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Evaluation of corncob hemicellulosic hydrolysate for xylitol production by adapted strain of *Candida tropicalis*

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ABSTRACT

A maximum xylose extraction of 21.98 g/L was obtained in hydrolysate with a solid to liquid ratio of 1:8 (w/v) at 1% H_2SO_4 and treated for 30 min. The optimized and treated corncob hemicellulosic hydrolysate medium supplemented with (g/L) yeast extract 5.0, KH_2PO_4 2.0, $MgSO_4 \cdot 7H_2O$ 0.3 and methanol 10 mL whose pH was adjusted to 4.5 acts as production medium. Under this condition; the adapted strain of *C. tropicalis* resulted in 1.22-fold increase in xylitol yield and 1.70-fold enhancement in volumetric productivity was obtained as compared to parent strain of *C. tropicalis*. On concentrating the hydrolysate under vacuum using rotavapor proves to be efficient in terms of improved xylitol yield and productivity over microwave assisted concentration using adapted strain of *C. tropicalis*. The immobilized cells of *C. tropicalis* resulted in more than 70% efficiency up to third cycle. The xylitol production could be scaled up to 10 L fermentor.

1. Introduction

The utilization of lignocellulosic wastes for industrial purposes is very attractive and promising as these are inexpensive, renewable and widely available in nature. Hemicelluloses are a plant cell wall polysaccharide and the third most abundant renewable polymer in nature. The main component of the hemicellulosic fraction is xylan, a heteropolysaccharide with homopolymeric backbone of xylose units (Saha, 2003). In this context, extensive research has been undertaken for bioconversion of hemicellulosic hydrolysate derived carbohydrates, particularly xylose, into several value added products (Chandel & Singh, 2011). It is a naturally occurring five-carbon sugar alcohol with outstanding organoleptic and anti-cariogenic properties (Mäkinen, 2000; Rao, Jyothi, Prakasham, Sarma, & Rao, 2006). Besides this, it prevents osteoporosis and can be used in diabetic food products which thereby make xylitol as an attractive sucrose substitute. Due to its properties, xylitol has become an attractive option in food and pharmaceutical industries. The production of xylitol involves established commercial process based on catalytic hydrogenation of highly purified xylose derived from hydrolysates of hemicellulosic rich materials, a high production cost process that uses elevated pressure and temperature, and requires extensive xylose purification steps (Liaw, Chen, Chang, & Chen, 2008). For these reasons, several researchers have explored the alternative route, wherein the existence of xylose-fermenting microorganisms *i.e.* bacteria, yeasts and fungi opened the possibility to produce xylitol by fermentation using xylose present in hydrolysates derived from agro-industrial lignocellulosic residues. This alternative route proves to be interesting as it requires use of mild conditions of pressure and temperature, and very little xylose purification thus making the process economical (Tada, Horiuchi, Kanno, & Kobayashi, 2004; Wang et al., 2011). The most studied xylitol producers are yeasts, with strains of the genus *Candida* and *Debaromyces* being the best natural producers (Sampaio et al., 2004; West, 2009). Xylose fermenting yeasts reduces xylose to xylitol by the NAD (P) H-dependent xylose reductase (XR) (Winkelhausen & Kuzmanova, 1998).

The global process from the raw material (agro residues) to the final product has the following sequential steps: reduction of size, acid hydrolysis, neutralization, detoxification, fermentation, recovery and purification. The hemicellulosic fraction can easily be hydrolyzed using dilute acids (Sarrouh, Santos, & Silva, 2007). Hydrolysis of hemicellulose yield sugars which are rapidly degraded to fermentation inhibitors *i.e.* furfural, hydroxymethylfurfural and other condensation byproducts (Rao et al., 2006). The amount of sugar released during hydrolysis is dependent on the type of material and operating conditions including temperature, reaction time and acid concentration (Rahman, Choudhury, Ahmad, & Kamaruddin, 2007). Nolleau, Preziosi Belloy, Delgenes, and Navarro (1993) pointed out that xylose in higher amounts favors xylitol production by yeasts. Thus, the hydrolysate needs to be concentrated before being used as a culture medium. However, at the same time, the concentrations of by-products originally

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present in the hydrolysate also increases, leading to the reduction on the xylose fermenting capability of the yeast (Felipe, Vitolo, Mancilha, & Silva, 1997). In order to ferment the hemicellulosic hydrolysates, the substrates must be detoxified using suitable methods to enable fermentative microorganisms to grow and ferment the substrate (Rao et al., 2006). Some examples of chemical treatments capable of inducing precipitation of the toxic compounds are pH adjustment (Martinez et al., 2001), detoxification with ion exchange resins (Rao et al., 2006) and adsorption on activated charcoal (Canilha, Carvalho, Giulietti, Felipe, & Almeida e Silva, 2008; Carvalheiro et al., 2005).

Activated charcoal treatment is an efficient and economic method for reduction in the amount of phenolic compounds, acetic acid, aromatic compounds (Canilha et al., 2008; Carvalheiro et al., 2005), furfural and hydroxymethylfurfural (Mussatto & Roberto, 2001) mainly found in hemicellulosic hydrolysates. The pH adjustment employing a combination of bases and acids is also a low cost treatment for the reduction of furfural to furfuryl alcohol (Roberto, Felipe, Lacys, Silva, & Mancilha, 1991). Cells previously adapted to the culture medium have shown improvement in the fermentative efficiency for xylitol production (Carvalho, Silva, Vitolo, Felipe, & Mancilha, 2002; Rodrigues et al., 2006). This low cost technique provides an alternative to the detoxification methods used for the removal of inhibitory compounds. In addition, the use of adapted inocula makes it possible to schedule a series of batch cultures so that the whole plant can be operated almost continuously with a concomitant reduction in the overall operation time (Silva & Roberto, 2001). Keller, Bates, Ruiz, and Ngriyen (1998) reported that even when the hydrolysate is highly concentrated, the adapted strain could produce the desired product, whereas, unadapted cells produce very little product or were not able to survive at all in the crude hydrolysate.

The initial cell concentration is also very important. Increasing the initial biomass concentration resulted in both higher fermentation efficiency and limitation of inhibition by toxic substances present in hydrolysates (Purwadi, Brandberg, & Taherzadeh, 2007). High cell densities can be achieved with immobilized systems but it is necessary to select a technique which has sufficient immobilizing capacity to avoid cell leakage into the medium (Yahashi, Horitsu, Kawai, Suzuki, & Takamizawa, 1996). Amongst the different immobilization methods, the use of solid supports or the use of gel entrapment methods (alginate and carrageenan) are useful (Yahashi et al., 1996). Calcium alginate is the widest support used for this purpose due to its rapid gelification and to the mild immobilization conditions (Carvalho et al., 2004). Immobilized cells are sheltered from inhibitory compounds present in the hydrolysate and can easily be separated from culture medium, thus facilitating the reuse of the biocatalyst for extended period of time (Webb & Atkinson, 1992).

This paper demonstrates that the xylose fermenting capability of *C. tropicalis* can be affected by the cell adaptation in treated (pH adjustment followed by activated charcoal) corncob hemicellulosic hydrolysate. The bioconversion process by adapted strain of *C. tropicalis* was also studied in treated and concentrated corncob hemicellulosic hydrolysate under flask level and in 10 L fermentor.

2. Materials and methods

2.1. Materials

Corncobs were provided by Tata Chemicals Ltd., Mumbai, India under the joint industrial research work being conducted in University of Delhi South Campus. These were air dried and milled into particles with 1–5 mm long and 1 mm thick. Aminex HPX 87H column (dimension 300 mm × 7.8 mm; average particle size 25 m) was purchased from Bio-Rad Laboratories, Hercules, CA, USA. All the chemicals were analytical grade.

2.2. Corncob composition

Corncob contains various components such as lignin, cellulose, hemicellulose, various extractives and inorganic components. The composition of lignocellulosic biomass of corncob which was chemically characterized for the determination of its concentration has been carried out by the following methods wherein cellulose was estimated by Updegraff method (Updegraff, 1969); lignin was estimated by acetyl bromide method (Fukushima & Hatfield, 2004) and the moisture content was estimated by gravimetric method. Similar procedures were followed for the estimation of lignocellulosic biomass of corncob in the present investigation. All the analysis was carried out in triplicates.

2.3. Preparation of the corncob hydrolysate

10.0 g of corncob hydrolyzed with 100 mL of 1.0% dil. H_2SO_4 in a glass batch reactor at 121 °C for 60 min with a solid/liquid ratio of 1:10 (w/v). After the hydrolytic reaction, the material was filtered and the pH of the hydrolysate was estimated. Now, 1.0 mL of this filtered crude hydrolysate was analyzed on HPLC for the amount of xylose extracted in the hydrolysate.

2.4. Optimization for maximum xylose extraction from corncob

In order to get maximum xylose extracted in the hydrolysate from corncob, different parameters were examined during the acid hydrolysis. Concentration of H_2SO_4 (0.25–2.5%); Solid to liquid ratio (1:6, 1:8, 1:10, 1:12, w/v) and reaction time (15–75 min).

2.5. Detoxification of the corncob hydrolysate

Crude hydrolysate (50 mL) thus obtained after acid hydrolysis was treated through pH adjustment. Wherein; pH of the hydrolysate was raised to 10.0 with NaOH and was brought back to 4.5 using 1 N HCl. After overtitration, the overtitrated sample was centrifuged and the supernatant thus obtained was mixed with 1.0% activated charcoal and incubated at 55 °C, 200 rpm for 60 min. After incubation, the contents were centrifuged at 10,000 rpm for 15 min at 4 °C in a refrigerated centrifuge (Sigma 4X15, Germany). The supernatant (liquid phase) was separated by centrifugation and was filtered through 0.45 μm filters. Now, 1.0 mL of the filtrate was analyzed for xylose along with other acid hydrolysate components *i.e.* Glucose, arabinose, acetic acid, furfural concentration by HPLC using Aminex HPX-87H column. Phenolic compounds were analyzed by HPLC at 280 nm.

2.6. Concentration of corncob hemicellulosic hydrolysate

The corncob hemicellulosic hydrolysate was concentrated two-fold by concentration at low temperature under vacuum using rotavapor and microwave assisted concentration.

2.7. Preparation of adapted strain for xylitol production

The inoculum was prepared by growing this selected parent strain of Candida in the medium containing (g/L): yeast extract 10.0 and xylose 20.0 for 48 h at 30 °C, 200 rpm. The inoculum thus obtained was centrifuged and the cell pellet suspended in the appropriate amount of sterilized double distilled water was transferred to the optimized and treated hydrolysate medium supplemented with yeast extract 5.0, KH_2PO_4 2.0, $MgSO_4{\cdot}7H_2O$ 0.3, methanol 10 mL. Then, pH of the medium was maintained at 4.5.

After inoculation and incubation for 48 h at 30 °C, 200 rpm, the yeast cells were centrifuged and the suspended cell pellet was resuspended in the fresh sterilized optimized and treated production medium. This yeast strain was maintained in the optimized and treated medium up to six successive cycles.

The strain thus developed after xylose enrichment technique was evaluated for xylitol production.

2.8. Xylitol production from corncob hydrolysate

The xylitol production media were prepared from the original and concentrated detoxified hydrolysate using corncob containing 20.91 g/L of xylose (original) and 40.16 g/L and 52.71 g/L of xylose (hydrolysate concentrated through rotavapor and microwave respectively) as the main sugar in the hydrolysate and was supplemented with yeast extract (5.0 g/L), KH_2PO_4 (2.0 g/L), and $MgSO_4 \cdot 7H_2O$ (0.3 g/L). The pH of the medium was adjusted to 4.5 and incubated at 30 ± 1 °C, 200 rpm. The culture of the adapted yeast strain was inoculated with 4.0% (v/v) inoculum in the fermentation medium prepared from original and concentrated detoxified corncob hydrolysate Aliquots of the medium were withdrawn after every 12 h and 6 h of interval and centrifuged at 10,000 rpm for 15 min respectively.

2.9. Whole cell immobilization of the selected organism for xylitol production

2.9.1. Inoculum preparation

A loop full of the selected organism was maintained on nutrient agar slants and was inoculated in the 50 mL of growth medium (nutrient broth) contained in 250 mL Erlenmeyer flasks. The inoculum was raised for 48 h. Now, the entire culture broth was centrifuged at 10,000 rpm for 10 min. The pellet thus obtained was washed twice with sterilized double distilled water and pelleted again. The suspended cell pellet thus obtained was used for immobilization. All the immobilization experiments were carried out under sterile conditions.

2.9.2. Immobilization using calcium alginate

An adequate volume of the cell suspension was added to a sterile solution of 3.0% (w/v) sodium-alginate previously heated to 111 °C for 15 min. The mixture was extruded drop wise using a sterile 5.0 mL syringe from a height of about 20 cm into a chilled solution of 0.2 M calcium chloride ($CaCl_2$) with mild constant stirring. The beads of calcium alginate containing entrapped cells were allowed to harden at 4 °C for 24 h in $CaCl_2$ solution. The beads thus formed were washed thoroughly (3 times) with sterile distilled water. These beads were then used for xylitol production as the yeast cells gets entrapped in calcium alginate. The average diameter of the calcium alginate beads was approx. 2.7 mm.

2.9.3. Biomass estimation

Biomass was estimated by dissolving the known amount of beads in 5.0% (w/v) EDTA solution (Bucke, 1987) followed by drying at 80 °C to constant weight.

2.9.4. Xylitol production using immobilized cells and its efficiency

Xylitol production was carried out in 250 mL Erlenmeyer flasks containing 50 mL of the production medium inoculated with immobilized beads (alginate) containing approximately equal number of cells *i.e.* 4.0% per 50 mL medium, similar to that used for free cells. The culture was incubated at 30 °C, 200 rpm. After each run/cycle, the solid carrier with immobilized cells were carefully drained and gently washed with water in order to eliminate all non-adhering yeast cells. The spent broth was replaced with fresh optimized medium for the next cycle. At the end of each cycle amount of xylitol produced was estimated and the process was carried out using the same immobilized cells for successive cycles until a sharp decline in xylitol production was recorded.

2.10. Scale up of xylitol production in a 10 L fermentor

Xylitol production was scaled up in a 10 L fermentor using 5 L of optimized and treated corncob hemicellulosic hydrolysate medium (New Brunswick Sci. Inc., Fermentor Bioflow IV, USA). The optimized and treated corncob hemicellulosic hydrolysate medium containing (g/L): 20.92 of xylose, along with other medium components (%): yeast extract 0.5, $MgSO_4 \cdot 7H_2O$ 0.03, KH_2PO_4 0.2 and methanol 1.0. The appropriate medium was sterilized *in situ* at 110 °C for 20 min and inoculated with 5.0% of the optimized inoculum (adapted strain). Fermentation was carried out at 30 °C. Agitation and aeration rate were adjusted to 400 rpm with a constant rate of 0.7 vvm (up to 24 h) and then shifted to 200 rpm and 0.3 vvm for rest of the fermentation run. Foaming was controlled by adding silicon antifoam agent (50%, v/v prepared in distilled water). Samples were withdrawn for 60 h at regular time intervals of 6 h, centrifuged and were analyzed for xylitol production, leftover xylose and cell mass.

3. Results and discussion

3.1. Compositional analysis of corncob

It is clearly evident from the compositional analysis of corncob that 42.59 ± 0.62% acid detergent fiber (ADF); 81.75 ± 0.52% neutral detergent fiber (NDF); 7.12 ± 0.21% lignin; 1.47 ± 0.20% ash; 39.16 ± 0.44% hemicellulose; 34.01 ± 0.86% cellulose was present.

Several researchers reported the importance of using agricultural wastes containing hemicellulose as a potential substrate for microbial xylitol production in presence of the enzyme xylose reductase. Since, use of microorganisms is regarded as cost effective process and has a low environmental impact due to the effective utilization of renewable resources such as agricultural wastes (Cho et al., 2000).

3.2. Xylose extraction from hemicellulosic materials

Corncob was evaluated for the quantification of xylose in the hydrolysate which resulted in 16.36 g/L of xylose. The optimized conditions obtained for extraction of xylose are 1.0% H_2SO_4 with a solid to liquid ratio of 1:8 (w/v) at 121 °C for 30 min which resulted in (g/L): xylose 21.98, glucose 3.56, arabinose 2.17, acetic acid 1.31, furfural 0.21 from corncob. A 1.34-fold increase in xylose concentration along with a reduction in the treatment time up to 30 min was achieved after optimization as against initial unoptimized hydrolysate. Contrary to our results, Rao et al. (2006) and Tada et al. (2004) reported maximum xylose extraction from corncob using 1.0% H_2SO_4 at a treatment temperature of 121 °C with an increased reaction time to 60 min as the optimal conditions with a solid to liquid ratio of 1:5 (w/v) and 1:10 (w/v) respectively.

3.3. Detoxification of the corn cob hemicellulosic hydrolysate

The detoxification of corn cob hydrolysate through overtitration followed by activated charcoal could effectively remove furfural (100.0%), acetic acid (73.28%) and phenolic compounds (97.16%). The sugar losses were low. The percent reduction in sugars as observed in the present study was xylose (4.82%), glucose (3.65%), arabinose (1.84%) as against optimized hydrolysate. Our results are in accordance with the reports of Ge et al. (2011) wherein, over liming and activated charcoal treatment resulted in the complete removal of furfural, while, acetic acid and phenolic

compounds could be decreased by 62.4 and 96.6%, respectively. Contrary to our results, they also reported that the losses of glucose, xylose and arabinose were higher *i.e.* 28.4, 18.3 and 8.6%, respectively. Chandel, Singh, Chandrasekhar, Rao, and Narasu (2011) reported that detoxification using an overliming process for sugarcane bagasse hydrolysate resulted in a similar effect of reduction in inhibitory compounds in the form of phenolics (33.21%) and furfurals (41.75%) along with 7.61% reduction was observed for reducing sugars. Certain reports suggest that the presence of activated charcoal in the hydrolysate mainly helps in the reduction of phenolic compounds generated during acid hydrolysis (Ge et al., 2011; Mustapa Kamal, Mohamad, Abdullah, & Abdullah, 2011).

3.4. Xylitol production using detoxified corncob hemicellulosic hydrolysate

The optimized and treated (detoxified) hydrolysate medium containing (g/L): xylose 20.92, glucose 3.43, arabinose 2.13, acetic acid 0.35 when supplemented with yeast extract 5.0, KH_2PO_4 2.0, $MgSO_4{\cdot}7H_2O$ 0.3, methanol 10 mL act as a xylitol production medium for the parent strain of *Candida tropicalis*. Results showed that 10.29 g/L of xylitol was produced in 60 h from 20.92 g/L of xylose with a xylitol yield of 0.50 g/g under optimized hydrolysate conditions with a combination of pH adjustment and activated charcoal treatment. Mussatto and Roberto (2001) reported that pH adjustment and activated charcoal treatment when used in combination produces better detoxification of hydrolysate with an efficient conversion of xylose into xylitol. On the other hand, Dominguez, Cruz, Roca, Dominguez, and Parajo (1999) and Canilha et al. (2008) reported that the use of only activated charcoal for detoxification of the hydrolysate yielded 0.47 g/g and 0.54 g/g of xylose respectively.

3.5. Adaptation of C. tropicalis on xylose by enrichment technique

In the present investigation, the adapted strain produced 7.46 g/L of xylitol in 48 h from 21.98 g/L of initial xylose present in untreated corncob hemicellulosic hydrolysate with a yield of 0.37 g/g. However, 12.23 g/L of xylitol was produced in 42 h with a volumetric productivity of 0.29 g/L/h and a yield of 0.61 g/g from the optimized and treated corncob hemicellulosic hydrolysate in presence of 20.92 g/L of initial xylose. An increase in xylitol yield by 1.64-fold was observed in optimized and treated corncob hemicellulosic hydrolysate as compared to crude hydrolysate. This increase in xylitol production was accompanied by reduction in the incubation period up to 6 h on treating hydrolysate. Sánchez, Bravo, Castro, Moya, and Camacho (1998) and Zou, Qi, Chen, Miao, and Zhong (2010) reported 0.61 g/g of xylitol from *Hansenula polymorpha* and *Pichia guilliermondii* respectively. Similarly, Carvalho et al. (2004) reported a xylitol yield of 0.62 g/g from *C. guilliermondii* in sugarcane bagasse hemicellulosic hydrolysate. It could be concluded that adaptation improves the bioconversion efficiency and yield for xylitol production. Similar to our findings, Silva and Roberto (2001) had also observed that yeast adaptation in hydrolysate increases the xylitol yield from 0.35 to 0.65 g/g. Rao et al. (2006) also reported that after adaptation up to 20th cycle, the xylitol production was improved to 0.65 g/g as against 0.43 g/g of xylose utilized in corn fiber hydrolysate after hydrolysate neutralization and treatments with activated charcoal and ion exchange resins. These reports also suggested that adaptation of microorganisms is an effective and inexpensive approach for increasing the xylitol yield in hydrolysate medium. However, Silva and Roberto (2001) gave a hypothesis which states that the improved performance of the adapted strain may be due to the yeasts ability to maintain the initial pH value, which in turn leads to inactivation of toxic compounds present in the hydrolysate.

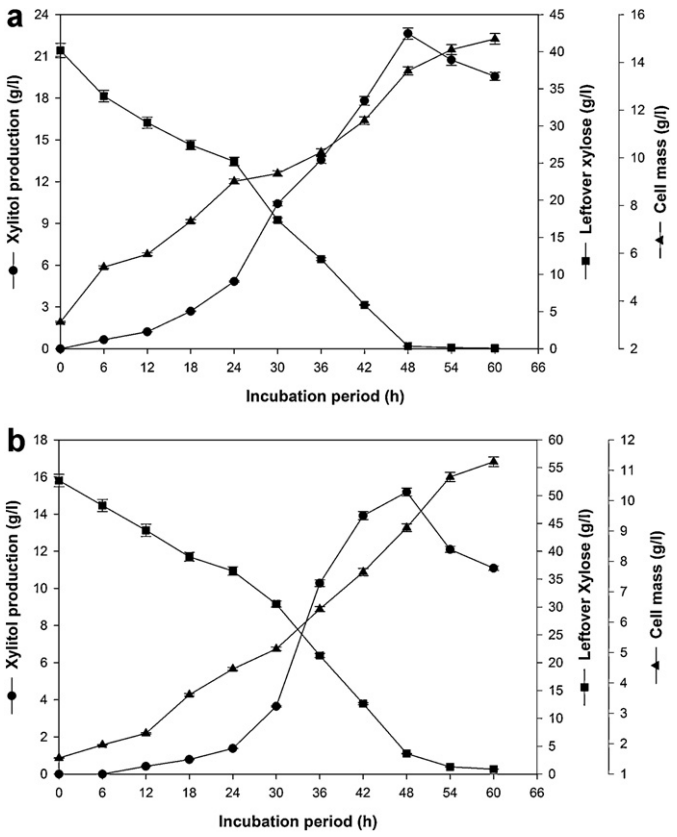

Fig. 1. Fermentation profile of xylitol production by adapted strain of *C. tropicalis* in concentrated corncob hemicellulosic hydrolysate medium in relation to time. (a) Rotavapor (b) microwave assisted concentration.

The corncob hemicellulosic hydrolysate was concentrated, since dilute hydrolysates can be a burden for the separation process. Lower substrate concentration limits both the productivity and yield of the fermentation step as has also been reported by Winkelhausen and Kuzmanova (1998). In the present investigation, the hemicellulosic hydrolysate was concentrated under vacuum at low temperature by using rotavapor and microwave. The hydrolysate was concentrated using two approaches, wherein in the first approach, the hydrolysate was concentrated under vacuum at low temperature using rotavapor which resulted in the xylitol production of 22.63 g/L in 48 h in presence of 40.16 g/L with a volumetric productivity of 0.471 g/L/h and a xylitol yield of 0.57 g/g of xylose (Fig. 1a). This is 1.2-fold increase in yield with 1.8-fold increase in productivity as observed on adapting the *C. tropicalis* on xylose contained in optimized corncob hydrolysate medium using enrichment technique. In the second approach, the hydrolysate was concentrated using microwave assisted concentration with an initial xylose concentration of 52.71 g/L which resulted in 15.19 g/L of xylitol with a productivity of 0.316 g/L/h and a yield of 0.31 g/g (Fig. 1b). This is 1.06-fold increase in yield with 1.47-fold increase in productivity as compared to the parent strain of *C. tropicalis*. On comparing with parent strain, it was observed that more or less same yield was obtained using adapted strain in presence of microwave assisted concentrated hydrolysate. Our results also showed that the concentration of corncob hemicellulosic hydrolysate through rotavapor is more favorable for xylitol production as compared to microwave assisted concentration. Rodrigues, Felipe, Silva, Vitolo, and Gómez (2001) reported that hydrolysate concentrated through vacuum evaporation after pH adjustment and charcoal treatment removes the remaining volatile compounds such as furfurals and acetic acid, meanwhile,

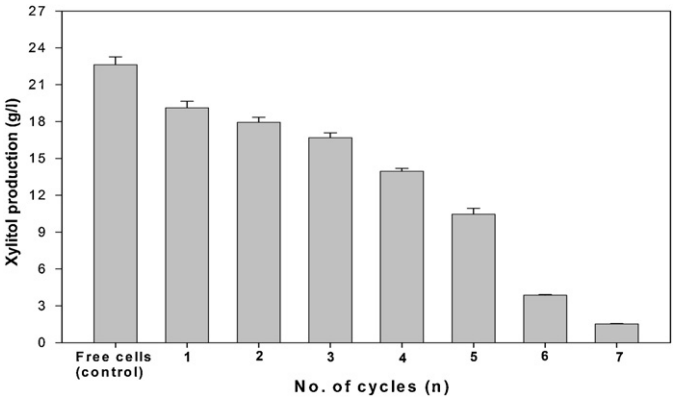

Fig. 2. Amount of xylitol produced in different cycles by immobilized cells using corncob hemicellulosic hydrolysate in 48 h.

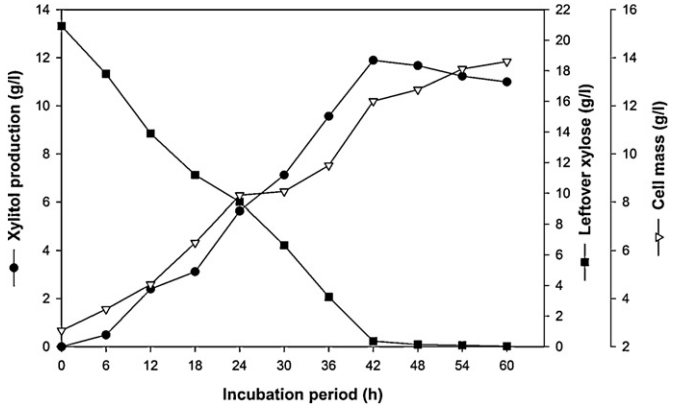

Fig. 3. Fermentation profile of xylitol production by adapted strain of *C. tropicalis* using optimized and treated corncob hemicellulosic hydrolysate medium in a 10 L fermentor at 30 °C.

concentrates the non volatile compounds such as sugars. This thereby improves the cell growth of the microorganism and its productivity in the hydrolysate. Since, in rotavapor concentration the temperature required for concentrating the hydrolysate is lower as compared to microwave and can be controlled. It was also observed that the process of rotavapor concentration does not cause instant charring or increase in the viscosity of the medium and allows the organism to grow in the medium and produces xylitol by utilizing xylose as a carbon source. Tada et al. (2004) reported comparable xylitol production rate of 0.51 g/L/h from corncob hemicellulosic hydrolysate using *C. magnoliae* with an initial xylose concentration of 25.0 g/L. Prakash, Varma, Prabhune, Shouche, and Rao (2011) reported the xylitol volumetric productivity of 0.28 g/L/h obtained from *D. hansenii* in sugarcane hemicellulosic hydrolysate. Cunha, Converti, Santos, Ferreira, and Da Silva (2009) and Rao et al. (2006) reported a xylitol yield of 0.49 g/g and 0.45 g/g by *C. guilliermondii* and *C. tropicalis* from sugarcane bagasse hydrolysate respectively.

3.6. Whole cell immobilization of *C. tropicalis* for xylitol production

Cells of *C. tropicalis* were successfully immobilized on calcium alginate. Our results are in agreement with the findings of several researchers, who reported that calcium alginate as the best support for cell immobilization for xylitol production (Carvalho et al., 2004). Immobilized cells when used in vacuum concentrated corn cob hydrolysate medium using rotavapor has resulted in more than 70.0% efficiency up to third cycle in *C. tropicalis* (Fig. 2). Dominguez et al. (1999) also reported that in successive cycles a decline in xylitol production was observed due to Al^{3+} which severely hindered the fermentation process through inhibiting xylose reductase system of *D. hansenii*. The Al^{3+} solution was used for improving the mechanical strength of alginate beads and to avoid cell leakage into the medium (Dominguez et al., 1999). To overcome the problem of cell leakage, they found that a higher percentage of Na-alginate *i.e.* 4% was optimal (Dominguez et al., 1999). It has also been reported that the optimal concentration of alginate may differ with respect to the organism and the product of interest (Nampoothiri & Pandey, 1998). Silva, Santos, Carvalho, Aracava, and Vitolo (2003) reported that maximum productivity and yield was attained at the first and second batches and in the subsequent cycles drop in these values was observed with a fermentation medium elaborated with commercial xylose and *C. guilliermondii* cells immobilized on porous glass. However, it could be hypothesized that decline in the xylitol production with subsequent cycles could possibly be due to reduction in the number of immobilized cells (*i.e.* lower inoculum level than the optimal concentration for optimal production) due to rupture or leakage of cells into the fermentation medium. The rupture of cells could be due to instability of calcium alginate beads in presence of phosphate of potassium ions present in the fermentation medium as has also been reported by Prabakaran and Hoti (2008). Even though in order to overcome cell leakage, if the biomass concentration (cell loading) per bead is increased concomitantly the nutrient/cell ratio will decrease which might adversely affect the process performance through an increase in cell leakage into the fermentation medium along the cycles.

3.7. Scale up for xylitol production in fermentor

Xylitol production was scaled up in a 10 L fermentor with a working volume of 5 L of corncob hemicellulosic hydrolysate which resulted in 11.89 g/L of xylitol with a yield of 0.58 g/g and the volumetric productivity of 0.283 g/L/h. The fermentation efficiency was 63.73% by the adapted strain of *C. tropicalis* (Fig. 3). More or less similar results were obtained as earlier observed during flask studies which thereby proves that the process could successfully be scaled up for xylitol production. It was also observed that two-phase aeration proves to be beneficial for xylitol production over one-phase aeration. Two-phase aeration process is widely acceptable in batch and continuous fermentation processes (Shue, Duan, Jou, Chen, & Chen, 2003). The phenomenon involved behind this process is that higher aeration rates leads to excess availability of oxygen which thereby causes NADH to be oxidized to NAD^+, and a high ratio of NAD^+/NADH leads to oxidation of xylitol to xylulose, which is further metabolized to cell material, and as a result, less xylitol and more cells are produced. Therefore, higher aeration rates at the initial stages are beneficial for the growth of the microorganism (Sampaio et al., 2004). While, at later stages of fermentation, lower aeration rates or under a limited oxygen supply, the electron transfer system is unable to reoxidize all of the produced NADH by respiration and/or fermentation; thus, the intracellular NADH level increases and the reaction of xylitol to xylulose decreases, and consequently xylitol accumulates (Zhang, Geng, Yao, Lu, & Li, 2012). Therefore, conditions of two-phase aeration were translated for corncob hemicellulosic hydrolysate (optimized and treated) for xylitol production in a 10 L fermentor. Similar has been observed by Ding and Xia (2006) and Zhang et al. (2012) wherein they reported that two-phase aeration is more effective over one-phase aeration process in xylitol production using hemicellulosic hydrolysate from Candida sp. ZUO4 and *C. athensensis* SB 18 respectively.

4. Conclusions

An optimized and treated hydrolysate resulted in (g/L): xylose 20.92, glucose 3.43, arabinose 2.13, acetic acid 0.35. A percent

reduction in furfural (100.0%), acetic acid (73.28%) and phenolic compounds (97.16%) was also observed. An efficient conversion of xylose to xylitol in original and concentrated hydrolysate was observed using adapted strain of *C. tropicalis*. The cells of *C. tropicalis* could successfully be immobilized and is efficient for xylitol production up to third cycle. Realizing, the importance of this high valued compound, as a sugar substitute, the process for xylitol production, will certainly be a boon to food and pharmaceutical industries which requires fermentative derived xylitol. This biobased process will lead to a paradigm shift of industries from chemical to biobased.

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