Microbial Production of Xylitol from Corn Cob Hydrolysate Using *Pichia* sp

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ABSTRACT

Xylitol (C₅H₁₂O₅) a five carbon sugar polyalcohol, is found in various fruits and some vegetables [28]. Because of its high sweetening power and no insulin requirement for its digestion, xylitol has recently become an attractive as an alternative sweetener for the treatment of diabetics [11]. It is anticariogenic prevents the formation of acids that attack the tooth enamel [15,24] and can be used as a sugar substitute in dietetic food for diabetics [18]. Xylitol can also be used in the treatment of osteoporosis since it considerably improves the biomechanical properties of the bones and prevents reduction both in their density and in their contents of minerals, calcium and phosphorus [16]. Moreover xylitol inhibits the growth of the bacterial species like *Streptococcus pneumoniae* and *Haemophilus influenzae*, which cause acute medium otitis, so it could be employed, instead of antibiotics, to combat this disease [9,26]. Owing to all these characteristics, xylitol is a feedstock of particular interest to the food odontological and pharmaceutical industries.

Introduction

Xylitol is currently produced on an industrial scale by a catalytic reduction (hydrogenation) of xylose obtained from wood sources such as white birches. However, alternative processes have been extensively explored because of the high production cost and environmental impact associated with the excessive utilization of natural wood sources [13]. Lignocellulosic materials are mainly composed of cellulose, hemicellulose and lignin. Hemicellulose is composed of linear and branched heteropolymers of L-arabinose, D-galactose, D-glucose, D-mannose and D-xylose. The composition of hemicellulose varies according to the plant species, for instance in wheat straw (32%), barley straw (32%), rice straw (25%), corn cobs (37%), sugarcane (22%) and eucalyptus wood (15-22%) [25]. The hemicellulose fraction can be hydrolyzed and used for fermentation of xylose, with a view of producing xylitol [4].

Microbial xylitol production from agricultural wastes containing hemicelluloses could be a possible candidate because it has the potential to realize cheaper production of xylitol with low environmental impact by the effective utilization of renewable resources such as agricultural wastes [20,6]. Among agricultural resources (wastes) corn cobs are...
regarded as a promising agricultural resource for microbial xylitol production because corn is widely cultivated and corn cobs are rich in hemicelluloses but are not effectively utilized. In microbial xylitol production from corn cobs the cobs are first hydrolysed to produce xylose from hemicelluloses by acid hydrolysis and the corn cob hydrolysate is then used as the medium for xylitol production by xylose utilizing organisms [17]. Therefore, to evaluate the feasibility of microbial xylitol production from corn cobs it is essential to examine the hydrolysis conditions of corn cobs and the xylitol production yield using the corn cob hydrolysate. The objective of the present study was to evaluate the bioconversion of xylose to xylitol by *Pichia* sp., in the corn cob hydrolysate.

**Materials and Methods**

**Raw material:**

Corn cobs were locally collected from pudukkottai, Tamil Nadu, India, dried in the sunlight, milled to give a particle size of approximately 1 mm thickness and used as a raw material in this study. Their average composition determined according to standard methodology [12] was 32% cellulose, 35% hemicelluloses, 20% lignin, 4% ash and 3.4% of acetyl groups (oven-dry basis).

**Preparation of Corn Cob Hydrolysate:**

100 g\(^{-1}\) corn cobs were hydrolyzed under various sulfuric acid concentrations (1%-4%) at different temperatures (121°C) and for different reaction times in an autoclave. After hydrolysis, the liquid fraction (corn cob hydrolysate) was filtered through Whatman No: 1 filter paper and the pH was raised to 9 with calcium oxide and decreased to pH 5.5 with sulfuric acid. The hydrolysate was concentrated under vacuum at 70°C to increase xylose concentration. After these treatments, the hydrolysate was mixed with 10% activated charcoal, agitated (200 rpm, 30°C, 1 h) and then filtered. The filtrate (corn cob hydrolysate) was used as fermentation medium for xylitol production.

**Microorganism and Culture Conditions:**

The yeast strain used in this study was isolated from a soil sample of agricultural fields, Pudukkottai, Tamil Nadu, India. The selected strain was identified as *Pichia* sp. according to taxonomic identification. Taxonomic identification was carried out by Department of Microbiology, JJ college of Arts and Science, Pudukkottai, Tamil Nadu, India. The strain was maintained on YPD agar containing (10 g\(^{-1}\) yeast extract, 20 g\(^{-1}\) bacteriological peptone, 20 g\(^{-1}\) glucose and 15 g\(^{-1}\) agar) at 30°C for 24 h, maintained at 4°C and subcultured at regular intervals.

**Inoculum Development:**

From the subculture, one loopful of yeast cells were inoculated into 30 ml test tubes containing 5 ml of preculture medium (30 g\(^{-1}\) xylose, 10 g\(^{-1}\) yeast extract, 20 g\(^{-1}\) peptone and pH 6.0) and cultivated at 30°C for 24 h on a rotary shaker (Orbitek, India) at 200 rpm. Inoculum (25 ml) was prepared in 100 ml Erlenmeyer flasks containing 30 g\(^{-1}\) xylose, 10 g\(^{-1}\) yeast extract, 20 g\(^{-1}\) peptone, 0.5 g\(^{-1}\) K\(_2\)HPO\(_4\), 0.5 g\(^{-1}\) KH\(_2\)PO\(_4\), 2 g\(^{-1}\) (NH\(_4\))\(_2\)SO\(_4\), 0.5 g\(^{-1}\) MgSO\(_4\).7H\(_2\)O and pH 6.0 and it was inoculated with 5 ml of preculture and incubated for 24 h on a rotary shaker (200 rpm) at 30°C. After 24 h, the flask culture was centrifuged and washed twice with distilled water and then used as inoculum for fermentation.

**Fermentation:**

In shaking flask batch fermentations were performed in 250 ml Erlenmeyer flask containing 100 ml of corn cob hydrolysate medium by adding 10 g\(^{-1}\) yeast extract, 20 g\(^{-1}\) peptone, 0.5 g\(^{-1}\) K\(_2\)HPO\(_4\), 0.5 g\(^{-1}\) KH\(_2\)PO\(_4\), 2 g\(^{-1}\) (NH\(_4\))\(_2\)SO\(_4\), 0.5 g\(^{-1}\) MgSO\(_4\).7H\(_2\)O and pH 6.0 and cultivated under aerobic condition on rotary-shaker at 200 rpm, 30°C for 96 hours. It was inoculated to a final concentration of 10\(^7\) cells/ml. The samples were withdrawn at regular intervals such as 24, 48, 72 and 96 h of incubation. The cell growth was determined spectrophotometrically (OD) at 600 nm. The periodically drawn samples were centrifuged at 12000 rpm for 10 mins and the supernatant was used for HPLC analysis for determining the xylose consumption and xylitol concentration in fermented broth.

**Analytical Procedures:**

Xylose and Xylitol concentrations were determined using High Pressure Liquid Chromatography (HPLC) with an Aminex HPX-87H, (Biorad, USA) carbohydrate column (300X7.8mm) at 45°C, using 5mM H\(_2\)SO\(_4\) as an eluent. A flow rate of 0.6 ml/min and a sample volume of 20µl were maintained. The eluate was monitored with Refractive Index (RI) detector. The peaks were identified and quantified by comparing with retention times of authentic standards (Xylose and Xylitol) [1]. The cell growth (Biomass) was determined by measuring optical density (OD) at 600 nm and correlated with dry weight (One OD unit = 1.55 g dry cell/l).
Optimization of Fermentation conditions:

Effect of Acid Concentration:

Different concentrations of sulphuric acid such as 1%, 2%, 3% and 4% were used for corncob hydrolysate preparation to determine the highest yield of xylose during acid hydrolysis. After pretreatment powdered corn cobs were hydrolysed under different temperature based on the acid concentration such as 1% H₂SO₄ concentration at 100°C for 30 mins, 2% at 110°C for 60 mins, 3% at 120°C for 90 mins, and 4% at 126°C for 120 mins in stainless steel pressure cooker.

Effect of Temperature:

The general method was repeated accordingly for the optimization of incubation temperature. The culture flasks were incubated at different temperature like 28°C, 33°C and 37°C, at 120rpm for 96 h. The culture supernatant was taken after 24, 48, 72 and 96 h of incubation and then biomass xylose consumption and xylitol concentrations were determined.

Effect of pH:

To determine the effect of pH, the fermentation media was prepared with various pH such as 5, 6, and 7. All flasks were inoculated and incubated at 30°C, 200 rpm for 96 h. The culture supernatant was taken after 24, 48, 72 and 96 h of incubation and and then biomass xylose consumption and xylitol concentrations were determined.

Effect of Agitation:

To determine the effect of agitation on the growth of yeast and xylitol production, the fermentation media were incubated at various shaking speeds such as 100, 150 and 200 rpm at 28°C for 96 h. The culture supernatant was taken after 24, 48, 72 and 96 h of incubation and and then biomass xylose consumption and xylitol concentrations were determined.

Results and Discussion

Batch fermentation of corncob hemicellulose hydrolysate by the yeast *Pichia sp* for xylitol production was performed using shaking flask experiments. Cell growth (OD), sugar utilization (xylose consumption) and xylitol concentration were monitored during fermentation.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Raw Material</th>
<th>Xylitol yield (gl⁻¹)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pichia sp.</em></td>
<td>Corn cobs</td>
<td>35</td>
<td>Our work</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>Corn cobs</td>
<td>15.0</td>
<td>Farooq Latif &amp; Mohamed Ibrahim, 2001</td>
</tr>
<tr>
<td><em>Candida sp</em></td>
<td>Corn cobs</td>
<td>70</td>
<td>Dominguuez et al., 1997</td>
</tr>
<tr>
<td><em>C. guilliermondi</em></td>
<td>Sugarcane bagasse</td>
<td>13.5</td>
<td>Alves et al., 1998</td>
</tr>
<tr>
<td><em>C. guilliermondi</em></td>
<td>Eucalyptus wood</td>
<td>8.5</td>
<td>Canettieri et al., 2001</td>
</tr>
<tr>
<td><em>D. hansenii</em></td>
<td>Corn cobs</td>
<td>64</td>
<td>Rivas et al., 2001</td>
</tr>
</tbody>
</table>

Effect of acid concentration on xylitol production:

The xylose concentration in the corncob hydrolysates resulting from the hydrolysis of 100 gl⁻¹ corn cobs under various sulphuric acid concentrations is shown in Figure.1. In the ranges of 1.0-3.0% sulphuric acid, the xylose concentration were approximately 40gl⁻¹ xylose and constant. The xylose yield was 0.25g xylose/g corn cobs. Since, hemicellulose account for approximately 35% of the dry corn cobs, the xylose yield for the hemicelluloses in the corn cobs becomes 0.71g xylose/g hemicelluloses (Kiyosi Tada et al., 2004). Naturally this is because the increase in sulphuric acid concentration enhanced the decomposition of lignin and the sugars released from the corn cobs during acid hydrolysis. The maximum xylose concentration of 25gl⁻¹ xylose was obtained at 2% sulphuric acid concentration. However the sugar concentration (xylose) dropped with a further increase in the acid concentration of 3-4%. Hence, microbial xylitol production was then performed using the hydrolysates obtained by 2% sulphuric acid treatment.

To determine the effect of pH, the fermentation media was prepared with various pH such as 5, 6, and 7. All flasks were inoculated and incubated at 30°C, 200 rpm for 96 h. The culture supernatant was taken after 24, 48, 72 and 96 h of incubation and and then biomass xylose consumption and xylitol concentrations were determined.

Effect of temperature on xylitol production:

Batch fermentation was performed at 4 different temperatures (28°C, 32°C, 36°C and 40°C) to determine the effect of temperature on xylitol production. The highest biomass 9.72g was obtained at the temperature 28°C followed by 8.3g at 32°C at 72 h. The growth was absent at 40°C. The maximum xylitol production (24.8 gl⁻¹, 21.2 gl⁻¹ and 18 gl⁻¹) was obtained at 28°C, 32°C and 36°C respectively (Fig.2 A,B,C). According to our results 28°C was an optimal temperature for xylitol production by *Pichia* sp from corncob hydrolysates. Cao et al., found that xylitol production by Candida sp.B-22 kept relatively constant in the temperature range 35–40°C and appreciably decreased over 45°C. A similar trend was observed for *D. hansenii* NRRL Y-7426 by Dominguez, et al., in the range 28–37°C, where 100 gl⁻¹ xylitol were produced from 130 gl⁻¹ xylose, while at 44°C xylitol production decreased down to 41.9 gl⁻¹. Barbosa, et al., observed for *C. guilliermondi* maximum xylitol formation (23gl⁻¹) and specific growth rate (0.78 h⁻¹) within 30–35°C and that both parameters decreased over 40°C.
Effect of pH on xylitol Production:

Batch fermentation was performed using 3 different pH values (4, 5 & 6) to determine the effect of pH on xylitol production (Fig.3A,B,C). The xylitol yield 30 g/l was obtained in pH 6 (Fig.3C). The highest biomass concentration 17.8 g/l was obtained after 72 hours in pH of 6. The xylose consumption rate was the same for all the pH value evaluated, it increased slightly when the pH was raised. The balance between xylitol production and cell growth indicates that the xylose is consumed at pH 5, 6 and 7. According to our results, 6 was an optimal pH value for xylitol production by Pichia sp from corncob hydrolysates. In previous reports [23] also attained very high xylitol concentration at a pH varying from 4 to 6. Vongsuvanlert and Tani (1989) reported that Candida boidinii grew best at initial pH of 6.5 but gave a maximum xylitol yield (0.13g/g) at pH 7.

Fig. 1: Effect of Acid Hydrolysis on Corncob Hydrolysate.

Fig. 2: Biomass, xylose consumption and xylitol production during batch fermentation of corncob hydrolysate using Pichia sp at different temperatures (A)28°C (B) 32°C (C) 36°C.
Effect of Agitation on xylitol Production:

The effect of agitation on xylitol production at different shaking speed (100, 150 and 200rpm) was performed (Fig.4A,B,C). The maximum xylitol production (35.2 g l⁻¹) was observed in flasks maintained at agitation 150rpm for 72 hours. The highest biomass (16.3g l⁻¹) was obtained at 150rpm (Fig.4B). The cell density and xylitol concentration increased with xylose consumption and remained constant. The xylose consumption was completely reduced after 72 hours. Similar observation has been reported for xylitol production from xylose by Candida guilliermondii [3]. They reported that maximum xylitol yield was observed at agitation speed of 150rpm. The xylitol yield sharply decreased with increasing agitation speed. This is probably due to oxygen mediated NADH consumption that lowers the level of NAD⁺, thus accelerating the further metabolism of xylose to xylitol [21].

The bioconversion of xylose into xylitol concerning our work and the work of other authors are in Table 1. A xylitol production of 35 g l⁻¹ was achieved in the present study, were lower than those obtained in corn cob hydrolysates using Candida sp (70 g l⁻¹) and D.hansenii (64 g l⁻¹). At the same time, the values obtained in our work were higher than those observed in corn cobs (15 g l⁻¹), sugarcane bagasse (13.5 g l⁻¹) and eucalyptus hydrolysates (8.5 g l⁻¹). It is difficult to compare the results obtained by different researchers since many variables influence the xylose to xylitol bioconversion in hemicellulosic hydrolysates. While a considerable high bioconversion yield could be achieved in the corn cob hydrolysate using a relatively low initial cell concentration (0.5 g l⁻¹), the poor bioconversions observed in the eucalyptus hydrolysate were attributed to a very high concentration of inhibiting hydrolysis by-products. As a whole, the present study points to the fact that the corn cobs hydrolysate is a potential raw material to be used as a source of xylitol for xylitol bioproduction using Pichia sp. The toxic compounds present in the hydrolysate can be efficiently removed by a simple detoxification strategy based on pH alteration and active charcoal adsorption. Instead of a complex chemically defined medium. Xylitol production from corn cob has some advantages such as the reduction in the cost of the medium, cheaper and easily available raw material and the ease of purification in the culture broth.
Fig. 4: Biomass, xylose consumption and xylitol production during batch fermentation of corn cob hydrolysate using Pichia sp at different agitations A) 100 rpm B) 150 rpm C) 200 rpm.

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References


