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Xylitol production from corncob hydrolysate using polyurethane foam with immobilized *Candida tropicalis*

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ABSTRACT

Polyurethane foam (PUF) was used as a carrier for *Candida tropicalis* (*C. tropicalis*) in the multi-batches fermentation of xylitol from xylose-containing corncob hemicellulose hydrolysate. After washing and sterilization, PUF (density of 320 kg m^{-3}, specific surface area of 1.5–2.0 × 10^5 m^2 m^{-3}, average porosity of 95%, pore diameter of 0.03 mm and cubic length of 5 mm) was mixed with the culture medium at appropriate proportion followed by the inoculation. The fermentation parameters such as initial cell concentration, PUF dosage, pH value and temperature were controlled to study the effects on xylitol fermentation. In the 21-day durability tests, the optimal xylitol yield and volumetric productivity reached to 71.2% and 2.10 g L^{-1} h^{-1} respectively. Moreover, the average xylitol yield and volumetric productivity were 66.3% and 1.90 g L^{-1} h^{-1} for ten batchwise operations. The current research demonstrated that the PUF immobilization could serve as an efficient method for improving the cells vitality and enzyme reactivity in the continuous operation of fermentation.

1. Introduction

Corncob is a common agricultural waste produced in large quantities every year. However, it has not been well re-utilized and causes environmental problems (Pepper & Olinger, 1988). On the other hand, it is also a renewable source of fibrous lignocellulosic material that contains about 30% hemicelluloses, a carbohydrate polymer mainly constituted by xylose units with a β-(1→4) linkage (Saha, 2003), which could be hydrolyzed to yield a xylose solution and then used as fermentation media to obtain xylitol, ethanol and other useful products (Mussatto & Roberto, 2004; Wang et al., 2011; Yuan, Zhang, Qian, & Yang, 2004).

Xylitol is a five-carbon sugar alcohol which has been widely used in the food, healthcare, and chemical industry (Pepper & Olinger, 1988; Sreenivas-Rao, Pavana-Jyothi, Prakasham, Sarma, & Venkateswar-Rao, 2006; Domínguez, Gong, & Tsao, 1997). As a sweetener, xylitol has the advantage that it does not cause dental caries, and can be used in the treatment of illnesses such as diabetes, disorders in lipid metabolism and parenteral and renal lesions, and for the prevention of otitis, lung infection and osteoporosis (Pereira, Mussatto, & Roberto, 2011). In industrial processes, xylitol is produced by the chemical reduction of xylose crystal which is obtained from the hemicellulose hydrolysate with dilute sulfuric acid (Liaw, Chen, Chang, & Chen, 2008). However, the chemical reduction process has many disadvantages including high pressure and high temperature, expensive separation and purification procedure, and the potential damage to environment. Bioconversion with moderate reaction conditions and low energy requirement was proposed as an alternative method for xylitol production (Nolleau, Preziosi-Belloy, Delgenes, & Navarro, 1993; Tada, Horiuchi, Kanno, & Kobayashi, 2004).

In this bioconversion process, many microorganisms such as: *Candida tropicalis* (*C. tropicalis*), *Debaryomyces hansenii*, *Candida guilliermondii*, *Candida subtropicalis* were used in the to production of xylitol (Liaw et al., 2008; Martínez, Silva, Silva, Solenzal, & Felipe, 2003; Rivas et al., 2003; Sreenivas-Rao et al., 2006). Among these, *C. tropicalis* represents a desirable microorganism for xylitol production with high yield and volumetric productivity from the xylose-containing hydrolysate by high temperature steaming (HTS) (Converti, Perego, Sordi, & Torre, 2002; Wang et al., 2011).

When compared with the free cell fermentation, the immobilized microorganism systems can be used to improve the performance of fermentation and reduce the overall production costs (Roberto, Felipe, Lacis, Silva, & Mancilha, 1991). The main merit is that the immobilized systems allow reutilization of the cells and direct most of the energy consumed to the desired bioconversion. In addition, the immobilization could enhance the stability of microorganism against adverse conditions, reduce the washing-out

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rate of cells and improve in the efficiency of bioconversion (Chen et al., 2006; Pandey, Soccol, Nigam, & Soccol, 2000). Therefore, many research efforts have been devoted to maintain high and durable cells reactivity with different cell immobilization systems for efficient and continuous fermentation operation (Branco et al., 2007; Carvalho, Silva, & Converti, 2002; Liaw et al., 2008; Zhou, Li, Bai, & Zhao, 2009).

Different immobilization strategies adopting various carriers were used for cell immobilization in xylitol production (Carvalho et al., 2004; Lebeau, Jouenne, & Junter, 1998; Na et al., 2000), such as sodium alginate, ceramics, nonwoven fabrics, microporous glass beads, zeolite and polyvinyl alcohol membrane (Martínez et al., 2003; Carvalho, Silva, Converti, & Vitolo, 2002; Carvalho, Silva, Converti, Vitolo, et al., 2002). In these carriers, polyurethane foam (PUF), which is a class of polymers with many intrinsic properties such as low toxicity, large surface area, high mechanical strength and resistance to organic solvents and microbial corrosion, has been widely applied to many territories such as medicine, biosensor, auto-motor, and building materials (Zheng et al., 2009; Zia, Barikani, Zuber, Bhatti, & Sheikh, 2008). PUF could be used as an excellent carrier candidate by virtue of the merits of excellent biocompatibility, satisfactory mechanical strength, moderate mass transfer rate and low mechanical friction factor, which could improve cells vitality and enzyme reactivity (El-Meligy, Mohamed, & Mahani, 2010; Zhu, Zhai, Deng, & Li, 2004). Moreover, the pore size of the PUF is tunable, which could be applied for different microorganism with varied diameters (Na et al., 2000). Therefore, PUF has been used in many studies for the microbial bioprocess (Domíngo, Radway, Wilde, Hermann, & Hazen, 1997; Quezada, Carballeira, & Sinisterra, 2009; Raghukumar, Mohandass, Kamat, & Shailaja, 2004; Zheng et al., 2009; Zhu et al., 2004). However, to our best knowledge, it has not been used as the carrier for *C. tropicalis* in the multi-batches xylitol fermentation from corncob hemicellulose hydrolysate (CHH) prepared by HTS.

In our research, the xylitol multi-batches fermentation using PUF immobilized *C. tropicalis* was studied. The fermentation parameters such as initial cell concentration, PUF dosage, initial pH value and culture temperature were controlled and optimized to investigate their effects on the xylitol fermentation. Moreover, the comparison of the multi-batches fermentation with and without immobilization was studied. It was indicated that the PUF immobilization is efficient for improving cell vitality and enzyme reactivity in the fermentation. The process of PUF immobilization is simple, low cost and environmental benign, which could find application in other fermentation processes.

2. Materials and methods

2.1. Preparation of CHH with HTS

Corncob was collected from Southern suburb of Beijing. It was milled into 20 mesh particles, washed by running water and dried at 50 °C to constant weight. In the pretreatment, 300 g corncob was soaked in 1 L dilute sulfuric acid (0.1%, v v^{-1}) at room temperature for 24 h. Then, the suspension was centrifuged, and the supernatant was recycled. The collected solids were washed with distilled water until the washing solution reached pH = 6.0. After the pretreatment, the corncob was hydrolyzed in a 5 L reactor with one turbine with a four-plane blade and which was capable of operating at high pressures and temperatures. The actual working volumes used for hydrolysis by HTS were adjusted by adding deionized water to 3.5 L at 165 °C (pressures of 0.6–0.8 MPa) for the residence time of 120 min. After hydrolysis, the hydrolysate was neutralized with lime to pH = 6.0–7.0, then filtered to obtain the CHH. Finally, the CHH was concentrated with vacuum evaporation at 55 °C for storage and fermentation.

2.2. Microorganism maintenance and inoculum preparation

C. tropicalis As 2.1776 was purchased from China General Microbiological Culture Collection Center (Beijing, China). After UV-mutagenesis as previously described (Cassier-Chauvat & Fabre, 1991), *C. tropicalis* JA 309, one of the mutated strains, was used in this research. The strain was cultured on an agar slant (pH = 6.0) containing 8 g L^{-1} yeast extract, 4 g L^{-1} glucose, 10 g L^{-1} xylose and 20 g L^{-1} agar at 30 °C for 48 h and stored at 4 °C. Cells from the slant were aseptically inoculated to 50 mL of inoculum medium in 250 mL flasks at 30 °C for 24 h on 200 rpm shaker. The inoculum medium (initial pH = 5.0–6.0) includes 20 g L^{-1} xylose, 10 g L^{-1} glucose, 10 g L^{-1} yeast extract, 3 g L^{-1} KH_2PO_4 and 2 g L^{-1} $(NH_4)_2HPO_4$. The prepared inoculum was directly added to the culture medium with different initial cell concentrations for xylitol fermentation.

2.3. PUF immobilization

PUF was purchased from Heng Xing Yue foam Ltd. (Beijing, China). It had a density of 320 kg m^{-3} and specific surface area of 1.5–2.0 × 10^5 m^2 m^{-3} with the average porosity and pore diameter of 95% and 0.03 mm respectively. After cutting into cubic pieces with a side length of 5 mm, the PUF was alternately eluted by 2 M NaOH solution and 2 M HCl. Then it was washed with distilled water until the pH value reached to 7.0 and dried at 55 °C for 24 h. Finally, PUF was mixed with the medium by an appropriate dosage and sterilized at 115 °C for 30 min in the autoclave.

2.4. Multi-batches fermentation in bioreactor

2.4.1. Conditions of the multi-batches xylitol fermentation

The batch fermentation was carried out in a 5 L bioreactor (B. Braun Biotech International, Germany) provided with temperature, stirring, aeration, pH value controller, dissolved oxygen (DO) value controller and two turbines with six-plane blades. The medium volume was fixed at 3.5 L and stirred under 200 rpm. DO conditions: in the first 24 h the aeration was set at 1.2 v v^{-1} min^{-1}, afterward it was turned down to 0.6 v v^{-1} min^{-1} for the later fermentation. The fermentation was carried out with the culture medium of 12 g L^{-1} yeast extract, 3 g L^{-1} KH_2PO_4, 2 g L^{-1} $(NH_4)_2HPO_4$, 0.1 g L^{-1} $MgSO_4 \cdot 7H_2O$ and the concentrated CHH by HTS mainly including xylose of 140 g L^{-1}, glucose of 14.14 g L^{-1}, arabinose of 12.02 g L^{-1} and acetic acid of 6.98 g L^{-1}. The initial cells concentration, quantity addition of PUF, initial pH value and culture temperature were investigated in the same medium. The experiment was monitored with periodic sampling to determine the parameters.

2.4.2. The multi-batches xylitol fermentation with PUF immobilization

At the beginning of the first batch fermentation, 0.2 L inoculum which were grown to 2.5 (OD 550) was inoculated into the 3.3 L CHH-based medium with a certain dosage of PUF in the bioreactor. The cells were immobilized in PUF by natural adsorption. At the end of the first batch, the broth removed from the bioreactor, leaving the PUF carrier with immobilized cells for the second batch. After adding the fresh culture medium to 3.5 L, the second batch started in the same conditions as described above. The rest batches were operated in the same manner.

2.4.3. The multi-batches xylitol fermentation without PUF immobilization

At the beginning of the first batch fermentation, 0.2 L inoculum which were grown to 2.5 (OD 550) was inoculated into the 3.3 L

CHH-based medium without PUF immobilization in the bioreactor. At the end of the first batch fermentation, the broth was centrifuged to separate the cells. Afterwards, the free biomass as the same dosage of the PUF-immobilized cells (referred to Section 2.4.2) was inoculated for the second batch. The rest batches fermentation was operated in the same manner.

2.5. Analytical methods

2.5.1. The measurement of cells weight and concentration in the broth

To measure the cells weight, 5 mL of the samples were firstly filtered with 0.45 μm pre-weighed filters (Gelman Sciences, AnnArbour, MI, USA). Washed with 15 mL ultra-pure water and filtered, then after drying at 55 °C for 24 h to a constant weight, the cells were weighed. The cell optical density was determined at 550 nm using a UV spectrophotometer (Shimadzu UV-1700, Jiangsu, China). The blank control was the culture medium. The calibration curve was used to determine the cells concentration in the medium or broth.

2.5.2. Measurement of the PUF immobilization

The broth with yeast and PUF carrier in the bioreactor was extracted from the fermentation system and weighed (labeled as "W_{all}"). The free cells could be measured based on the equation of $3.5 \times C$ and labeled as "W_{free}". Afterwards, the extraction was thoroughly washed with water to obtain PUF cubes, and the PUF was further treated with 0.05 M NaOH under ultrasonification to remove the immobilized biomass. All the washing solutions were filtered to separate the cells. After several washings, the cells were dried and weighed (labeled as "W_{bm}"). The PUF dosage was labeled as "W_{PUF}" after the washing and drying. The PUF immobilization efficiency was determined by $(W_{bm} - W_{free})/W_{PUF}$ (Zhou et al., 2009). Results represent the average of three independent experiments conducted with two replicates for each condition.

2.5.3. The measurement of main components in the hydrolysate and broth

All samples were filtered through 0.22 μm filters and diluted prior for HPLC analysis. The analysis was performed using a Hitachi HPLC system (Hitachi, Tokyo, Japan) and the N2000 software (Ejer Technol. Co. Ltd., Zhejiang, China). Xylitol and individual sugars in the hydrolysate and broth were measured on a Sugar-pak1 column (Waters, Milford, MA, USA) at 80 °C. As the mobile phase, ultra-pure water was supplied at a flow rate of 0.5 mL min^{-1}. Xylitol yield (%) is expressed as the ratio between the final xylitol concentration and the initial xylose concentration in the broth.

2.5.4. The observation of cells and carrier by SEM

For SEM analysis, the dehydrated PUF carrier, the carrier with immobilized *C. tropicalis*, cells in the immobilized and free cells in the non-immobilized fermentation broth were fixed in a 5.0% (w v^{-1}) glutaraldehyde solution for 2 h at 40 °C and dehydrated in an ascending water–ethanol series. The PUF as the carrier were dehydrated using a JFD-310 freeze-drying device (JEOL, Tokyo, Japan). The dried specimens were carefully mounted onto aluminum stubs using conductive adhesive. The mounted specimens were sputter-coated with gold and examined using a scanning electron microscope (JSM-6380 LV, Japan).

3. Results and discussion

3.1. Initial cell concentration

In the beginning of the PUF immobilized xylitol fermentation, various initial cell concentrations were inoculated into the medium and immobilized in PUF by natural adsorption. In Table 1, with the increase of initial cell concentration from 0.5 to 1.5 g L^{-1}, the yield and volumetric productivity of xylitol increased from 59.6 to 68.7% and 1.51 to 2.08 g L^{-1} h^{-1} respectively, the final cell concentration was elevated from 6.9 to 11.7 g L^{-1} and the incubation time of batch fermentation decreased from 55 to 46 h. However, Table 1 also showed that xylitol yield and final cell concentration slightly decreased on further increasing the initial cell concentration from 1.5 to 2.5 g L^{-1}. The amount of inhibitory factors in CHH-based medium was invariable. When the initial cell concentration was low, the adverse factors exerted more negative effects on cells, which resulted in a long lag phase (Converti et al., 2002). However, with the increase of initial cell concentration over 2.0 g L^{-1}, the dissolved oxygen in the medium decreased severely and more nutrient supplementation and xylose were consumed by cells proliferation instead of xylitol bioconversion. Moreover, the concentration of fermentation culture medium was diluted by the excess inoculums. Therefore, the optimal initial cell concentration was 1.5 g L^{-1} for the PUF immobilized fermentation.

3.2. PUF dosage

In the batch fermentation without PUF immobilization, the optimal xylitol yield (64.4%) and biomass (15.2 g L^{-1}) were obtained at 48 h as the control in our experiments. Fig. 1 shows that when

Table 1
Effect of the initial cell concentration on the PUF-immobilized xylitol fermentation from the CHH.[a]

X_0 (g L^{-1})[b]	S_0 (g L^{-1})[c]	P_0 (g L^{-1})[d]	t (hrs)[e]	S (g L^{-1})[f]	P (g L^{-1})[g]	X (g L^{-1})[h]	Q_P (g L^{-1} h^{-1})[i]	$Y_{P/S}$ (%)[j]
0.5	139.3 ± 0.2	2.6 ± 0.05	55 ± 0.5	3.2 ± 0.05	83.5 ± 0.2	6.9 ± 0.1	1.51 ± 0.03	0.60 ± 0.02
1.0	141.2 ± 0.3	3.5 ± 0.05	48 ± 0.5	3.6 ± 0.05	92.6 ± 0.3	9.1 ± 0.2	1.91 ± 0.02	0.65 ± 0.03
1.5	140.3 ± 0.3	3.7 ± 0.05	46 ± 0.5	3.2 ± 0.05	96.7 ± 0.4	11.2 ± 0.1	2.09 ± 0.03	0.69 ± 0.02
2.0	141.2 ± 0.2	3.9 ± 0.05	50 ± 0.5	4.4 ± 0.05	94.1 ± 0.3	11.7 ± 0.2	1.87 ± 0.02	0.67 ± 0.02
2.5	140.2 ± 0.2	4.2 ± 0.05	54 ± 0.5	3.1 ± 0.05	90.8 ± 0.4	12.4 ± 0.2	1.68 ± 0.03	0.64 ± 0.03

Results represent the average of two independent experiments conducted with three replicates for each condition.

[a] Xylitol bioconversion from the CHH by *C. tropicalis* at different initial cell concentrations in the first batch fermentation with PUF immobilization (culture temperature of 30 °C, initial pH of 6.0, PUF dosage of 10 g L^{-1} and DO conditions: in the first 24 h the aeration was set at 1.2 v v^{-1} min^{-1}, afterward it was turned down to 0.6 v v^{-1} min^{-1} for the later fermentation).

[b] Initial cell concentration.

[c] Initial xylose concentration.

[d] Initial xylitol concentration.

[e] Batch-fermentation time, the criteria of batches fermentation stop was the time when the concentration of xylitol no longer increased in the broth.

[f] Residual xylose concentration.

[g] Final xylitol concentration.

[h] Final cell concentration.

[i] Volumetric productivity of xylitol.

[j] Product yield of xylitol, the ratio of xylitol production and initial xylose concentration.

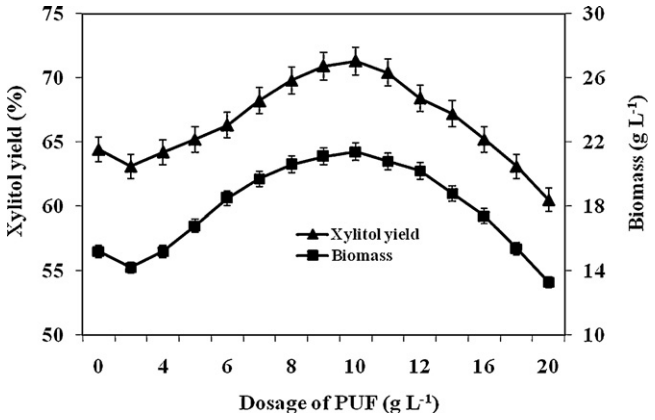

Fig. 1. Effect of the PUF dosage on the immobilized xylitol fermentation by *C. tropicalis* from the CHH. Xylitol bioconversion from the CHH by *C. tropicalis* at different PUF dosages in the culture medium of the multi-batches immobilized fermentation for 48 h (culture temperature of 30 °C, initial pH of 6.0, initial cell concentration of 1.5 g L^{-1}, initial xylose concentration of 140 g L^{-1}, and constant aeration of 1.0 v v^{-1} min^{-1}). Results represent the average of three independent experiments conducted with two replicates for each condition. Standard deviations were below 2.5%.

the PUF dosage in culture medium was less than 8 g L^{-1}, the PUF immobilization efficiency was lower than 30%, with the xylitol yield lower than 70% and the biomass less than 18 g L^{-1}. When the PUF dosage increased to 10 g L^{-1}, the PUF immobilization efficiency increased to more than 35% (the immobilization efficiency of each batch as the same value), the xylitol yield and biomass reached the highest values of 71.5% and 20.4 g L^{-1} respectively, since an increasing amount of carrier provided greater surface area, which was improved the immobilization efficiency and fermentation level. When the dosage increased to 12 g L^{-1}, the xylitol yield and biomass decreased dramatically. The reason was that excessive carrier occupied large space and increased volume of the medium. Therefore, the efficiencies of mass transfer and circulation decreased during the fermentation, resulting in the decrease of nutrient supply capacity and the DO level of medium (Cao, Tian, Zhao, Qian, & Yang, 2003). Therefore, it was demonstrated that the optimal dosage of PUF in culture medium was 10 g L^{-1} (Fig. 1).

3.3. Culture temperature and Initial pH value

In Fig. 2, when the culture temperature was kept in the range of 25–35 °C, the xylitol yield and biomass increased from 43.0 to 73.2% and 11.2 to 18.1 g L^{-1} respectively, and the xylose residual reduced from 42.3 to 4.5 g L^{-1} in the PUF immobilized fermentation at 48 h. By contrast, the xylitol yield and biomass were improved from 37.8 to 69.0% and 10.3 to 16.5 g L^{-1} respectively, and the xylose residual decreased from 48.8 to 5.3 g L^{-1} in the fermentation without immobilization. It was found that the optimal culture temperatures of fermentations with and without immobilization were both 30 °C. It probably resulted from the action of xylose reductase (XR), a key enzyme supplied by cells for the bioconversion of xylose to xylitol, had the highest activity at 30 °C (Converti & Domínguez, 2001; Wang et al., 2011). In Fig. 3, it also showed that, when the initial pH value increased from 5.5 to 6.5, the ranges of xylitol yield, biomass and xylose consumption varied smaller in the PUF immobilized fermentation than that of the fermentation without immobilization. Moreover, the optimal initial pH values were both 6.0.

Although the fermentations with and without immobilization had similar optimal culture temperature and initial pH value, the immobilized fermentation obtained a higher xylitol yield and

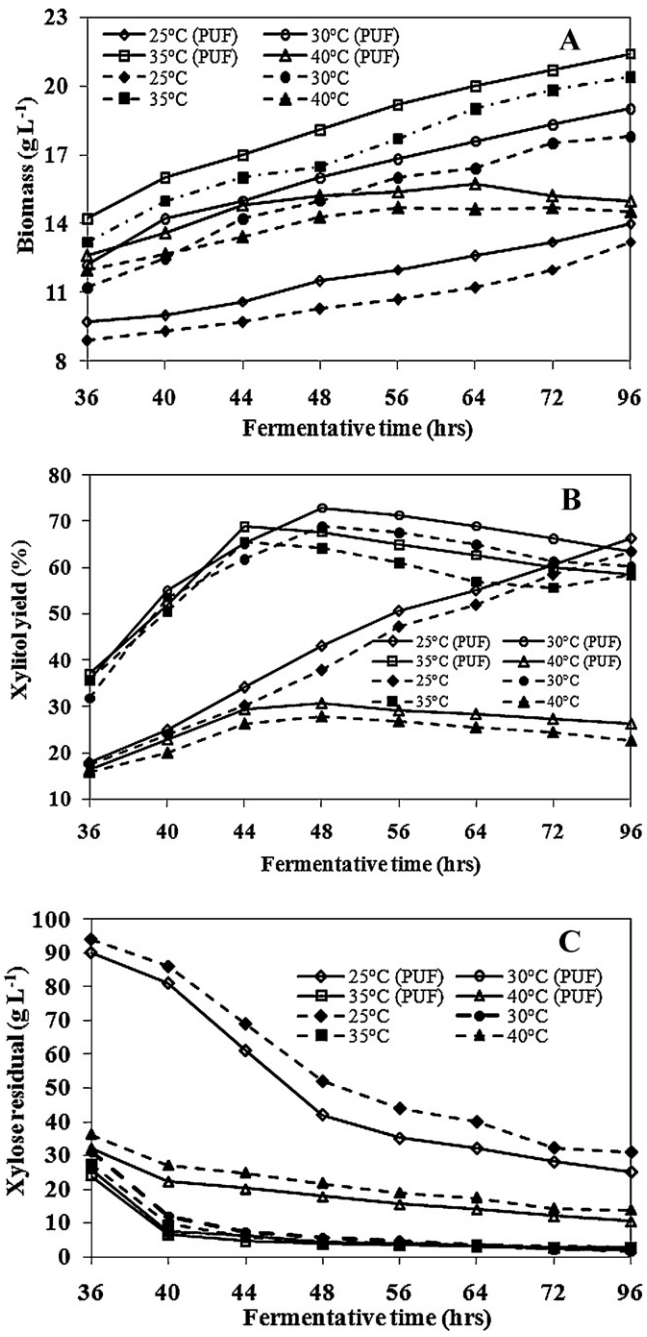

Fig. 2. Effect of the culture temperature on xylitol fermentation from the CHH using *C. tropicalis* with and without immobilization in PUF. Results of xylitol fermentation from the CHH at different culture temperatures using *C. tropicalis* with and without PUF immobilization (initial pH of 6.0, initial xylose concentration of 140 g L^{-1}, PUF dosage of 10 g L^{-1} and DO conditions: in the first 24 h the aeration was set at 1.2 v v^{-1} min^{-1}, afterward it was turned down to 0.6 v v^{-1} min^{-1} for the later fermentation in the bioreactor). Adding "(PUF)" represents the xylitol fermentation with PUF immobilization. Results represent the average of three independent experiments conducted with three replicates for each condition. Standard deviations were below 2.3%.

biomass, and lower xylose residue than those without immobilization (Figs. 2 and 3). It could be attributed to the immobilized cells exhibiting a higher tolerance and adaptability toward the adverse factors in the CHH medium than that without PUF protection. In addition, because of the purifying function of PUF, the concentration of inhibitors around PUF was greatly reduced (Zheng et al., 2009). Therefore, during the multi-batches PUF immobilized fermentation, the optimal xylitol yield could be achieved at the

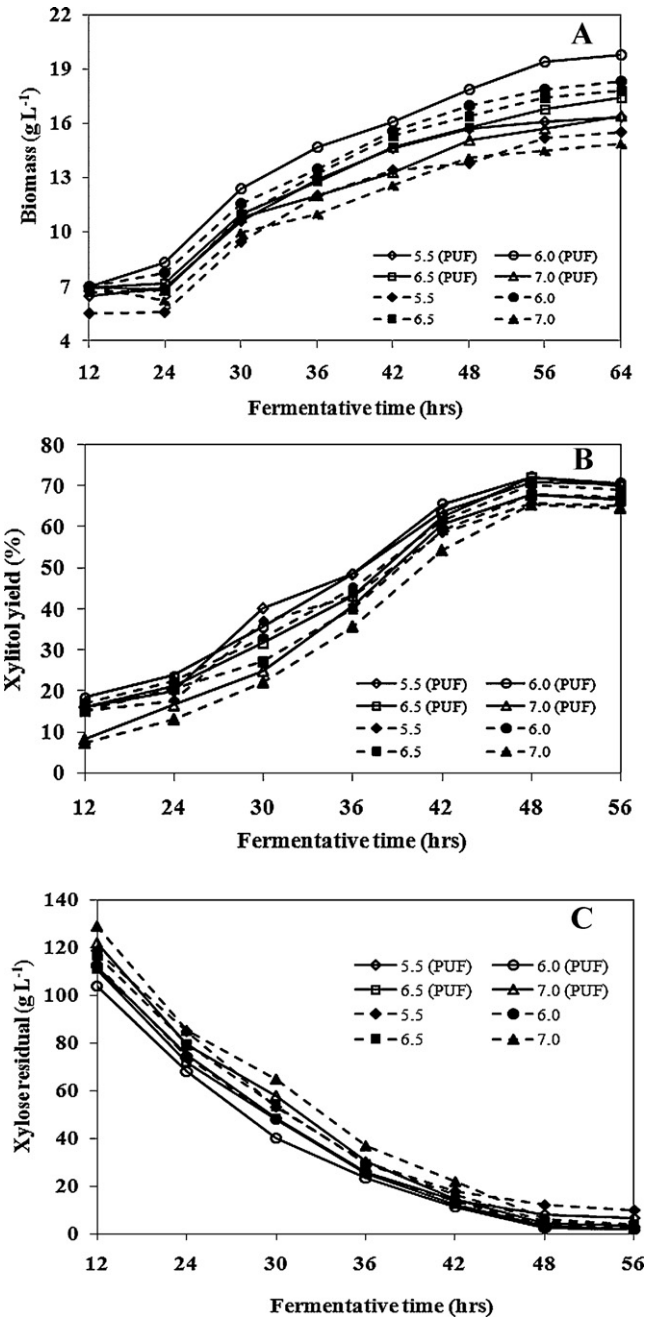

Fig. 3. Effect of the initial pH value on xylitol fermentation from the CHH using *C. tropicalis* with and without the PUF immobilization. Results of xylitol fermentation from the CHH at different initial pH values between using *C. tropicalis* with and without PUF immobilization (culture temperature of 30 °C, initial xylose concentration of 140 g L^{-1}, PUF dosage of 10 g L^{-1} and the constant aeration of 1.0 v v^{-1} min^{-1} in the multi-batches fermentation). Adding "(PUF)" represents the xylitol fermentation with PUF immobilization. Results represent the average of three independent experiments conducted with three replicates for each condition. Standard deviations were below 2.6%.

conditions of a wider culture temperature (25–35 °C) and initial pH range (5.5–6.5), which permitted an easier operation in the real industrial producing.

3.4. Multi-batches fermentation

Table 2 shows the comparative study on multi-batches fermentation with and without PUF immobilization. In the fermentation

Table 2
Comparative results of the two processes of multi-batches fermentation from the CHH using *C. tropicalis* with and without immobilization in PUF.[a]

Batch[b]	1 (48 h)	2 (46 h)	3 (46 h)	4 (46 h)	5 (48 h)	6 (50 h)	7 (52 h)	8 (54 h)	9 (56 h)	10 (60 h)
S (g L^{-1})/	3.3 ± 0.1/	2.8 ± 0.1/	2.3 ± 0.1/	2.5 ± 0.2/	3.6 ± 0.2/	4.5 ± 0.2/	6.2 ± 0.2/	7.3 ± 0.2/	8.7 ± 0.2/	10.6 ± 0.1/
S' (g L^{-1})	3.5 ± 0.1	3.2 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	4.1 ± 0.1	5.7 ± 0.2	7.3 ± 0.1	8.7 ± 0.2	11.2 ± 0.3	14.5 ± 0.2
P (g L^{-1})/	96.6 ± 0.3/	99.2 ± 0.4/	101.4 ± 0.5/	98.1 ± 0.5/	95.6 ± 0.5/	93.7 ± 0.3/	91.1 ± 0.4/	86.7 ± 0.4/	84.2 ± 0.3/	79.9 ± 0.3/
P' (g L^{-1})	95.2 ± 0.4	95.4 ± 0.5	93.7 ± 0.4	91.2 ± 0.5	88.3 ± 0.4	86.9 ± 0.4	82.6 ± 0.3	77.0 ± 0.3	71.5 ± 0.3	64.5 ± 0.2
$Y_{P/S}$ (%)/	69.2 ± 0.1/	71.1 ± 0.1/	71.2 ± 0.2/	70.3 ± 0.02/	68.2 ± 0.2/	67.1 ± 0.2/	65.3 ± 0.1/	62.2 ± 0.2/	60.4 ± 0.1/	56.8 ± 0.02/
$Y'_{P/S}$ (%)	67.8 ± 0.2	68.0 ± 0.1	66.9 ± 0.2	65.1 ± 0.01	62.8 ± 0.1	62.3 ± 0.2	58.8 ± 0.1	55.3 ± 0.2	51.2 ± 0.1	45.3 ± 0.01
Q_P (g L^{-1} h^{-1})/	2.01 ± 0.03/	2.07 ± 0.02/	2.11 ± 0.03/	2.04 ± 0.02/	1.99 ± 0.03/	1.95 ± 0.03/	1.89 ± 0.02/	1.81 ± 0.03/	1.76 ± 0.02/	1.66 ± 0.03/
Q'_P (g L^{-1} h^{-1})	1.98 ± 0.02	1.99 ± 0.03	1.95 ± 0.02	1.89 ± 0.03	1.84 ± 0.03	1.81 ± 0.02	1.72 ± 0.03	1.60 ± 0.02	1.49 ± 0.02	1.33 ± 0.02
X_0 (g L^{-1})/	4.4 ± 0.05/	9.3 ± 0.05/	9.8 ± 0.05/	10.4 ± 0.05/	10.8 ± 0.05/	10.7 ± 0.05/	10.8 ± 0.05/	10.3 ± 0.05/	9.6 ± 0.05/	8.9 ± 0.05/
X'_0 (g L^{-1})	4.3 ± 0.05	7.7 ± 0.05	8.1 ± 0.55	8.2 ± 0.5	8.4 ± 0.05	8.7 ± 0.05	8.3 ± 0.05	8.0 ± 0.05	7.2 ± 0.05	6.3 ± 0.05
X (g L^{-1})/	14.2 ± 0.1/	14.8 ± 0.1/	15.6 ± 0.2/	16.3 ± 0.1/	16.2 ± 0.2/	16.5 ± 0.2/	16.7 ± 0.2/	15.8 ± 0.2/	15.2 ± 0.1/	14.3 ± 0.1/
X' (g L^{-1})	14.7 ± 0.1	15.7 ± 0.1	15.4 ± 0.1	15.7 ± 0.1	15.5 ± 0.1	15.2 ± 0.1	15.1 ± 0.1	14.0 ± 0.1	12.4 ± 0.1	11.0 ± 0.1
A_{550}/	1.13 ± 0.02/	1.04 ± 0.02/	0.95 ± 0.02/	0.98 ± 0.02/	1.03 ± 0.02/	1.08 ± 0.03/	1.15 ± 0.02/	1.24 ± 0.03/	1.28 ± 0.02/	1.31 ± 0.03/
A'_{550}[c]	1.29 ± 0.02	1.23 ± 0.02	1.25 ± 0.02	1.26 ± 0.03	1.31 ± 0.02	1.37 ± 0.03	1.46 ± 0.04	1.50 ± 0.03	1.54 ± 0.04	1.57 ± 0.03

Adding "/" or not represented whether the xylitol fermentation with PUF immobilization or without, respectively.
Results represent the average of two independent experiments conducted with three replicates for each condition.

[a] Results of xylitol multi-batches fermentation from the CHH using *C. tropicalis* with and without PUF immobilization (culture temperature of 30 °C, initial pH of 6.0, initial xylose concentration of 140 g L^{-1}, PUF dosage of 10 g L^{-1} and DO conditions): in the first 24 h the aeration was set at 1.2 v v^{-1} min^{-1}, afterward it was turned down to 0.6 v v^{-1} min^{-1} for the later fermentation.

[b] Times of the batch fermentation.

[c] Absorbance at 550 nm of fermentation supernatant after centrifugation.

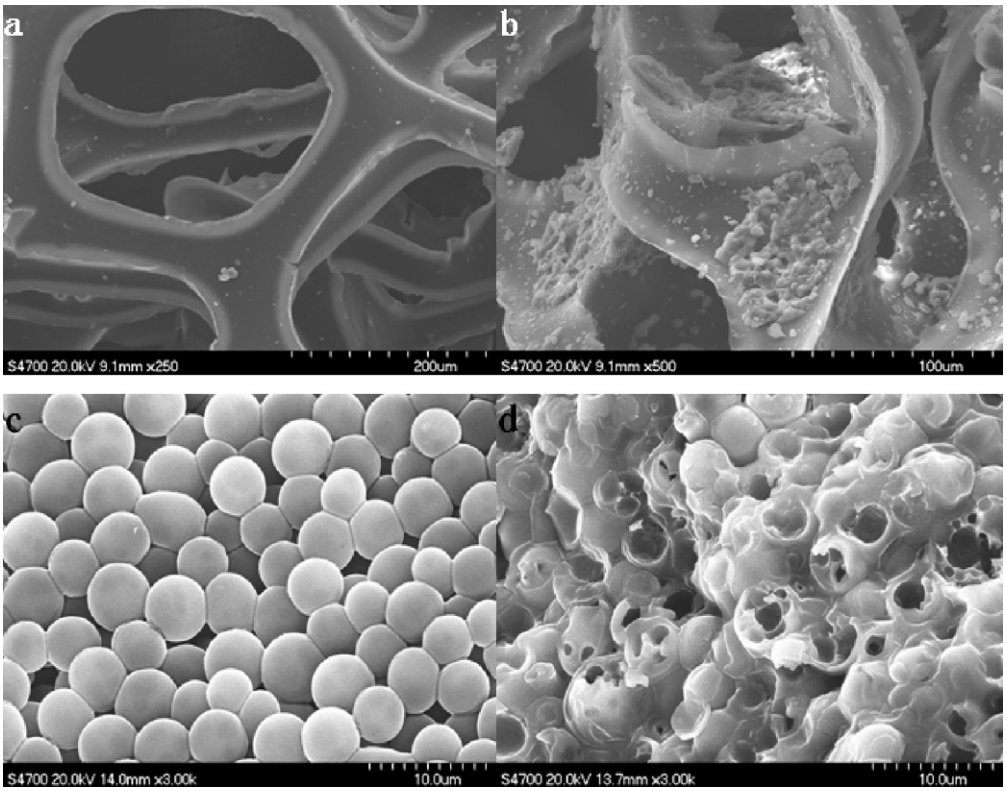

Fig. 4. SEM images of PUF carrier and immobilized *C. tropicalis*. (a) PUF carrier without *C. tropicalis*; (b) PUF carrier immobilized with *C. tropicalis*; (c) free *C. tropicalis* in the PUF immobilized fermentation; (d) free *C. tropicalis* in the non-immobilized fermentation. Results of multi-batches xylitol fermentation from the CHH using *C. tropicalis* with and without PUF immobilization (culture temperature of 30 °C, initial pH of 6.0, initial xylose concentration of 140 g L^{-1}, PUF dosage of 10 g L^{-1} and DO conditions: in the first 24 h the aeration was set at 1.2 v v^{-1} min^{-1}, afterward it was turned down to 0.6 v v^{-1} min^{-1} for the later fermentation).

with and without immobilization, the optimal xylitol yields were dropped from 71.2 to 57.0% and 67.8 to 45.1% respectively, and the volumetric productivities were dropped from 2.10 to 1.66 g L^{-1} h^{-1} and 1.98 to 1.33 g L^{-1} h^{-1} respectively after ten batches. Moreover, the yield and volumetric productivity of xylitol in immobilized fermentation were 5–10% higher, and the average batch period was 7–15% shorter than those without immobilization. With the increase of batch times, the immobilized fermentation exhibited the higher xylitol yield and more stable performance than those without immobilization.

In addition, it also showed that the color of the immobilized fermentation was lighter than that of without immobilization. The reason was that PUF carrier had a strong pigment-adsorption capacity and provided a suitable fermentative environment for higher cells vitality to assimilate the pigment. However, at the end of 8th batch fermentation, the difference of broth chrominance between the fermentations with and without immobilization was narrowed which may ascribe to the physical pigment-adsorption capacity of PUF carrier was saturated and the cells vitality became weak in the later batches of fermentation (Zhu et al., 2004).

It was noteworthy that in the second or third batch in the immobilized fermentation, the xylitol yield and volumetric productivity were higher than that of the first batch, suggesting the immobilized cells exhibits appropriate and continuous proliferation to maintain high enzyme activity and xylitol production. After the 8th batch without immobilization and the 10th batch of immobilized fermentation, the autolysis and plasmolysis of cells began to intensify, which led to the decrease of cells viability and xylitol yield. Moreover, from the optical microscopy observation of the cells morphology, the size and shape of cells became irregular which resulted in decreased enzyme activity and xylitol yield. It was remarkable that high cells viability could be maintained and the cells life could be extended in the multi-batches immobilized fermentation (Santos, Sarrouh, Rivaldia, Converti, & Silva, 2008). With the aid of PUF as the immobilized carrier, the fermentation performance was substantially improved and the average yield and volumetric productivity of xylitol increased to 66.3% and 1.90 g L^{-1} h^{-1} respectively. It was demonstrated that the toxicity of inhibitors in the CHH-based medium could be alleviated by adopting the PUF immobilization fermentation (Converti et al., 2002; Zhou et al., 2009).

Fig. 4 shows the SEM images of the dehydrated PUF carrier (Fig. 4a), PUF carrier immobilized with *C. tropicalis* (Fig. 4b), free *C. tropicalis* in the PUF immobilized fermentation (Fig. 4c) and free *C. tropicalis* in the non-immobilized fermentation (Fig. 4d). The surface of blank carrier (Fig. 4a) was clean and with many channels and holes. Whereas for the carrier immobilized with *C. tropicalis* (Fig. 4b), the surface became rough with many cells adhered due to the affinity between the PUF carrier and yeast. In Fig. 4c, the cell was well-preserved with smooth surface. According to the uniform shapes and size of cells, it could be concluded that the growth had the similar period. Therefore, it was indicated that cells immobilized in PUF could renew and transpose with each other and a dynamic balance could be reached by the similar rate of the proliferation and death of the immobilized cells (Cao et al., 2003). Moreover, the immobilized cells were able to renew and exchange with the free cells in the broth through the continuous adsorption–desorption dynamic equilibrium at the interface, providing the cells with high vitality for xylitol fermentation (Na

et al., 2000; Ory de, Romero, & Cantero, 2004). As a result, there were more cells that achieved the immobilized experience in the PUF to obtain the better environment of metabolism and growth (Yamaguchi, Ishida, & Suzuki, 1999). By contrast, in Fig. 4d, in the fermentation without PUF, most of the cells were broken and autolyzed, suggesting low vitality of the cells.

Moreover, from the optical microscopy, in the multi-batches immobilized fermentation, the proportion of filamentous cells immobilized in PUF was 10–15%, and the proportion of filamentous cells was 3–4% free in the broth. On the contrary, the proportion of filamentous cells in the broth was only 1–2% in the multi-batches fermentation without immobilization, much less than that of in the immobilized fermentation. It is reported that the higher cells vitality and enzyme activity were preformed when *C. tropicalis* was in the filamentous form during the fermentation (Chen et al., 2006). Therefore, with the PUF immobilization, the cells vitality could be activated and then resulted in a good fermentation performance.

4. Conclusions

In this paper, PUF was used as *C. tropicalis* carrier in the multi-batches xylitol fermentation. The fermentation parameters such as initial cell concentration, PUF dosage, initial pH value and culture temperature were controlled and optimized to investigate their effects on the xylitol fermentation. The results showed that, with the PUF immobilization, the optimal fermentation conditions could be extended to 25–35 °C for culture temperature and 5.5–6.5 for the initial pH range. In the multi-batch tests, the maximum xylitol yield and volumetric productivity respectively reached to 71.2% and 2.10 g L^{-1} h^{-1}. Moreover, the average xylitol yield and volumetric productivity were 66.3% and 1.90 g L^{-1} h^{-1} for ten batches operation. It was indicated that the PUF immobilization is an efficient method for enhancing cell vitality and enzyme reactivity for xylitol fermentation. Furthermore, the morphology of cells was monitored to study the mechanism of the improved cells vitality with PUF immobilization. The strategy for PUF immobilization is simple, low cost and environmental benign, which could find further application in other fermentation processes.

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