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Economical Production of Xylitol from *Candida magnoliae* TISTR 5663 Using Sugarcane Bagasse Hydrolysate

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ABSTRACT

Xylitol is a popular sweetener in food, pharmaceutical and oral hygiene products due to its many advantages for health such as preventing tooth decay and suitability for diabetes patients. In this research, economical production of xylitol was obtained using the high-xylitol-producing, osmotolerant yeast, *Candida magnoliae* TISTR 5663, compared with two lower cost culture media (0.15 and 0.64 USD.L^{-1}) with the defined medium for *C. magnoliae* TISTR 5663 (approximately 1 USD.L^{-1}). Among these media, the specific growth rates, xylitol yields and productivities obtained were not significantly different ($P > 0.05$, 0.001 and 0.001, respectively). Thus, the cheapest minimal medium with $(NH_4)_2HPO_4$ as the nitrogen source (0.15 USD.L^{-1}) was used for the production of xylitol from sugarcane bagasse hydrolysate as a carbon source. It was found that *C. magnoliae* TISTR 5663 gave a noticeably xylitol yield and productivity of 0.295 $g.g^{-1}$ and 0.024 $g.L^{-1}.hr^{-1}$, respectively. This indicated that the culture medium containing these inexpensive carbon and nitrogen sources was feasible for lowering the production cost of xylitol. In addition, the xylitol production was modeled using either xylose or sugarcane bagasse hydrolysate as the main carbon source. The model fitted well with the observations for cell growth, xylose consumption and xylitol production from xylose as a carbon source, but was less suitable using the sugarcane bagasse hydrolysate data.

Keywords: xylitol, xylose, production media, *Candida magnoliae*, fermentation, model

INTRODUCTION

Xylitol is a five-sugar alcohol derived from xylose (Sirisansaneeyakul *et al*., 1995; Winkelhausen and Kuzmanova, 1998). It is attractive as a popular sweetener in confectionery and chewing gum, pharmaceuticals and oral hygiene products because of its sweetening power property like sucrose (Emodi, 1978; Parajó *et al*., 1998). Moreover, it has many advantages for health such as preventing tooth decay and is suitable for diabetes patients since it is insulin-independent and causes only limited increases in the glucose and insulin blood levels (Emodi, 1978; Parajó *et al*., 1998). Xylitol can be produced by many methods including direct extraction from fruit and vegetables (Washüttl *et al*., 1973), chemical hydrogenation (BeMiller and Stapley, 2009; Rafiqul and Mimi Sakinah, 2012) and fermentation from microorganisms (Sirisansaneeyakul *et al*., 1995; Nakano *et al*., 2000). Chemical hydrogenation is commercial used for the xylitol

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production process, however, fermentation is recommended as the potential process for xylitol production (Sirasansaneeyakul *et al*., 1995, 2013). Xylitol fermentation is performed under mild fermentation conditions and can reduce the production cost (Sirasansaneeyakul *et al*., 2013). Recently, effective xylitol fermentation was studied using the high-xylitol-producing osmotolerant yeast, *Candida magnoliae* TISTR 5663 (Sirisansaneeyakul *et al*., 2013). However, the medium used was quite expensive and resulted in a high cost of xylitol production. Due to the higher prices of the nitrogen source (casamino acid) and trace elements (yeast nitrogen base without amino acid and ammonium sulfate) according to Nakano *et al*. (2000), the medium cost was 1.0 USD.L^{-1}. Therefore, in this study, two medium formulas, provided using xylitol production with *C. mogii* TISTR 5892 (Sirisansaneeyakul *et al*., 1995) and *C. tropicalis* HDY-02 (Ling *et al*., 2011), with reported production costs of 0.15 and 0.64 USD.L^{-1}, respectively, were investigated to produce yeast biomass and xylitol, compared with the defined medium of *C. magnoliae* TISTR 5663. The best production medium that efficiently produced xylitol from *C. magnoliae* TISTR 5663 was used to produce xylitol using sugarcane bagasse hydrolysate as feedstock.

MATERIALS AND METHODS

Microorganism and culture media

The yeast *Candida magnoliae* TISTR 5663 was used throughout this study. The microorganism was grown on yeast malt peptone (YMP) agar slants at 37 °C for 24 hr. The three growth and xylitol production media contained the chemicals shown in Table 1.

Preparation of inoculum

The inoculum was prepared in each production medium. Two loopfuls of cells from YMP agar slants were inoculated into 10 mL of the three media containing 10 g.L^{-1} glucose and 5 g.L^{-1} xylose as mixed carbon sources in three 250 mL Erlenmeyer flasks. The flasks were incubated aerobically on a rotary shaker at 250 rpm at 30 °C for 24 hr. All three culture broths were scaled up by transferring into 500 mL Erlenmeyer flasks containing 90 mL of each medium. These inocula were incubated for 24 hr under the conditions described above. Finally, the flask culture broths were employed as the seeds for the experiments.

Biomass and xylitol production in shake flask culture

The experiments were conducted in 500 mL Erlenmeyer flasks cultivated on a rotary shaker. Twenty five milliliters of each inoculum were inoculated separately into each medium. The experiments were carried out in duplicate. After inoculation, the culture flasks with an initial 250 mL working volume and pH 4.0 were incubated at 30 °C on a rotary shaker at 250 rpm (Wannawilai *et al.,* 2009). When the carbon sources were exhausted, the high cell density cultures were performed with an initial 30 g.L^{-1} xylose and varied nitrogen sources (Table 1). These were: 1) 2.3 g.L^{-1} urea and 1.0 g.L^{-1} casamino acids (medium 1); 2) 6.0 g.L^{-1} $(NH_4)_2HPO_4$ (medium 2); and 3) 4.6 g.L^{-1} yeast extract and 5.0 g.L^{-1} $(NH_4)_2SO_4$ (medium 3). Thereafter, all flasks contained the same initial working volume of 270 mL, and were subjected to the same conditions as described previously.

Subsequently, for the xylitol production phase, all flasks were started with an initial 30 g.L^{-1} xylose and those defined nitrogen sources (Table 1). The experiments were carried out with an initial 300 mL working volume and pH 7.0 on a rotary shaker controlled at 150 rpm and 30 °C (Wannawilai *et al.,* 2009).

Biomass and xylitol production in fermenter culture

The biomass and xylitol production with media 1 and 2 were placed in a 5 L stirred-tank fermenter (Biostat B, B. Braun

Biotech International; Goettingen, Germany). The experiment started with 10% inoculum (300 mL) and the growth medium (2.7 L) with a final total working volume of 3 L. Growth media 1 and 2 contained various nitrogen sources, trace elements (Table 1) and initial concentrations of glucose and xylose at 10 and 5 $g.L^{-1}$, respectively. The fermentation was controlled at 30 °C and a dissolved oxygen concentration of 70–80% of air saturation by adjustment of the aeration rate and agitation speed and a pH of 4.0. Then, xylose was added to the fermenter to achieve an initial concentration of 30 $g.L^{-1}$ after the xylose and glucose in the growth medium had been exhausted.

Table 1 Composition and cost of three production media for xylitol production.

Chemical	Production medium		
	1[a]	2[b]	3[c]
1. Trace elements ($g.L^{-1}$)			
1.1 Biotin	0.000002	0.000018	
1.2 Calcium pantothenate	0.0004	0.0182	
1.3 Folic acid	0.000002		
1.4 Inositol	0.002	0.0365	
1.5 Niacin	0.0004		
1.6 p-Amino benzoic acid (PABA)	0.0002		
1.7 Pyridoxine hydrochloride	0.0004	0.0009	
1.8 Riboflavin (vitamin B2)	0.0002		
1.9 Thiamine hydrochloride	0.0004	0.00366	
1.10 $B(OH)_3$	0.0005		
1.11 $CuSO_4$	0.00004	0.00146	
1.12 KI	0.0001		
1.13 $FeCl_3$	0.0002	0.0091	
1.14 $MnSO_4$	0.0004	0.0064	
1.15 Na_2MoO_4	0.0002		
1.16 $ZnSO_4$	0.0004	0.00546	
1.17 KH_2PO_4	1.0	18.75	1.3
1.18 $MgSO_4.7H_20$	0.5	1.13	0.6
1.19 NaCl	0.1		
1.20 $CaCl_2$	0.1	0.10	
2. Nitrogen sources ($g.L^{-1}$)			
Urea	2.3		
Casamino acids	1.0		
Yeast extract			4.6
$(NH_4)_2SO_4$			5.0
$(NH_4)_2HPO_4$		6.00	
3. Cost (Baht.L^{-1})	32.01	4.79	20.52
Cost (USD.L^{-1}) [d]	1.00	0.15	0.64

[a] = Yeast nitrogen base without amino acids and ammonium sulfate composed of 20 chemicals of trace elements.

[b, c] = Production media of *Candidus mogii* TISTR 5892 (Sirisansaneeyakul *et al*., 1995) and *C. tropicalis* HDY-02 (Ling *et al*., 2011), respectively.

[d] Conversion rate of 32 baht per USD (7 April 2015).

The fermentation was switched to the xylitol production phase by controlling the agitation speed and aeration rate at 300 rpm and 1.0 gas flow volume per unit liquid volume per minute (vvm), respectively. The pH in this production phase was controlled at 7.0. The temperature was controlled at 30 °C during the fermentation.

Biomass and xylitol production from sugarcane bagasse hydrolysate

The sugarcane bagasse hydrolysate was produced using 1.5% H_2SO_4 and a liquid-to-solid ratio at 10:1, which was treated at 105 °C for 60 min. The supernatant was collected from filtration, neutralized with NaOH and concentrated in a rotary vacuum evaporator. Then, the sugarcane bagasse hydrolysate without detoxification was supplemented with the nitrogen source and trace elements (production medium 2). The fermentation was operated in 500 mL Erlenmeyer flasks containing 250 mL production medium as an initial working volume, together with 10% inoculum. The growth and xylitol production phases were operated under aerobic and microaerobic conditions, respectively, as described above. The experiments were conducted in duplicate.

Modelling xylitol production

The growth of yeast cells was related to the specific growth rate (μ) and xylose concentration (*S*) as described by the Monod equation (Equation 1). The consumption of xylose for growth, maintenance and xylitol production at various times is given by Equation 2. The production of xylitol was expressed using the Luedeking-Piret equation (Equation 3):

$$\frac{dC_X}{dt} = \frac{\mu_{max} \cdot C_S}{K_S + C_S} \cdot C_X \quad (1)$$

$$\frac{dC_S}{dt} = -\left[\left(\frac{1}{Y_{X/S}}\right)\left(\frac{dC_X}{dt}\right) + m_S C_X + \left(\frac{1}{Y_{P/S}}\right)\left(\frac{dC_P}{dt}\right)\right] \quad (2)$$

$$\frac{dC_P}{dt} = (\alpha\mu + \beta)C_X \quad (3)$$

where μ_{max} is the maximum specific growth rate, K_S is the Monod constant, $Y_{X/S}$ and $Y_{P/S}$ are the theoretical yields of biomass and xylitol from xylose, respectively, $Y_{X/S}$ is 0.452 $g.g^{-1}$ by estimation (Roels, 1983; Voleskey and Votruba, 1992; Tochampa *et al*. 2005), $Y_{P/S}$ is 0.912 $g.g^{-1}$ as described by Barbosa *et al*. (1988), m_S is the maintenance coefficient, α is the growth-associated coefficient and β is the nongrowth-associated coefficient.

Analytical methods

Samples were periodically removed from the shake flask and fermenter cultures for analyses. The cell mass concentration was determined by measuring both the dry cell weight and the optical density of the culture broth at 660 nm using a spectrophotometer (UV-1201; Shimadzu; Kyoto, Japan). For dry weight measurement, cells were centrifuged at 4,000 revolutions per minute (rpm; 1,700×*g*) for 10 min, washed twice with distilled water and dried at 105 °C for 24 h to obtain the constant dry cell weight. The supernatants from broth samples (xylose as substrate) after filtration were used to measure xylose, xylitol and reducing sugar. Xylose, xylitol and reducing sugar were determined by the methods of Deschatelets and Yu (1986), Adler and Gustafsson (1980) and the dinitrosalicylic acid method (Miller, 1959), respectively. The glucose concentration was determined by calculating the difference between the reducing sugar and xylose. The supernatants of the broth samples (sugarcane bagasse hydrolysate as substrate) were prepared to measure the mixtures of sugars (xylose, glucose, arabinose and fructose), sugar alcohols (xylitol and arabitol) and fermentation inhibitors (furfural, hydroxymethylfurfural, acetic acid and phenolic compounds) using high performance liquid chromatography. The fermentation results and kinetic parameters were calculated as described by Sirisansaneeyakul *et al.* (2013). Statistically significant differences between the three culture media were determined using an *F*-test. Moreover,

the production cost of xylitol was estimated based on raw material cost which was assumed to be 30% of the total production cost. The remaining 70% of the total production cost included maintenance, utilities, depreciation and labor (Franceschin *et al.,* 2011). Accordingly, the xylitol production cost was estimated as shown in Equations 4–6:

$$\text{Cost (USD.g}^{-1}_{\text{xylitol}}) = \frac{\text{Raw materials cost (USD.L}^{-1}) + \text{Other cost (USD.L}^{-1})}{\text{Xylitol concentration (g.L}^{-1})} \quad (4)$$

$$\text{Raw materials cost (USD.L}^{-1}) = \sum_{i=0}^{i=n} [\text{chemical concentration i (g.L}^{-1}) \times \text{cost i (USD.g}^{-1})] \quad (5)$$

$$\text{Other cost } \left(\text{USD.L}^{-1}\right) = \frac{70}{30} \times \text{Raw material cost (USD.L}^{-1}) \quad (6)$$

where cost is measured in USD per gram of xylitol, raw materials and other costs are measured in USD per liter, chemical concentration is measured in grams per liter and chemical cost is measured in USD per gram.

Finally, the model parameters—μ_{max}, K_S, m_S, α and β—were optimized from the best fit of kinetic expressions to the experimental data with using the Berkeley Madonna™ program (http://www.berkeleymadonna.com/). The coefficient of determination (R^2) was used to test the difference between the model-prediction and the observations (Kumar, 2007; Lang *et al.,* 2009) as shown in Equation 7. The non-normalized root mean square was used to determine the actual error at all points (Yang and Al-Duri 2005; Lang *et al.* 2009) as defined in Equation 8.

$$R^2 = \frac{\sum_{i=1}^{N} (C_{cal} - \overline{C}_{exp})^2}{\sum_{i=1}^{N} (C_{cal} - \overline{C}_{exp})^2 + \sum_{i=1}^{N} (C_{cal} - C_{exp})^2} \quad (7)$$

$$\text{Non-normalized RMS} = \sqrt{\frac{\sum_{i=1}^{N} (C_{cal} - C_{exp})^2}{N}} \quad (8)$$

where C_{cal} is the theoretical cell, xylose or xylitol concentrations, C_{exp} and $\overline{C}_{exp}$ are the experimental and the average experimental cell, xylose or xylitol concentrations, respectively, and N is the number of measurements.

RESULTS AND DISCUSSION

Biomass and xylitol production from shake flask culture

The biomass and xylitol production profiles from the three production media are shown in Figure 1. In the first phase (Figure 1, a1), the medium contained mixed carbon substrates of 10 g.L^{-1} glucose and 5 g.L^{-1} xylose. Glucose was used to produce the biomass and xylose was provided to induce the enzyme which converts xylose to xylitol (Sirisansaneeyakul *et al*., 1995; Tochampa *et al.*, 2005). This phase was operated under aerobic conditions which were optimal for biomass production at initial pH 4.0 and 30 °C on a rotary shaker at 250 rpm and an initial working volume of 250 mL. The initial volumetric oxygen transfer coefficient (k_La) 18.95 hr^{-1} could be estimated under these conditions (Schiefelbein *et al*., 2013). Production medium 1 produced a biomass concentration (C_X), specific growth rate (μ) and biomass yield ($Y_{X/S}$) higher than those obtained from the other two production media (Table 2). However, it was found that these observations were not significantly different at $P > 0.05$, 0.05 and 0.01, respectively.

Afterwards, xylose and nitrogen sources were added and cultivated under initial pH 4.0 at 30 °C on the rotary shaker at 250 rpm and an initial working volume of 270 mL to produce a high biomass concentration during a cultivation time of 25–84 hr (Figure 1, a2). The effects of biomass and initial working volume on k_La value were found. The biomass generated viscosity in the fermentation broth, while the high working volume influenced the oxygen transfer which decreased the k_La value (Schiefelbein *et al*., 2013). Thus, this second period had a lower k_La than the first phase providing the microaerobic condition which was the optimal condition for xylitol production. Therefore, all fermentation kinetic parameters estimated for xylitol production were higher than those estimated for the biomass production as shown in Table 2.

In the xylitol production phase (Figure 1, b), the experiments were started by adding xylose and nitrogen sources. Fermentation was switched into microaerobic conditions on the rotary shaker at 150 rpm, with an initial working volume of 300 mL, a temperature of 30 °C and initial pH 7.0. The initial k_La was estimated to be 9.40 hr^{-1} (Schiefelbein *et al*., 2013). Under these conditions, all production media produced higher xylitol concentrations and yields as shown in Table 2. Moreover, the statistical analysis using an *F*-test showed the volumetric production rate of xylitol (Q_P), the xylitol yield ($Y_{P/S}$) and the specific rate of xylitol production (q_P) were not significantly different at $P > 0.001$.

Therefore, production medium 2 was the cheapest medium (estimated at 0.15 $USD.L^{-1}$) which was selected for further confirmation of the xylitol production from xylose in the fermenter and also used as a xylitol production medium

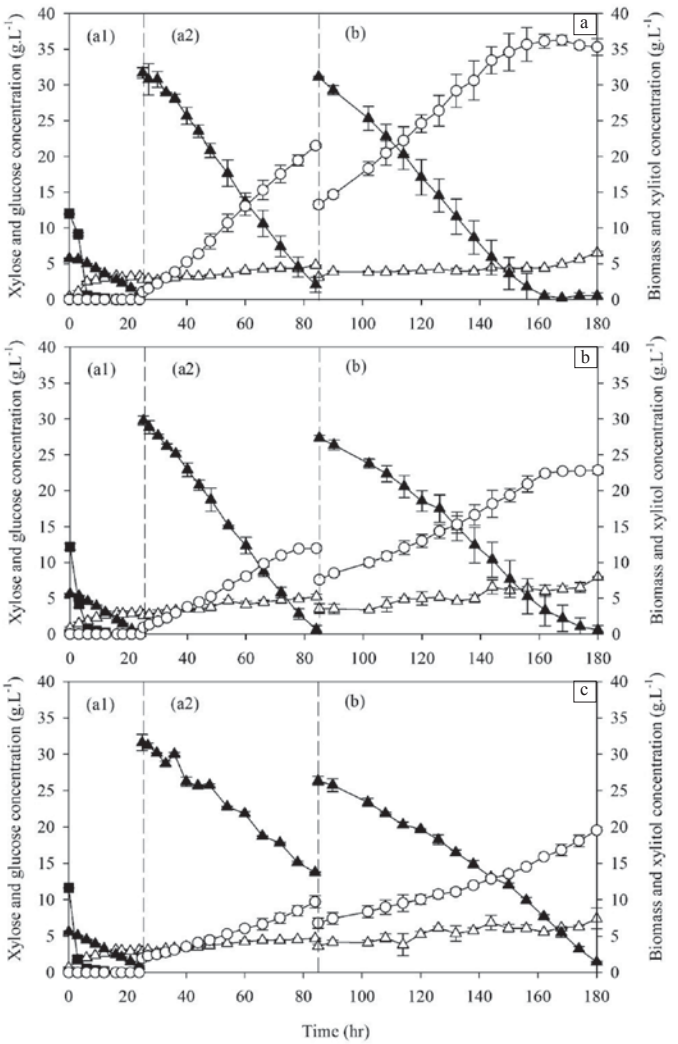

Figure 1 Profiles of biomass (a1, a2) and xylitol (b) production of *C. magnoliae* TISTR 5663 by three production media: (a) Medium 1 (control); (b) Medium 2; (c) Medium 3. ▲ = Xylose; ■ = Glucose; △ = Biomass; ○ = Xylitol. In the subfigures "(a1)" and "(a2)" show biomass production and "(b)" shows xylitol production. Error bars show ± SD.

containing sugarcane bagasse hydrolysate as substrate.

Biomass and xylitol production using xylose as substrate in fermenter culture

The comparison of biomass and xylitol production from medium 1 and 2 in fermenter cultures are shown in Figure 2. The yeast could rapidly grow in production medium 2 (Figure 2a) which was very different from the shake flask cultures. All growth kinetic parameters obtained from production medium 2 were higher than

Table 2 Summary of the biomass and xylitol production with *Candida magnoliae* TISTR 5663 from three production media in shake flask culture.

Fermentation kinetics	Production medium		
	1	2	3
Biomass production (0–24 hr) [a]			
C_X (g.L^{-1})	2.966±0.038^A	2.634±0.177^A	2.796±0.051^A
μ (hr^{-1})	0.130±0.036^A	0.074±0.008^A	0.093±0.011^A
Q_S (g.L^{-1}.hr^{-1})	0.950±0.004	1.003±0.026	0.937±0.006
$Y_{X/S}$ (g.g^{-1})	0.169±0.016^B	0.114±0.004^B	0.144±0.004^B
Biomass production (25–84 hr) [b]			
C_X (g.L^{-1})	3.654±0.040	3.811±0.110	3.559±0.040
μ (hr^{-1})	0.004±0.000	0.007±0.001	0.004±0.000
$Y_{X/S}$ (g.g^{-1})	0.028±0.002	0.043±0.008	0.037±0.004
C_P (g.L^{-1})	16.346±0.196	9.258±0.082	7.370±0.636
Q_P (g.L^{-1}.hr^{-1})	0.259±0.003	0.157±0.001	0.092±0.015
Q_S (g.L^{-1}.hr^{-1})	0.511±0.000	0.520±0.001	0.358±0.018
q_P (g.g^{-1}.hr^{-1})	0.080±0.001	0.049±0.000	0.029±0.005
$Y_{P/xyl}$ (g.g^{-1})	0.507±0.005	0.302±0.001	0.258±0.054
Xylitol production (85–180 hr) [c]			
C_P (g.L^{-1})	29.133±1.932	17.927±0.261	14.643±0.039
Q_P (g.L^{-1}.hr^{-1})	0.223±0.034^C	0.134±0.007^C	0.083±0.008^C
Q_S (g.L^{-1}.hr^{-1})	0.418±0.023	0.321±0.018	0.266±0.005
q_P (g.L^{-1}.hr^{-1})	0.066±0.009^C	0.033±0.003^C	0.018±0.001^C
$Y_{P/xyl}$ (g.g^{-1})	0.533±0.052^C	0.418±0.000^C	0.311±0.035^C

[a, b] = Biomass production phases were incubated in a rotary shaker at 250 rpm, initial pH 4.0, temperature 30 °C and initial working volumes of 250 and 270 mL, respectively.

[c] = Xylitol production phase was incubated in a rotary shaker at 150 rpm, initial pH 7.0, temperature 30 °C and initial working volume 300 mL.

[A, B, C] Means within rows followed by the same letter are not-significantly different at $P > 0.05$, 0.01 and 0.001, respectively. Values shown as means±S.D.

C_X and C_P are concentrations of final biomass and highest xylitol, respectively; Q_S and Q_P are volumetric rates of substrate consumption and xylitol production, respectively; $Y_{X/S}$ and $Y_{P/xyl}$ are yields of biomass from substrate and xylitol from xylose, respectively; q_P is the specific rate of xylitol production; μ is the specific growth rate.

those of production medium 1. Thus, production medium 2 was suitable for growing *C. magnoliae* TISTR 5663.

However, the xylitol production using medium 1 gave better fermentation kinetic parameters than those obtained from production medium 2 (Figure 2b). Although these results were similar to those from the shake flask culture, production medium 2 was decided as suitable for use as the xylitol production medium due to its lower cost. The xylitol production costs estimated from both production media were 0.21 and 0.04 USD.g^{-1} xylitol, calculated using Equations 4–6, with the highest obtainable xylitol yield at 16.143 and 13.562 g.L^{-1}, respectively.

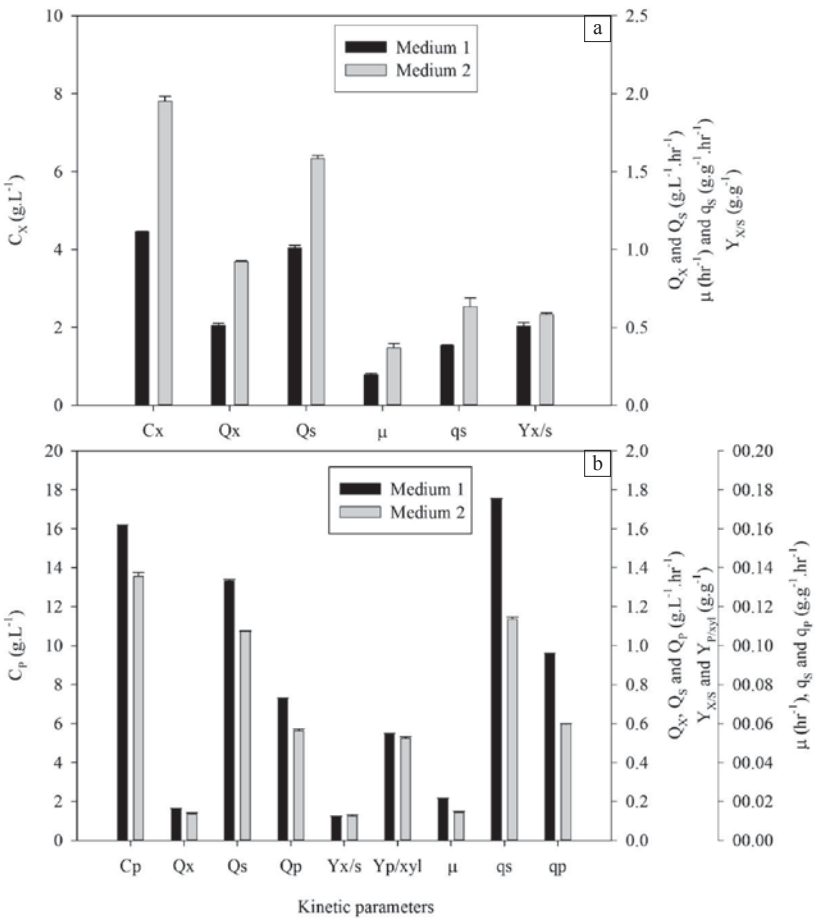

Figure 2 Kinetic parameters of biomass and xylitol production by *C. magnoliae* TISTR 5663 from production medium 1 and 2 in fermenter culture: (a) Biomass production phase (pH controlled at 4.0, dissolved oxygen concentration 70–80% air saturation, 30 °C); (b) Xylitol production phase (pH controlled at 7.0, agitation speed 300 rpm, aeration rate 1.0 vvm, 30 °C). C_X and C_P are concentrations of final biomass and highest xylitol, respectively; Q_X, Q_S and Q_P are volumetric rates of biomass production, substrate consumption and xylitol production, respectively; $Y_{X/S}$ and $Y_{P/xyl}$ are yields of biomass from substrate and xylitol from xylose, respectively; q_S, q_P are the specific rates of substrate consumption and xylitol production, respectively; μ is the specific growth rate. Error bars show ± SD.

Biomass and xylitol production from sugarcane bagasse hydrolysate

The profiles for biomass and xylitol production of *C. magnoliae* TISTR 5663 using production medium 2 with sugarcane bagasse hydrolysate are shown in Figure 3.

The growth phase (Figure 3a) involved cultivation under aerobic conditions (200 rpm, 30 ºC, pH 4.0) using the hydrolysate composed of (mean ± SD) 8.888 ± 0.117 g.L^{-1} xylose, 1.019 ± 0.027 g.L^{-1} glucose, 0.968 ± 0.162 g.L^{-1} fructose, 1.496 ± 0.067 g.L^{-1} arabinose, 3.439 ± 0.162 g.L^{-1} acetic acid, 0.034 ± 0.0004 g.L^{-1} furfural, 0.118 ± 0.0028 g.L^{-1} hydroxymethylfurfural (HMF) and 0.004 ± 0.0006 g.L^{-1} phenolic compounds. The yeast specific growth rate obtained was 0.029 ± 0.000 hr^{-1}, resulting in the highest biomass concentration of 2.599 ± 0.118 g.L^{-1} (Table 3). The fermentation was then switched into the xylitol production phase with sugarcane bagasse hydrolysate and the nitrogen source (6.0 g.L^{-1} $(NH_4)_2HPO_4$) added at 48.25 hr of fermentation time (Figure 3b). The fermentation was conducted microaerobically on the rotary shaker at 150 rpm, pH 7.0 and 30 ºC, with an initial working volume of 300 mL. The hydrolysate contained 24.388 ± 0.603 g.L^{-1} xylose, 3.666 ± 0.435 g.L^{-1} glucose, 2.252 ± 0.125 g.L^{-1} fructose, 8.413 ± 0.502 g.L^{-1} arabinose, 3.501 ± 0.007 g.L^{-1} acetic acid, 0.101 ± 0.0003 g.L^{-1} furfural, 0.009 ± 0.0012 g.L^{-1} HMF and 0.021 ± 0.0002 g.L^{-1} phenolic compounds which indicated that xylose was not only used to produce xylitol, but also to maintain the yeast cells. The high maintenance coefficient (m_S) at 0.077 ± 0.008 g.g^{-1}.hr^{-1} was found at 48.25–88 hr of fermentation. The xylitol concentration of 4.833 ± 0.321 g.L^{-1} was highest at 288 hr of fermentation. The xylitol yield ($Y_{P/xyl}$) and productivity (Q_P) were 0.295 ± 0.010 g.g^{-1} and 0.024 ± 0.002 g.L^{-1}. hr^{-1}, respectively (Table 3). These results showed the preference for xylitol production rather than cell growth, which was affected especially by the inhibitory acetic acid which has been reported as a strong inhibitor for xylose metabolism by yeasts (Ferrari *et al*., 1992; Silva *et al*., 2004). In particular, concentrations higher than 3 g.L^{-1} acetic acid inhibited xylose consumption and xylitol production of *C. guilliermondii* completely (Felipe

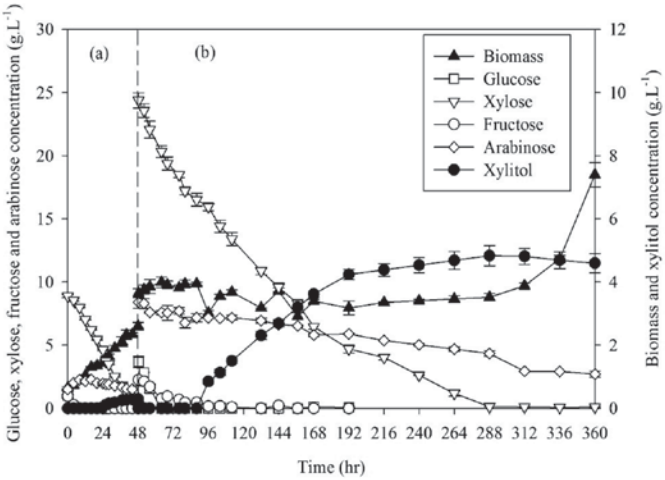

Figure 3 Batch production of xylitol in shake flask culture from production medium 2 using sugarcane bagasse hydrolysate as substrate with "(a)" indicating biomass production phase (initial pH 4.0, rotary shaker speed 250 rpm, 30 °C, initial working volume 250 mL, initial volumetric oxygen transfer coefficient 18.95 hr^{-1} and "(b)" showing the xylitol production phase (initial pH 7.0, rotary shaker speed 150 rpm, 30 °C, initial working volume 300 mL, initial k_La 9.40 hr^{-1}). Error bars show ± SD.

et al., 1995). In the current work, 3.439–3.501 $g.L^{-1}$ acetic acid was found to clearly inhibit yeast cell growth and xylitol production.

From the results, the production cost was estimated to be rather high at 0.10 $USD.g^{-1}$ xylitol from *C. magnoliae* TISTR 5663. However, detoxification of inhibitors is not attractive as it increases the production cost. Alternatively, a process using an inhibitor-tolerant yeast could be considered, with fermentation of lignocellulosic hydrolysate in a fed-batch operation, to obtain higher xylitol yield, productivity and concentration which would minimize the production cost. This might be competitive with the price of commercial xylitol at 4–5 $USD.kg^{-1}$ (Prakasham *et al*., 2009).

Kinetic modelling xylitol production from xylose and sugarcane bagasse hydrolysate

The kinetic parameters were estimated from experimental data describing growth, xylose consumption and xylitol formation from production medium 2 using xylose and sugarcane bagasse hydrolysate as substrates and the results are summarized in Table 4. The model fitted well with the observations of cell growth, xylose consumption and xylitol production from xylose with the determination coefficients (R^2) higher than 0.85 (Table 4). Nevertheless, the cell growth described with the Monod-type equation was not as applicable using sugarcane bagasse hydrolysate (R^2=0.51). This might have been due

Table 3 Fermentation kinetics for the biomass and xylitol production from *Candida magnoliae* TISTR 5663 with the production medium 2 containing sugarcane bagasse hydrolysate.

Fermentation kinetics	Biomass production	Xylitol production	
	0–48 hr	48.25–88 hr	>88–288 hr
Concentration ($g.L^{-1}$)			
C_X	2.599±0.118	3.942±0.047	3.517±0.115
C_P	0.298±0.051	0.000±0.000	4.833±0.321
Specific rates ($g.g^{-1}.hr^{-1}$)			
μ (hr^{-1})	0.029±0.000	0.002±0.001	0.000±0.000
q_S	0.167±0.012	0.097±0.007	0.026±0.001
q_P	0.006±0.001	0.000±0.000	0.006±0.000
q_{xyl}	0.094±0.007	0.052±0.002	0.022±0.000
Volumetric rates ($g.L^{-1}.hr^{-1}$)			
Q_X	0.041±0.002	0.007±0.002	0.000±0.000
Q_S	0.235±0.028	0.366±0.018	0.098±0.004
Q_P	0.011±0.001	0.000±0.000	0.024±0.002
Q_{xyl}	0.181±0.000	0.198±0.002	0.082±0.003
Yields ($g.g^{-1}$)			
$Y_{X/S}$	0.174±0.013	0.020±0.006	0.000±0.000
$Y_{P/xyl}$	0.063±0.004	0.000±0.000	0.295±0.010
Maintenance coefficient ($g.g^{-1}.hr^{-1}$)			
m_S	0.071±0.015	0.077±0.008	0.035±0.002

C_X and C_P are concentrations of final biomass and highest xylitol, respectively; Q_X, Q_S, Q_P and Q_{xyl} are volumetric rates of biomass production, substrate consumption, xylitol production and xylose consumption, respectively; $Y_{X/S}$ and $Y_{P/xyl}$ are yields of biomass from substrate and xylitol from xylose, respectively; q_S, q_P and q_{xyl} are the specific rates of substrate consumption, xylitol production and xylose consumption, respectively; μ is the specific growth rate; m_S is the maintenance coefficient for substrate.
Values shown as mean±SD.

to its complex substrates other than xylose. The yeast specific growth rate (μ) was not only affected by these substrates, but also by the hydrolysate inhibitors. As a result, the maximum specific growth rate obtained was rather low at 0.0015 hr^{-1} and the Monod constant (K_S) was fairly high when sugarcane bagasse hydrolysate was used as substrate. Additionally, inhibitors affect yeast cells which clearly increased the maintenance coefficient (Table 4).

A comparison between the experimental data and the model predictions at the xylitol production phase is shown in Figure 4. For production medium 2 with xylose (Figure 4a), the kinetic model accurately predicted the yeast cell growth. In addition, the xylose consumption was predicted reasonably well, with the exception of prediction in the 4–36 hr range of fermentation which was slightly inferior, whereas xylitol production was predicted rather poorly later than 30 hr fermentation. When the sugarcane bagasse hydrolysate was used as substrate (Figure 4b), the Monod kinetic model was able to predict the growth, however, its determination coefficient was low (R^2 = 0.51). The xylose concentration fitted well with the available data up to 192 hr fermentation. Therefore, the proposed kinetic models in the study could simply describe certain levels of change in growth, xylose consumption and xylitol production.

Table 4 Summary of kinetic parameters for xylitol production from production medium 2 with xylose and sugarcane bagasse hydrolysate.

Kinetic parameters	Substrate	
	Xylose [a]	Sugarcane bagasse hydrolysate [b]
Equation 1: $\frac{dC_X}{dt}=\frac{\mu_{max}\cdot C_S}{K_S+C_S}\cdot C_X$		
μ_{max} (hr^{-1})	0.023	0.0015
K_S ($g.L^{-1}$)	0.214	2.821
R^2	0.9472	0.5194
Non-normalized RMS	0.4199	0.3556
Equation 2: $\frac{dC_S}{dt}=-\left[\left(\frac{1}{Y_{X/S}}\right)\left(\frac{dC_X}{dt}\right)+m_S C_X+\left(\frac{1}{Y_{P/S}}\right)\left(\frac{dC_P}{dt}\right)\right]$		
m_S ($g.g^{-1}.hr^{-1}$)	0.000	0.019
R^2	0.9491	0.9827
Non-normalized RMS	1.9379	1.0983
Equation 3: $\frac{dC_P}{dt}=(\alpha\mu+\beta)C_X$		
α ($g.g^{-1}$)	2.018	2.018
β ($g.g^{-1}.hr^{-1}$)	0.003	0.006
R^2	0.8557	0.9298
Nonn-ormalized RMS	1.5944	0.6358

[a] = Cultivation in fermenter.

[b] = Cultivation in shake flask.

Equation parameters are defined where Equations 1–3 are included in the text.

R^2 = Coefficient of determination; RMS = root mean square.

CONCLUSION

The minimal medium containing (per liter of solution): 18.75 g KH_2PO_4, 6 g $(NH_4)_2HPO_4$, 1.13 g $MgSO_4.7H_2O$, 0.1 g $CaCl_2$, 36.5 mg myo-inositol, 18.2 mg calcium pantothenate, 3.66 mg thiamine-HCl, 0.9 mg pyridoxal-HCl, 0.018 mg biotin, 9.1 mg $FeCl_3$, 6.4 mg $MnSO_4.H_2O$, 5.46 mg $ZnSO_4.7H_2O$ and 1.46 mg $CuSO_4.5H_2O$ was determined as the best, which is useful for the production of biomass and xylitol using *C. magnoliae* TISTR 5663. The sugarcane bagasse hydrolysate also showed its potential as good feedstock for the production of xylitol. The xylitol concentration, yield and productivity obtained are promising for maximization using sugarcane bagasse hydrolysate. A simple, unstructured model provided a reasonable mathematical description of cell growth from sugarcane bagasse hydrolysate.

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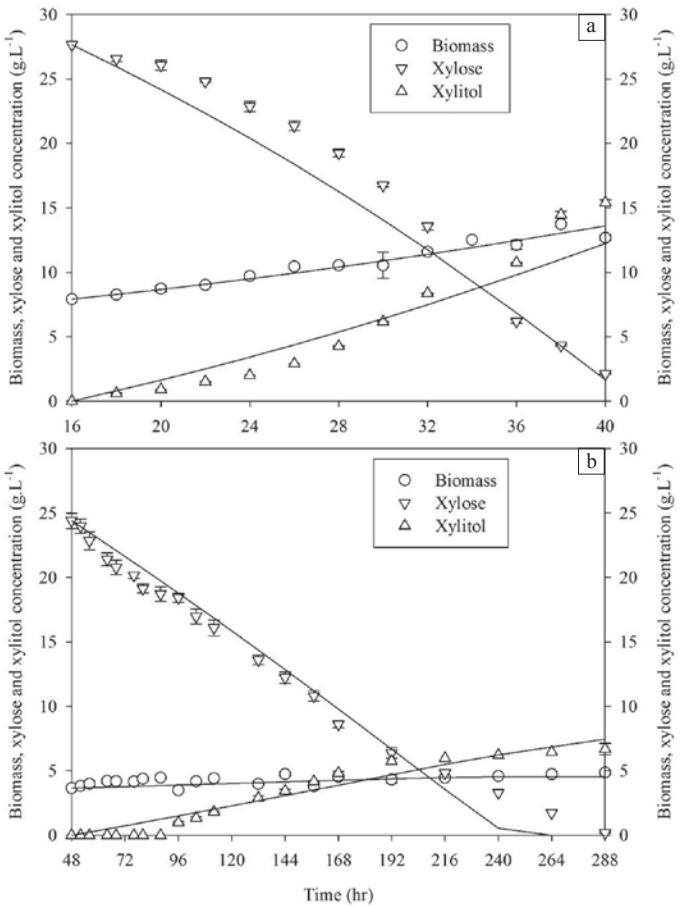

Figure 4 Comparison between observations (points) and model predictions (curves) for the xylitol production from production medium 2: (a) Xylose used as substrate in fermenter culture; (b) Sugarcane bagasse hydrolysate used as substrate in shake flask culture.

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