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Purification of Xylitol Obtained by Fermentation of Corncob Hydrolysates

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Hydrolysates obtained by autohydrolysis−posthydrolysis of corncobs were detoxified with charcoal, concentrated, supplemented with nutrients, and fermented with *Debaryomyces hansenii*. After biomass removal, the fermented media contained 0.1137 kg of nonvolatile components (NVC)/kg of liquor, which corresponded mainly to xylitol (0.6249 kg/kg of NVC) but also to minor amounts of inorganic components (measured as ashes), proteins, nonfermented sugars (xylose and arabinose), uronic acids, arabitol, and other nonvolatile components (ONVC). The media were subjected to further processing (sequential stages of adsorption, concentration, ethanol precipitation, concentration, and crystallization) to obtain food-grade xylitol. Adsorption experiments were carried out at various solid-to-liquor ratios. Under selected conditions (1 kg of charcoal/15 kg of liquors), the xylitol content increased to 0.6873 kg/kg of NVC, and almost total decoloration was achieved. The resulting liquor was concentrated by evaporation to increase its NVC content to 0.4032 kg/kg of liquor (corresponding to a xylitol concentration of 0.280 kg/kg of liquor), and ethanol was added to precipitate a part of the NVC (mainly proteins, but also uronic acids, ashes, and other nonvolatile compounds). Refined liquors (containing 0.7303 kg of xylitol/kg of NVC) were concentrated again, and ethanol was added (to reach 40−60% volume of the stream) to allow crystallization at −10 or −5 °C. Under selected conditions, 43.7% of xylitol contained in the initial fermentation broth was recovered in well-formed, homogeneous crystals, in which xylitol accounted for 98.9% of the total oven-dry weight. Material balances are presented for the whole processing scheme considered in this work.

KEYWORDS: Charcoal adsorption; crystallization; decoloration; ethanol precipitation; xylitol purification

INTRODUCTION

Xylitol is a pentitol with high sweetening power and anticariogenic properties and is suitable for diabetics, with technological and biological properties fostering its utilization in the food industry (*1*, *2*). Xylitol crystals are white, odorless, and hygroscopic, having a negative heat of solution (about −145 kJ/kg, causing a fresh feeling when it comes into contact with saliva), with a density of 1520 kg/m^3 and a melting point between 92 and 96 °C, exhibiting low solubility in methanol, ethanol, and 2-propanol and a high solubility in water (*3*).

Most of the xylitol produced in the world (≈30000 tons/year) is made in Finland. Recently, xylitol of Chinese origin was introduced in the market (*4*). Traditionally, xylitol has been produced from xylose by either chemical or biotechnological methods, although recently Roquette has developed a cereal-based process based on the bioconversion of dextrose into xylose and subsequent hydrogenation to xylitol. Xylose is produced by hydrolysis of the xylan contained in some lignocellulosic materials such as hardwoods or agricultural wastes and byproducts. For example, shells of peanuts have been cited as the starting feedstock for xylitol production in China (*4*).

Corncobs (the substrate utilized in this work) are potentially useful as raw materials for obtaining added-value products (*5*). The worldwide production of cereals in 2005 was 2.23 × 10^9 tons. Considering that corn represented ≈31% of this amount (*6*), the annual generation of corncobs can be estimated as >695 MM tons/year.

Xylose can be produced from corncobs by autohydrolysis−posthydrolysis. Autohydrolysis (in aqueous media) leads to hemicellulose solubilization, allowing its separation from cellulose and lignin. Sugar oligomers, monosaccharides, and acetic acid are the main autohydrolysis reaction products. The mild acidic reaction conditions result in liquors with reduced contents of undesired byproducts (particularly those from sugar decomposition and acid-soluble lignin), causing inhibition in subsequent fermentation steps. As the sugar oligomers produced in autohydrolysis cannot be assimilated by yeasts, they must be converted into sugars by a posthydrolysis stage catalyzed by acids or enzymes to produce suitable fermentation media. The lower cost, simplicity, and faster kinetics of the acid-catalyzed posthydrolysis make it preferable over enzymatic hydrolysis.

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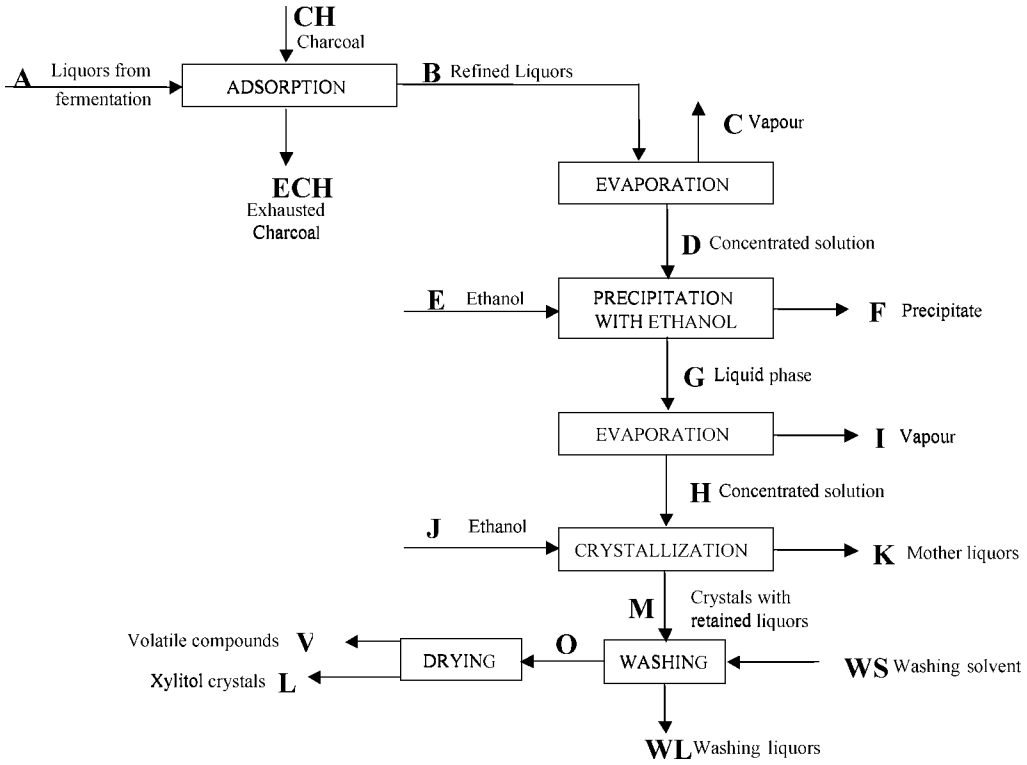

Figure 1. Scheme of the process considered in this work for xylitol purification.

Acid posthydrolysis of autohydrolysis liquors leads to xylose-rich solutions with small amounts of other sugars such as arabinose and glucose, with minimal decomposition of sugars into furfural and hydroxymethylfurfural, and limited amounts of products derived from acid-soluble lignin (*7*).

Xylose-containing hydrolysates can be used as fermentation media for xylitol production with *Debaryomyces hansenii* (*7*). Physicochemical refining (*8*−*11*) and/or biological detoxification (*12*−*14*) may be required to improve the bioconversion. The biotechnological production of xylitol from a variety of xylan-containing feedstocks (including corncobs) has been studied in recent years (*15*−*23*).

Xylitol purification from fermented broths is difficult because of the low product concentration and the complex composition of the media (*24*). Even though crystallization has been employed to recover xylitol from fermentation media formulated with commercial xylose, the complex composition of fermented hydrolysates does not allow a direct crystallization, requiring previous purification steps such as adsorption on activated charcoal and ethanol precipitation (*3*, *25*).

This work deals with the purification and recovery of xylitol present in the fermentation media resulting from the bioconversion of xylose-containing solutions produced by autohydrolysis−posthydrolysis of corncobs. The multistage process considered for this purpose (see **Figure 1**) includes sequential stages of adsorption, concentration, ethanol precipitation, concentration, and crystallization. The effects of the charcoal to liquor mass ratio in the adsorption step were assessed in selected experiments, as were the effects of the most influential variables of crystallization (proportion of ethanol in the media and temperature) on the amounts and properties of the resulting phases.

MATERIALS AND METHODS

Xylitol Manufacture. Corncobs locally collected were assayed for composition according to the TAPPI T13m method. The feedstock contained 31.7% cellulose, 34.7% hemicellulose (without acetyl groups), 20.3% lignin, and 3.4% acetyl groups (oven-dry basis).

Corncob samples were subjected to non-isothermal autohydrolysis in a Parr reactor at a water to solid ratio of 8 kg/kg to reach a maximum temperature of 202 °C, conditions reported as optimal for oligosaccharide production (*26*). After solid removal, sulfuric acid was added to autohydrolysis liquors to reach 0.5 wt % of the solution, and a postyhydrolysis step was carried out at 125 °C for 165 min to cleave the xylooligosaccharides into sugars (*7*). Liquors were neutralized with $CaCO_3$ to pH 6.5, and the precipitate was removed by vacuum filtration. Hydrolysates were detoxified by charcoal adsorption using 10 g of hydrolysate/g of charcoal (obtained from Carlo Erba, Milan, Italy) at room temperature under magnetic stirring for 1 h. The liquid phase was recovered by vacuum filtration. Vacuum evaporation of liquors was carried out at 30−40 °C to increase xylose concentration to 100 g/L without causing sugar degradation, supplemented with nutrients (*5*), and sterilized by filtration through 0.2 μm pore diameter membranes (Nalgene, Rochester, NY). Media were fermented with *D. hansenii* NRRL Y-7426 in 250 mL Erlenmeyer flasks containing 50 mL of medium at 30 °C with orbital agitation at 200 rpm. Microbial biomass was removed from the fermented hydrolysate by centrifugation.

Analysis of Liquors. Samples of liquors were filtered through 0.20 μm membranes and assayed for glucose, arabinose, arabitol, xylose, xylitol, acetic acid, and ethanol by high-performance liquid chromatography (using an HPLC Agilent, model 1100, Palo Alto, CA) fitted with a RI detector. Separation was achieved using a Supelcogel H59304-U column (Sigma Aldrich Corp., Bellefonte, PA) eluted with 0.005 M H_2SO_4 at a flow rate of 0.5 mL/min at 50 °C. The content of nonvolatile compounds (NVCs) of the broth was measured by oven-drying until constant weight. All determinations were made in triplicate. Uronic acids were determined according to the method of Blumenkrantz and Asboe-Hansen (*27*) using galacturonic acid as a standard for quantification. The protein content was estimated spectrophotometrically according to the Lowry method (*28*), and the ash content was determined by using the ISO 776 method. Metal ions were assayed for by atomic absorption spectrometry using a 220 Fast Sequential instrument (Varian, Palo Alto, CA). For color determination, samples were filtered through a 0.45 μm membrane, pH was adjusted to 7.0 ± 0.1, and transmittances were measured at 420 and 560 nm, using a UV−vis spectrophotometer (Perkin-Elmer model Lambda 25, Wellesley, MA). The International Commission for Uniform Methods of Sugar Analysis (ICUMSA) 4 color (*29*) was calculated by using the

equation

$$\text{ICUMSA } 4 = 1000(-\text{Log } T)/(bC)$$

where T is the transmittance, C is the concentration of solids (g/mL), and b is the path length of light (cm) (*29*).

Charcoal Adsorption of Fermented Liquors. Samples of the fermentation media were treated with charcoal at liquid to solid mass ratios in the range of 5−125 kg/kg for medium purification.

Concentration and Ethanol Precipitation of the Clarified Liquors. Liquors from the adsorption step were concentrated by vacuum evaporation at 30−40 °C to reach a xylitol concentration of 0.280 kg/kg of liquor. The resulting solution was mixed with ethanol at a volume ratio of 4 L of solvent/L of liquor, stirred, and kept for 1 h at 4 °C. The precipitate was separated by centrifugation (4000 rpm, 5 min), weighed, and redissolved in water. Separate aliquots from this solution were assayed for composition using the same methods employed for liquors. Assays were run in quadruplicate, and both average values and standard deviations (SD) are presented.

Crystallization. The liquid phase from the above treatments was vacuum-concentrated at 30−40 °C and mixed with the desired amount of ethanol (see below). Crystallization tests were performed for 72 h under slight agitation (40 rpm) at the desired temperatures (see below) in an ethylene glycol bath (Julabo, model F25, Seelbach, Germany). Finely ground commercial xylitol was added (up to 1.0 g/L) to improve the formation of xylitol crystals, which were separated by vacuum filtration and washed. Mother liquors from crystallization and xylitol crystals (after redissolution) were analyzed using the methods described above.

RESULTS AND DISCUSSION

Characterization of Liquors. In a previous work (*7*), xylose solutions were obtained by autohydrolysis−posthydrolysis of corncobs, detoxified, concentrated, supplemented with nutrients, and fermented by the yeast *D. hansenii* NRRL Y-7426. The fermented broth was filtered to remove biomass, giving a solution (stream A in **Figure 1**) containing volatile compounds (VC, including solvent and other volatile compounds generated in the chemical processing of the raw material) and nonvolatile compounds (NVC, mainly made up of xylitol). NVC also included unfermented sugars (xylose and arabinose), arabitol generated in fermentation, proteins, inorganic compounds (measured as ashes), and others (denoted ONVC, which corresponded mainly to acid-soluble lignin, uronic acid, and extractives). Calculations are referred to 100 kg of stream A, the properties (composition and ICUMSA 4 color) of which are given in **Table 1**. Ashes, mainly from the neutralization of posthydrolysis liquors, contained Ca^{2+} (0.4020 kg/kg of ashes), K^+ (0.2060 kg/kg of ashes), Mg^{2+} (0.02860 kg/kg of ashes), and Na^+ (0.02280 kg/kg of ashes). As the target compound in this study (xylitol) is nonvolatile under the drying conditions employed in this work, and considering that the VC fraction can be directly removed by oven-drying under the same conditions, our attention was focused on the processing of the NVC fraction to increase its xylitol content. Becasue food-grade xylitol should contain no less than 98.5 wt % (dry solid basis) of the substance xylitol ($C_5H_{12}O_5$) [according to the Food Chemicals Codex (1992), The United States Pharmacopeia (1990), and the European Guideline 95/31/EC], the objective of this work was to remove NVC components of stream A different from xylitol to achieve a final product fitting this specification.

Charcoal Adsorption. Gurgel et al. (*24*) reported that the presence of colored substances in a viscous solution is a factor that could interfere negatively in the separation of the crystals by centrifugation or filtration. Separation of colored substances present in fermented media was attempted by charcoal adsorption (see **Figure 1**).

Table 1. Properties of Stream A in **Figure 1**

(A) NVC Content of Stream A
0.1137 kg of NVC/kg of A

(B) Composition of NVC in Stream A

component	mass fraction (kg/kg of NVC)	SD
xylitol	0.6249	0.0002
xylose	0.0167	0.0002
arabinose	0.0653	0.0003
arabitol	0.0225	0.0053
proteins	0.0460	0.0001
uronic acids	0.0192	0.0005
ashes	0.1264	0.0033
ONVC (by difference)	0.0790	0.0001

(C) ICUMSA Color

4869	34.1

Preliminary adsorption assays were performed to assess the effects of the charcoal/liquor mass ratio (in the range from 1/125 up to 1/5 kg/kg) on the amount, composition, and color of liquors (see **Table 2**). For calculation purposes, considering that the liquors retained by charcoal could be recovered by washing and recycled to the autohydrolysis unit, and to provide a better estimate of the yields that could be reached in an integrated, industrial process, the amount of liquor leaving the adsorption stage (stream B in **Figure 1**) was calculated as the difference between the feed (stream A in **Figure 1**) and the NVC retained by charcoal. Adsorption was suitable for removing proteins, uronic acids, and ONVC. Data in **Table 2** showed that enhanced purification effects were achieved by operating at the highest charcoal/liquor mass ratios assayed. In the experiment performed with 1 kg of charcoal/125 kg of liquor, the xylitol content of the processed stream (0.6395 kg/kg of NVC) did not vary significantly with respect to the one of the feed stream, the most important effects being the removal of ICUMSA 4 color and proteins. The contents of sugars and polyols decreased slightly when adsorption was carried out with 1/125−1/15 kg of charcoal/kg of liquor, but their proportions decreased sharply when using 1/5 kg of charcoal/kg of liquor. Under these conditions, the xylitol content increased to 0.7030 kg/kg of NVC, proteins were extensively removed (94.0 wt % of the initial amount), and other purification effects took place (including 38.5% removal of uronic acids, 30.4% removal of xylose, 99.8% removal of ONVC, and 98.6% removal of ICUMSA 4 color). However, the xylitol loss (19.5% of the initial amount) was too high for practical purposes.

On the basis of the above considerations, an intermediate charcoal charge (1 kg/15 kg of liquor) was selected for further operation. Adsorption under these conditions led to stream B in **Figure 1**, the compositional data of which are shown in **Table 3**.

Remarkable purification effects were achieved (81.9% removal of proteins, 66.7% removal of ONVC, and 98.8% removal of ICUMSA 4 color), keeping the xylitol loss at an acceptable level (3.2% of the initial amount) and increasing the xylitol mass fraction to 0.6873 kg/kg of NVC. Therefore, further refining of stream B was needed to achieve the desired purity level. As almost total decoloration took place under the selected conditions, the ICUMSA 4 color was not measured in the products of the further process stages.

Evaporation and Ethanol Precipitation. Stream B in **Figure 1** was concentrated by vacuum evaporation to obtain a concen-

Table 2. Effects of the Charcoal/Liquor Mass Ratio on the Amount of Liquor, NVC Content, and NVC Composition

(A) Amount, Composition, and Color of Streams from the Charcoal Adsorption Stage

	charcoal to liquor mass ratio					
	1/125	1/90	1/60	1/30	1/15	1/5
kg of stream[a]	99.86	99.93	99.65	99.13	98.64	96.76
kg of NVC/kg of stream	0.1124	0.1131	0.1106	0.1059	0.1014	0.0840
ICUMSA color	1499	1341	978	483	59	67

(B) Composition of NVC Present in Streams from the Charcoal Adsorption Stage

	mass fraction (kg of component/kg of NVC) and SD											
	1/125		1/90		1/60		1/30		1/15		1/5	
	mass frac	SD	mass frac	SD	mass frac	SD	mass frac	SD	mass frac	SD	mass frac	SD
xylitol	0.6395	0.0004	0.6347	0.0008	0.6431	0.0005	0.6544	0.0006	0.6873	0.0005	0.7030	0.0006
xylose	0.0160	0.0008	0.0165	0.0005	0.0162	0.0006	0.0163	0.0007	0.0176	0.0006	0.0163	0.0007
arabinose	0.0651	0.0003	0.0636	0.0002	0.0649	0.0002	0.0656	0.0002	0.0702	0.0002	0.0732	0.0002
arabitol	0.0219	0.0013	0.0231	0.0014	0.0218	0.0017	0.0231	0.0018	0.0241	0.0019	0.0248	0.0023
proteins	0.0324	0.0011	0.0301	0.0010	0.0271	0.0012	0.0207	0.0013	0.0094	0.0013	0.0039	0.0016
uronic acids	0.0190	0.0006	0.0191	0.0008	0.0190	0.0007	0.0196	0.0008	0.0186	0.0008	0.0165	0.0010
ashes	0.1282	0.0008	0.1270	0.0009	0.1296	0.0011	0.1353	0.0012	0.1427	0.0012	0.1722	0.0015
ONVC (by difference)	0.0779	0.0011	0.0858	0.0013	0.0784	0.0015	0.0650	0.0013	0.0299	0.0007	0.0003	0.0000

[a] Values calculated by assuming total recovery of NVC present in liquors retained by charcoal (see text).

Table 3. Properties of Streams B, D, F, and G in **Figure 1**

(A) NVC Content and Amounts of Considered Streams

	stream B	stream D	stream F[a]	stream G
kg of stream	98.64	24.79	0.99	90.78
kg of NVC/kg of stream	0.1014	0.4036	1.0000	0.0993

(B) Composition of NVC Present in the Considered Streams

	stream B		stream D		stream F		stream G	
	mass frac (kg/kg of NVC)	SD	mass frac (kg/kg of NVC)	SD	mass frac (kg/kg of NVC)	SD	mass frac (kg/kg of NVC)	SD
xylitol	0.6873	0.0027	0.6968	0.0081	0.3878	0.0191	0.7303	0.0062
xylose	0.0176	0.0007	0.0177	0.0010	0.0185	0.0013	0.0176	0.0011
arabinose	0.0702	0.0009	0.0700	0.0025	0.0533	0.0034	0.0708	0.0024
arabitol	0.0241	0.0003	0.0265	0.0014	0.0261	0.0017	0.0254	0.0016
proteins	0.0094	0.0019	0.0076	0.0005	0.0212	0.0019	0.0057	0.0008
uronic acids	0.0186	0.0011	0.0177	0.0006	0.0766	0.0076	0.0104	0.0006
ashes	0.1427	0.0017	0.1439	0.0019	0.3468	0.0049	0.1229	0.0023
ONVC	0.0299	0.0037	0.0196	0.0066	0.0697	0.0331	0.0169	0.0045

[a] Data corresponding to the NVC fraction of stream F.

trated liquor (stream D in **Figure 1**) with a NVC content of 0.4032 kg/kg. The degree of concentration achieved in the evaporation stage corresponded to a compromise between high xylitol concentration, on the one hand, and ease of experimental handling and limited solvent utilization, on the other. The compositional data in **Table 3** show that no significant degradation effects occurred during concentration.

Preliminary solvent precipitation experiments were performed with methanol, ethanol, propanol, butanol, hexane, heptane, and acetone (data not shown) to explore their comparative ability for purification. These solvents were selected from literature information (*14*). On the basis of the experimental results, and considering that ethanol is considered to be a safe solvent by the U.S. Food and Drug Administration, this solvent was selected for further experimentation.

In the precipitation step, ethanol (stream E in **Figure 1**) was added to liquors at a volume ratio (4 L/L) selected on the basis of previous experimental results. The precipitate (stream F in **Figure 1**) contained 1.0 kg of NVC and volatile compounds. As before, the precipitate was considered to be exclusively made of NVC for calculation purposes (no retained mother liquors were considered), to provide a better estimate of an integrated, industrial process.The refined liquid phase is denoted stream G in **Table 1**. **Table 3** lists experimental data on the properties of streams F and G. The xylitol present in stream G accounted for 92.6% of the amount present in stream A. The small xylitol loss could be due to the formation of some hydrogen bonds between ethanol and xylitol (*30*). The xylitol content of stream G increased to 0.7303 kg/kg of NVC owing to the removal of proteins (45.8%), uronic acids (49.8%), ashes (22.4%), and ONVC (49.0%). As the purity of the product was below the target value, additional processing steps were performed.

Table 4. Properties of Stream H in **Figure 1**

Amount and Composition of Stream H Concentrated at Various Levels (Streams H1–H3)			
stream	H1	H2	H3
mass ratio J/H	0.1883	0.2582	0.3437
kg of stream	13.85	13.23	12.42
kg of NVC/kg of stream	0.6515	0.6818	0.7263

Table 5. Properties of Streams K and L in **Figure 1**

(A) Operational Conditions Leading to Streams K1–K6 and L1–L6

T (°C)	–5	–5	–5	–10	–10	–10
% ethanol (v/v)	40	50	60	40	50	60
stream K	K1	K2	K3	K4	K5	K6
stream L	L1	L2	L3	L4	L5	L6

(B) Amount and NVC Content of Streams K1–K6

stream	K1	K2	K3	K4	K5	K6
kg of stream	15.41	14.46	13.55	14.22	13.66	13.50
kg of NVC/kg of stream	0.5176	0.4725	0.4341	0.4757	0.4565	0.4523

(C) Composition of NVC Present in Streams K1–K6 (Expressed as Mass Fractions, kg/kg of NVC)

	K1		K2		K3		K4		K5		K6	
	mass frac	SD	mass frac	SD	mass frac	SD	mass frac	SD	mass frac	SD	mass frac	SD
xylitol	0.6972	0.0022	0.6491	0.0014	0.5920	0.0011	0.6451	0.0015	0.6155	0.0015	0.6076	0.0011
xylose	0.0196	0.0003	0.0223	0.0004	0.0264	0.0002	0.0225	0.0002	0.0242	0.0004	0.0246	0.0003
arabinose	0.0795	0.0005	0.0921	0.0003	0.1071	0.0003	0.0930	0.0005	0.1004	0.0005	0.1025	0.0002
arabitol	0.0283	0.0002	0.0326	0.0004	0.0373	0.0004	0.0328	0.0002	0.0353	0.0003	0.0361	0.0005
proteins	0.0060	0.0001	0.0065	0.0000	0.0079	0.0000	0.0065	0.0001	0.0068	0.0000	0.0069	0.0001
uronic acids	0.0115	0.0003	0.0131	0.0002	0.0155	0.0003	0.0132	0.0001	0.0141	0.0003	0.0144	0.0001
ashes	0.1390	0.0014	0.1623	0.0016	0.1885	0.0017	0.1639	0.0013	0.1778	0.0017	0.1816	0.0012
ONVC	0.0190	0.0006	0.0220	0.0009	0.0252	0.0009	0.0229	0.0006	0.0259	0.0009	0.0262	0.0006

(D) Amount and Xylitol Content of Streams L1–L6

stream	L1	L2	L3	L4	L5	L6
kg of stream	1.05	2.19	3.14	2.26	2.78	2.92
kg of xylitol/kg of NVC	0.9826	0.9835	0.9894	0.9856	0.9876	0.9871

(E) Xylitol Recovery in the Crystallization Stage and in the Whole Process Operating under the Crystallization Conditions Leading to Streams L1–L6

stream	L1	L2	L3	L4	L5	L6
crystallization yield	0.1562	0.3269	0.4714	0.3378	0.4173	0.4370
recovery yield	0.1448	0.3032	0.4372	0.3132	0.3869	0.4052

Concentration and Crystallization. Stream G was concentrated by vacuum evaporation, to give the concentrated stream H in **Figure 1**. Three different concentration degrees were considered (streams H1−H3 in **Table 4**), to obtain the same xylitol content in the crystallization media (0.400 kg/kg) when different amounts of ethanol were added. Stream H3 corresponds to an upper limit for concentration, because handling problems occurred when this threshold was exceeded.

Crystallization was performed by cooling (*31*) in the presence of a solvent suitable for both reducing the xylitol solubility (*25*) and improving the purity of crystals by keeping impurities in the mother liquor (*32*, *33*). Xylitol has a high solubility in water, but it is scarcely soluble in methanol, ethanol, and 2-propanol. Among these solvents, ethanol was selected for the reasons already indicated in the precipitation stage. According to the operational procedure shown in **Figure 1**, ethanol can be recovered and recycled, minimizing the operational costs and the environmental impact.

Crystallization was carried out at −10 or −5 °C starting from the three concentrated solutions (H1−H3), to which ethanol was added to obtain volume ratios ethanol/VC of 0.4, 0.5, or 0.6 L/L by keeping the xylitol content at the desired level of 0.400 kg/kg of stream (see **Table 4**). Six sets of mother liquors (streams K1−K6) and xylitol crystals (L1−L6) were obtained with this experimental plan. **Table 5** lists the results assessing the effects of ethanol concentration and crystallization temperature on the amounts and properties of the involved streams. Ethanol addition decreased the viscosity of the medium, making the crystallization easier. As expected, higher ethanol concentrations resulted in decreased xylitol concentrations in the mother liquors. In the experiments performed in media containing 60% ethanol, a reduction in temperature from −5 to −10 °C resulted in a small variation in the xylitol content of the mother liquors, with handling problems caused by increased viscosity and low crystallization rate.

Data on the crystallization yield (defined as the ratio between the amounts of xylitol in streams L1−L6 with respect to the amount of xylitol in stream H) and on the recovery yield (defined as the ratio between the amounts of in streams L1−L6 with respect to the amount of xylitol present in stream A) are listed in **Table 5**. Operating at −5 °C, increased ethanol contents in the crystallization media resulted in increased crystallization yields (from 0.1562 to to 0.4710 kg/kg obtained in the experiment corresponding to the medium with 60% ethanol). When the temperature was decreased to −10 °C, crystallization yields were improved significantly in media containing 40 and 50% ethanol, but not in the experiment containing 60% ethanol. On the basis of these findings, the preferred crystallization conditions corresponded to a temperature of −5 °C and a volumetric ethanol concentration of 60%. Under these conditions, stream K retained quantitatively the ashes contained in stream H, as well as 97.3% of uronic acids, 91.0% of proteins, 98.7% of arabinose, 97.8% of xylose, and 97.2% of ONVC contained in the same stream. Crystallization led to regularly shaped, well-formed, homogeneous crystals containing 98.9 wt % of xylitol, exceeding the purity threshold required for food applications. It can be noted that the xylitol yield achieved (0.4710 g/g) corresponds to a single crystallization stage and that this result could be improved by further processing of mother liquors, for example, by performing successive crystallization steps (*3*) or recycling a part of the stream.

LITERATURE CITED

(1) Emodi, A. Xylitol. Its properties and food applications. *Food Technol.* **1978**, *1*, 28−32.

(2) Pepper, T.; Olinger, P. M. Xylitol in sugar-free confections. *Food Technol.* **1988**, *42*, 98−106.

(3) Heikkilae, H.; Nygren, J.; Sarkki, M. L.; Gros, H.; Eroma, O. P.; Pearson, J.; Pepper, T. Crystallization of xylitol, crystalline xylitol product and use thereof. WO Patent 9959426, 1999.

(4) Tran, L. H.; Yogo, M.; Ojima, H.; Idota, O.; Kawai, K.; Suzuki, T.; Takamizawa, K. The production of xylitol by enzymatic hydrolysis of agricultural wastes. *Biotechnol. Bioprocess Eng*. **2004**, *9* (3), 223−228.

(5) Rivas, B.; Torres, P.; Domínguez, J. M.; Perego, P.; Converti, A.; Parajó, J. C. Carbon material and bioenergetic balances of xylitol production from corncobs by *Debaryomyces hansenii*. *Biotechnol. Prog*. **2003**, *19*, 706−713.

(6) FAO. *FAO Production Yearbook*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2005.

(7) Rivas, B.; Domínguez, J. M.; Domínguez, H.; Parajó, J. C. Bioconversion of post-hydrolyzed autohydrolysis liquors: an alternative for xylitol production from corncobs. *Enzyme Microb. Technol.* **2002**, *31*, 431−438.

(8) Parajó, J. C.; Domínguez, H.; Domínguez, J. M. Production of xylitol from concentrated wood hydrolysates by *Debaryomyces hansenii*: effect of the initial cell concentration. *Biotechnol. Lett.* **1996**, *18*, 593−598.

(9) Parajó, J. C.; Domínguez, H.; Domínguez, J. M. Improved xylitol production with *Debaryomyces hansenii* Y-7426 from raw or detoxified wood hydrolyzates. *Enzyme Microb. Technol.* **1997**, *21*, 18−24.

(10) Cruz, J. M.; Domínguez, J. M.; Domínguez, H.; Parajó, J. C. Solvent extraction of hemicellulosic wood hydrolysates: a procedure useful for obtaining both detoxified fermentation media and polyphenols with antioxidant activity. *Food Chem.* **1999**, *67*, 147−153.

(11) Larsson, S.; Reimann, A.; Nilvebrant, N. O.; Jönsson, L. F. Comparison of different methods for the detoxification of lignocellulose hydrolyzates of spruce. *Appl. Biochem. Biotechnol.* **1999**, *77*−*79*, 91−103.

(12) Palmqvist, E.; Hahn-Hägerdal, B.; Szengyel, Z.; Zacchi, G.; Rèczey, K. Simultaneous detoxification and enzyme production of hemicellulose hydrolysates obtained after steam pretreatment. *Enzyme Microb. Technol.* **1997**, *20*, 286−293.

(13) Jönsson, L. J.; Palmqvist, E.; Nilvebrandt, N. O.; Hahn-Hägeldal, B. Detoxification of wood hydrolysates with laccase and peroxidase from the white-rot fungus *Trametes versicolor*. *Appl. Microbiol. Biotechnol*. **1998**, *49*, 691−697.

(14) Parajó, J. C.; Domínguez, H.; Domínguez, J. M. Xylitol production from *Eucalyptus* wood hydrolyzates extracted with organic solvents. *Process Biochem.* **1997**, *7*, 599−604.

(15) Mayerhoff, Z. D. V. L.; Roberto, I. C.; Silva, S. S. Production of xylitol by *Candida mogii* from rice straw hydrolyzate: study of environmental effects using statistical design. *Appl. Biochem. Biotechnol.* **1998**, *70*−*72*, 149−159.

(16) Kim, S. Y.; Oh, D. K.; Kim, J. H. Evaluation of xylitol production from corn cob hemicellulose hydrolyzate by *Candida parapsilosis*. *Biotechnol. Lett.* **1999**, *21*, 891−895.

(17) Cruz, J. M.; Domínguez, J. M.; Domínguez, H.; Parajó, J. C. Xylitol production from barley bran hydrolyzates by continuous fermentation with *Debaryomyces hansenii*. *Biotechnol. Lett.* **2000**, *22*, 1895−1898.

(18) Preziosi-Belloy, L.; Nolleau, V.; Navarro, J. M. Xylitol production from aspenwood hemicellulose hydrolyzate by *Candida guilliermondii*. *Biotechnol. Lett.* **2000**, *22*, 239−243.

(19) Allam, R. F. Microbial production of xylitol from acid treated corn cobs. *Pakistan J. Sci. Ind. Res.* **2003**, *46* (6), 465−470.

(20) El-Batal, A. I.; Khalaf, S. A. Xylitol production from corn cobs hemicellulosic hydrolysate by *Candida tropicalis* immobilized cells in hydrogel copolymer carrier. *Int. J. Agric. Biol.* **2004**, *6* (6), 1066−1073.

(21) Tada, K.; Horiuchi, J. I.; Kanno, T.; Kobayashi, M. Microbial xylitol production from corn cobs using *Candida magnoliae*. *J. Biosci. Bioeng.* **2004**, *98* (3), 228−230.

(22) Fogel, R.; García, R.; Oliveira, R.; Palacio, D.; Madeira, L.; Pereira, N. Optimization of acid hydrolysis of sugarcane bagasse and investigations on its fermentability for the production of xylitol by *Candida guilliermondii*. *Appl. Biochem. Biotechnol.* **2005**, *121*−*124*, 741−752.

(23) Mussatto, S. I.; Dragone, G.; Roberto, I. C. Kinetic behavior of *Candida guilliermondii* yeast during xylitol production from brewer's spent grain hemicellulosic hydrolysate. *Biotechnol. Prog.* **2005**, *21* (4), 1352−1356.

(24) Gurgel, P. V.; Mancilha, I. M.; Peçanha, R. P.; Siqueira, J. F. M. Xylitol recovery from fermented sugarcane bagasse hydrolyzate. *Bioresour. Technol.* **1995**, *52*, 219−223.

(25) Vyglazov, V. V. Kinetic characteristics of xylitol crystallization from aqueous-ethanolic solutions. *Russ. J. Appl. Chem.* **2004**, *77* (1), 26−29.

(26) Garrote, G.; Domínguez, H.; Parajó, J. C. Manufacture of xylose-based fermentation media from corncobs by posthydrolysis of autohydrolysis liquors. *Appl. Biochem. Biotechnol*. **2001**, *95*, 195−207.

(27) Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54* (2), 484−489.

(28) Lowry, O. H.; Rosebrough, N. J.; Farra, L.; Randall R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

(29) ICUMSA. *Method 4 Official for the Determination of Color of Sugar in Solution*; International Commission for Uniform Methods of Sugar Analysis; Verlag Bartens: Berlin, Germany, 1978; pp 343−344.

(30) Solomons, T. W. G.; Fryhle, C. In *Organic Chemistry*, 7th ed.; Wiley: New York, 1999; Vol. 1, pp 53−79.

(31) De Faveri, D.; Perego, P.; Converti, A.; Del Borghi, M. Xylitol recovery by crystallization from synthetic solutions and fermented hemicellulose hydrolyzates. *Chem. Eng. J.* **2002**, *90*, 291−298.

(32) Dominguez de Paz, G. Crystallization kinetics for the sugar-water-ethanol system in a continuous MSMPR crystallizer. *Int. Sugar J.* **2002**, *104* (1237), 14−20.

(33) Gabas, N.; Laguerie, C. Batch crystallization of D-xylose by programmed cooling or by programmed adding of ethanol. *Chem. Eng. Sci.* **1992**, *47* (12), 3148−3152.

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