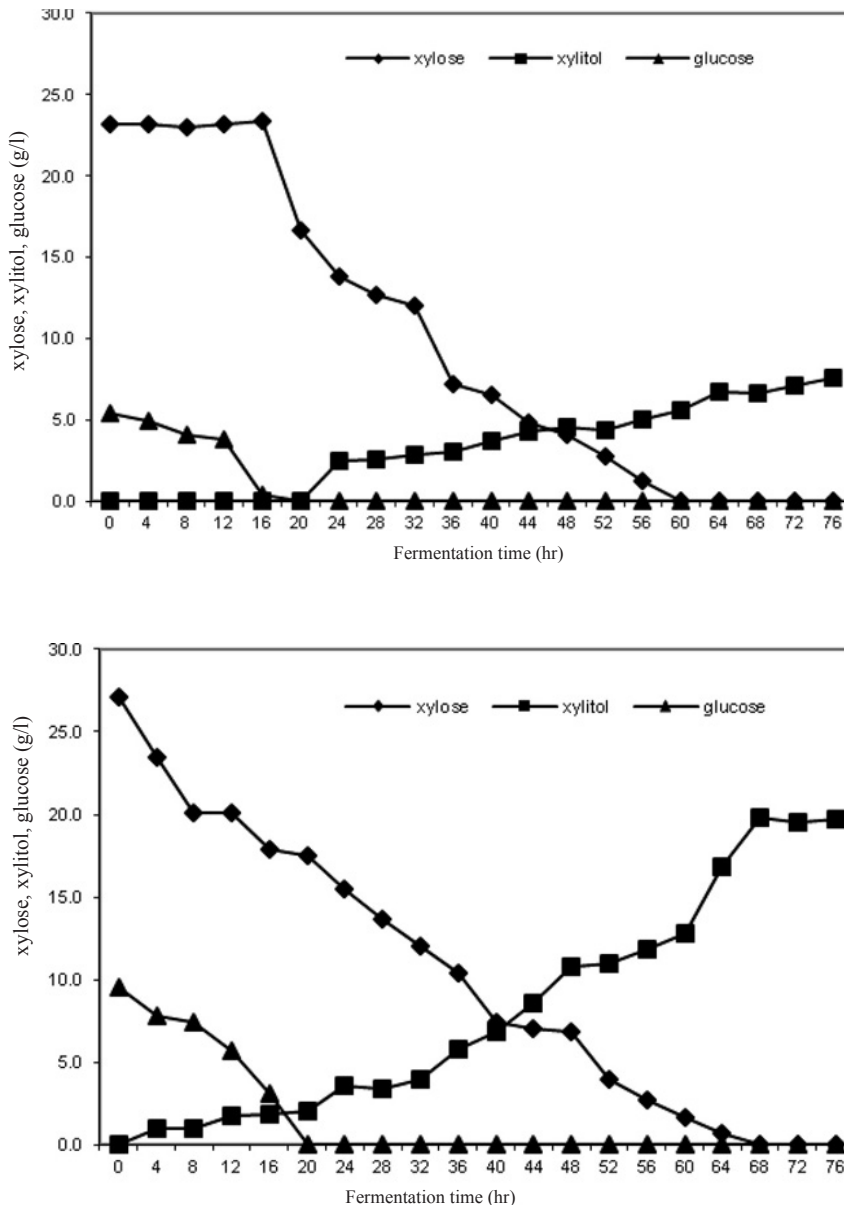(a) optical density at 600 nm, and (b) dry cell weight*

Fig. 3: Time courses for xylitol accumulation in (a) non-treated medium, and (b) overliming medium

of 5.039 g L^{-1} and yield of only 0.23 g xylitol/g xylose (Table 2). This results demonstrated the ability of yeast to convert xylose to high production of xylitol after the overliming process.

On the basis of the above results, overliming has been proven to be the selected method for the detoxification of hydrolysates with excellent yield; $Y_{p/s}$ was obtained (0.729 gg^{-1}) at good productivity (0.25 gL^{-1}h^{-1}) with the maximum xylitol production of 19.739 gL^{-1} (Table 2).

CONCLUSION

This study represented the main outcome of detoxification method and lime to improve the fermentability of hydrolysates by using *Candida tropicalis*. The results have been proven using the overliming method, while the volumetric productivity and yields were found to have increased to two folds compared to the non-treated medium. Thus, an optimal strategy should be carried out and taken into consideration since chemical detoxification degrades the sugar content of the hydrolysates, is cost extensive and produces waste as well. A combination of hydrolysate detoxification may be a general solution for dilute-acid hydrolysate continuous cultivation to achieve high xylitol productivity.

ACKNOWLEDGEMENT

REFERENCES

Cruz, J.M., Domínguez, J.M., Domínguez, H., & Parajó, J.C. (1999). Solvent extraction of hemicellulosic wood hydrolysates: A procedure useful for obtaining both detoxified fermentation media and polyphenols with antioxidant activity. *Food Chemistry*, *67*, 147-153.

Dominguez, J.M., Cao, N., Gong, C.S., & Tsao, G.T. (1997) Dilute acid hemicellulose hydrolysates from corn cobs for xylitol production by yeast. *Bioresource Technology*, *61*, 85-90.

Felipe, M.G.A., Vitolo, M., Mancilha, I.M., & Silva, S.S. (1997). Fermentation of sugar cane bagasse hemicellulosichydrolysate for xylitol production: Effect of pH. *Biomass and Bioenergy*, *13*, 11-14.

Millati, R., Niklasson, C., & Taherzadeh, M.J. (2002). Effect of pH, time and temperature of overliming on detoxification of dilute-acid hydrolyzates for fermentation by *Saccharomyces cerevisiae*. *Process Biochemistry*, *38*, 515-522.

Mohagheghi, A., Ruth, M., & Schell, D.J. (2006). Conditioning hemicellulose hydrolysates for fermentation: Effects of overliming pH on sugar and ethanol yields. *Process Biochemistry*, *41*, 1806-1811.

Mussatto, S.I., Dragone, G., & Roberto, I.C. (2005). Influence of the toxic compounds present in brewer's spent grain hemicellulosichydrolysate on xylose-to-xylitol bioconversion by *Candida guilliermondii*. *Process Biochemistry*, *40*, 3801-3806.

Mussatto, S.I., & Roberto, I.C. (2004). Alternatives for detoxification of diluted-acid lignocellulosichydrolyzates for use in fermentative processes: A review. *Bioresource Technology*, *93*, 1-10.

Palmqvist, E., & Hagerdal, B.H. (2000). Fermentatiom of lignocellulosichydrolysates. I: Inhibition and detoxificaton. *Bioresource Technology*, *74*, 17-24.

Parajó, J.C., Domínguez, H., & Domínguez, J. (1998). Biotechnological production of xylitol. Part 1: Interest of xylitol and fundamentals of its biosynthesis. *Bioresource Technology*, *65*, 191-201.

Parajó, J.C., Domínguez, H., & Domínguez, J.M. (1996). Xylitol from wood: Study of some operational strategies. *Food Chemistry*, *57*, 531-535.

Pushpamalar, V., Langford, S.J., Ahmad, M., & Lim, Y.Y. (2006). Optimization of reaction conditions for preparing carboxymethyl cellulose from sago waste. *Carbohydrate Polymers*, *64*, 312-318.

Rahman, K.A.A.A. (2009). Utilizing sago (*Metroxymmon* spp.) Bark waste for value added products. IEEE Xplore.

Rao, R.S., Jyothi, C.P., Prakasham, R.S., Sarma, P.N., & Rao, L.V. (2006). Xylitol production from corn fiber and sugarcane bagasse hydrolysates by *Candida tropicalis*. *Bioresource Technology*, *97*, 1974-1978.

Roberto, I.C., Felipe, M.G.A., Lacis, L.S., Silva, S.S., & de Mancilha, I.M. (1991). Utilization of sugar cane bagasse hemicellulosichydrolyzate by *Candida guilliermondii* for xylitol production. *Bioresource Technology*, *36*, 271-275.

Villarreal, M.L.M., Prata, A.M.R., Felipe, M.G.A., & Almeida E Silva, J.B. (2006). Detoxification procedures of eucalyptus hemicellulose hydrolysate for xylitol production by *Candida guilliermondii. Enzyme and Microbial Technology*, *40*, 17-24.

Wrolstad, R.Z. (Ed.). (2009). *Current protocols in food analytical chemistry.* (n.p.): John Wiley and Sons.

Yahashi, Y., Horitsu, H., Kawai, K., Suzuki, T., & Takamizawa, K. (1996). Production of xylitol from -xylose by *Candida tropicalis*: The effect of glucose feeding. *Journal of Fermentation and Bioengineering*, *81*, 148-152.