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## Effects of Temperature and pH on Xylitol Recovery from Oil Palm Empty Fruit Bunch Hydrolysate by *Candida tropicalis*

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**Abstract:** Oil Palm Empty Fruit Bunch (OPEFB) is composed of pentose that can be used as a raw material for the production of xylitol, a potential application in the food and medical areas. The effects of temperature and pH on xylitol bioconversion by yeast sp. *Candida tropicalis* were investigated. The optimum pH resulted to be in the range of 2-4. The percentage of xylose consumed for xylitol production progressively increased with pH, whereas those associated to both biomass growth and catabolic reaction through the TCA cycle decreased, reaching nearly constant values at pH 4. The optimum temperature range for xylitol production was 30-35°C. Xylitol formation became the most significant activity at 20°C, further increased up to 30-35°C and then decreased over 40°C. The results collected at variable temperature were finally used for estimation of the parameters of the fermentation system.

**Key words:** Xylose, xylitol, oil palm empty fruit bunch, *Candida tropicalis*

### INTRODUCTION

Xylitol is a five carbon sugar alcohol which is found naturally in small amounts of fruits and vegetables. It has similar sweetness as sucrose, is non-cariogenic, tolerated by diabetics and because of its negative heat of dissolution, used as a part of the coating of pharmaceutical products (Lim and Zaharah, 2000). Nowadays, xylitol is synthesized by hydrogenation of xylose but the solution produced by this process requires expensive purification and separation steps to obtain pure xylitol. It can alternatively be produced by biotechnological methods based on fermentation of agro-industrial residues, which could potentially compete with the traditional chemical way.

Oil Palm Empty Fruit Bunch (OPEFB) contains lignocellulosic materials which provide abundant and renewable energy sources. It is estimated that OPEFB biomass is comprised of 24% xylan, a sugar polymer made of pentose sugar xylose (Rahman *et al.*, 2006). Xylose can be used as substrate for production of xylitol. Conversion of D-xylose from biomass, especially OPEFB offers effective treatment of waste from oil palm industry. Oil Palm Empty Fruit Bunch (OPEFB) is one of the major waste products of the palm industry and used as organic mulch in young and mature oil palm plantations (Lim and Zaharah, 2000). Currently, there are more than 3 million hectares of oil palm plantations which generating an approximately 90 million tones of renewable biomass (i.e., trunks, fronds, shells, palm press fiber and EFB) on

each and every single year (Cheng *et al.*, 2007). In the present study xylose was produced by the acid hydrolysis.

Yeast is the most promising xylitol producer *Panhysoles tannophilus*, *Debarymyces hansenii* and *Candida* showed good performances as xylitol producers. The optimum temperature for *Candida* sp. usually in the range 28-30°C (Elina *et al.*, 2008) and 28-37°C for *D. hansenii* NRRL Y-7426 (Dominguez *et al.*, 1997). Yeast also grows well in acidic condition with pH between 3.5 and 4.0, but the tolerance is in the wide range 2.5-8.0. The optimum pH for xylitol production appears to be 5.5 for *D. hansenii* (Dominguez *et al.*, 1997), in the range of 4-6 for *Candida* sp. and 2.5 for *Candida tropicalis* DSM 7524 (Silva and Afscar, 1994).

The objective of this study is to obtain high yields and productivities by taking these two parameters as well as the potential of *Candida tropicalis* on hemicellulose bioconversion by this yeast.

### MATERIALS AND METHODS

**Raw material:** The OPEFB was collected from local plantation (Felda Air Tawar, Kota Tinggi, Johor), sun dried and ground to a particle size <1mm. The composition of OPEFB is shown in Table 1 by Rahman *et al.* (2006).

**Preparation of OPEFB hydrolysate:** Homogenized OPEFB was subjected to acid hydrolysis according to the method of Rahman *et al.* (2006). Operating temperature of

**Table 1: Main composition of OPEFB**

Main fraction	Composition (%)
Glucan	42.85
Xylan	24.01
Lignin (acid insoluble)	11.70
Ash	0.52
Others	20.92

Rehman *et al.* (2006)

hydrolysis was carried out at 119°C and samples collected after 60 min with 2% concentration of sulfuric acid. The solid and liquid were separated from supernatant by filtration.

**Detoxification:** The OPEFB hydrolysate was heated to 100°C and maintained for 15 min to reduce the volatile compound. The hydrolysate was then overlimed according to Cruz *et al.* (1999). The filtrate was used for xylitol production. The detoxified hydrolysate was concentrate under vacuum at 70°C to achieve 10-11% (w/v) of xylose concentration.

#### Microorganism and fermentation

**Pre-culture medium:** *Candida tropicalis* was grown on pre-culture medium containing (L<sup>-1</sup>): 2 g KH<sub>2</sub>PO<sub>4</sub>, 5 g (NH<sub>4</sub>)<sub>2</sub>PO<sub>4</sub>, 4 g yeast extract, 0.5 g MgSO<sub>4</sub>•7H<sub>2</sub>O, 20 g xylose. Solution of D-xylose, yeast extracts and the rest of salts were sterilized separately by autoclaving at 121°C for 20 min. Pre-culturing was conducted in a 250 mL Erlenmeyer flask containing 100 mL of medium, agitated at 200 rpm on a rotary platform shaker (CERTOMAT MOII) for 20 h at 35°C. The cells were centrifuged at 2000x g for 10 min and resuspended in 5 mL of sterile water to serve as inocula.

**Fermentation medium:** The detoxified OPEFB hydrolysate was complemented with (L<sup>-1</sup>): 2 g KH<sub>2</sub>PO<sub>4</sub>, 5 g (NH<sub>4</sub>)<sub>2</sub>PO<sub>4</sub>, 4 g yeast extract, 0.5 g MgSO<sub>4</sub>•7H<sub>2</sub>O, 20 g xylose and 1 g Peptone, was used as fermentation medium.

**Preparation of shake flask experiment:** The effects of pH on OPEFB hydrolysate to xylitol bioconversion was investigate. A set of 5 batch test was carried out by inoculating the cells into 125 Erlenmeyer flask containing 50 mL of OPEFB hydrolysate medium but having initial pH varying between 2 and 6. The pH was adjusted using 1 M NaOH.

The effect of temperature on the process, additional of 5 batch runs was performed varying this parameter between 20-34°C. All tests were performed in duplicate at 200 rpm and using a starting inoculums level of 1.36±0.06 g L<sup>-1</sup> cell dry weight.

**Analytical methods:** Aliquots of the culture were centrifuged at 6000x g for 10 min and supernatants were used for determination of D-xylose and xylitol. The

concentrations of xylose and xylitol were determined by HPLC, model Shimadzu, equipped with a refractive index detector, model and a 100×30 mm column, model SHODEX- NH<sub>2</sub> at 80°C with 75% acetonitrile and H<sub>2</sub>O as mobile phase at 1.0 mL min<sup>-1</sup>. Biomass concentration either in the broth or inoculums suspensions was determined by optical density measurements at 600 nm. A calibration curve was used to relate OD with cell dry weight.

## RESULTS AND DISCUSSION

**Effects of pH on xylitol bioconversion:** The experimental results and the kinetic parameters of a set of 5 batch xylitol bioconversion was performed using cells cultivated at initial pH varying from 2.0-6.0. The investigation of pH effects on OPEFB hydrolysate bioconversion processes its important to keep constant this parameter at the starting values. However, the pH variation consequent to carbon dioxide released by the fermentation was less than one unit. Therefore, it was preferred to perform test without any continuous control of pH (Sampaio *et al.*, 2006). The results demonstrate that optimum pH of the bioconversion was in the range 3-4 with the maximum production of xylitol 26.12 g L<sup>-1</sup> (Fig. 1).

From literature (Silva and Afscar, 1994) obtained the highest xylitol yield under pH 2.5. However, the result of study doesn't show the same conditions. When pH was increase from 2 to 4, a drastic increase in xylitol productivity from 6.28 to 26.12 g L<sup>-1</sup> after 54 h of fermentation, whereas, an evident drop of xylitol production after this threshold. Some hypotheses to explain this situation which involves; 1) transport of xylose into the cell, 2) reduction of xylose to xylitol under consumption of NADPH, 3) regeneration of NADH by the respiratory chain and 4) transport of xylitol out of the cell (Converti and Dominguez, 2001). An increase in external pH (from 4-6) could affect the activity of this transporter, making xylose transport from the bulk to inside the cell which phenomenon limiting the formation of xylitol. Since, low pH influences the maintenance requirement of the cell, the productivity decrease observed with decreasing pH from 4 to 2 could be the result of pH incidence on the redox balance of this bioreduction (June 11, 2009) (Converti and Dominguez, 2001).

Another possibility which been assumed by Converti and Dominguez (2001), the ineffective of counterbalancing of external pH variation which could lead to internal yeast homeostasis. The extracellular pH shift could lead to slight but significant changes of intracellular pH.

**Effects of temperature on OPEFB hydrolysate bioconversion:** Table 2 shows the influence of temperature on experimental results and kinetic parameters

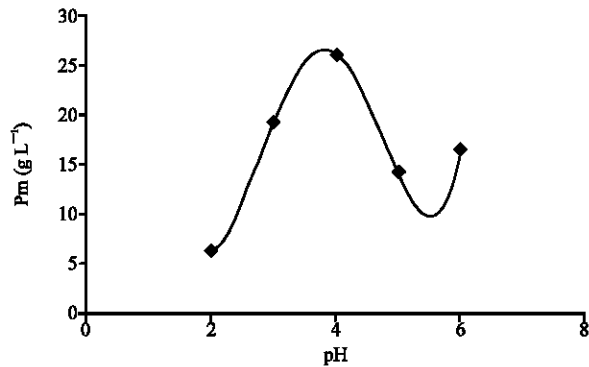


Fig. 1: Maximum production values for batch xylitol productions from OPEFB hydrolysates by *Candida tropicalis*, carried out at different pH

Table 2: Results of temperature effects for xylitol bioconversion by *Candida tropicalis*

T (°C)	Q <sub>p</sub> <sup>a</sup> (g L <sup>-1</sup> h <sup>-1</sup> )	q <sub>p</sub> <sup>b</sup> (g g <sup>-1</sup> h <sup>-1</sup> )	Y <sub>PF</sub> <sup>c</sup> (g g <sup>-1</sup> )
20	0.137	0.033	0.34
25	0.118	0.018	0.24
30	0.318	0.020	0.51
35	0.411	0.018	0.84
40	0.280	0.014	0.78

<sup>a</sup>Volumetric productivity; <sup>b</sup>specific productivity; <sup>c</sup>xylitol yield on consume xylose

referred to *Candida tropicalis* cultivation on xylose. This result suggests the optimum temperature range for OPEFB hydrolysate to xylitol bioconversion was 30 to 35°C. The above temperature range was shown to be optimal for the yield of xylitol on consumed xylose 0.84 g g<sup>-1</sup> while the highest volumetric productivity (Q<sub>p</sub>) was 0.411 g/L/h were observed at 35°C. While for the lowest and highest temperature, the microorganism was not able to grow at all (results not shown). At 10 and 45°C, xylitol accumulation in the medium was negligible, which means that it was not representative of cell metabolic response under these thermal conditions.

The influence of temperature on the flux of carbon addressed gives evident at low temperature (20°C). The largest percentages of xylose were consumed by the catabolic reaction through the TCA cycle and for biomass production. Only 5% of substrate being addressed to xylitol formation. This activity became more progressively with increasing temperature, turned to metabolic activity and further increased up to 30 to 35°C and then decreased. The percentage of xylose utilized for biomass production progressively decreased with temperature. This statement can describe the reduction of xylitol accumulation at 40°C. According to Sampaio *et al.* (2006) about 2% of starting xylose was converted to ethanol formation.

Previously had demonstrated that xylitol formation from xylose by pentose fermenting yeasts can kinetically

be described assuming a direct dependence of productivity on the activity of enzyme controlling xylitol accumulation. On the basis of this assumption, the thermodynamic behavior of the system can be described according to which the rate increase with temperature, which is consistent with the principal theory of chemical kinetics, would be contrasted by a progressive decrease in enzyme activity due to thermal inactivation. However, the thermodynamic behavior was not included in this study.

## CONCLUSION

In our previous study, *Candida tropicalis* can produce 26.12 and 22.22 g L<sup>-1</sup> xylitol from both pH and temperature effects using OPEFB hydrolysate as the sole carbon source. However, this value is much lower than previous study as the hydrolysate obtained some leftover toxic components in the treated acid hydrolysate that negatively affected the fermentation performance on discovering best pH and temperature effects on *Candida tropicalis*. As general conclusion, the experimental data regarding temperature and pH effects on xylitol production could be very useful for industrial applications. It could be more effective to use Response Surface Methodology (RSM) for optimization of these both parameters, contributing to make the process economically and more profitable.

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