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Strategies for Xylitol Purification and Crystallization: A Review

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This paper reviews the literature on the main aspects of purification and crystallization of xylitol produced either by chemical or biotechnological routes. Different strategies have been used to clarify media containing xylitol: activated charcoal, pH adjustment, ion-exchange resins, membrane separation, chromatographic methods, liquid-liquid extraction, and precipitation, or a combination of these techniques. This study explores the most recent results of research work carried out in this field as well as the main approaches to recover and crystallize xylitol in a pure form. The effects of impurities, temperature, supersaturation, crystal seed amount, and size on xylitol crystal growth are also discussed.

Keywords xylitol; chemical production; biotechnological production; purification; crystallization

INTRODUCTION

Xylitol (CH_2OH-CHOH-HOCH-CHOH-HCOH-CH_2OH) is a particular type of sugar alcohol, or polyol, which is used in food and pharmaceutical industries as a sucrose substitute for people affected by diabetes and obesity, to prevent ear infections in young children, and to reduce gingivitis, and control halitosis (1-3). It can be produced by chemical or biotechnological techniques using pure xylose or xylose-containing hemicellulosic hydrolyzates (4). Figure 1 shows a flow chart of processes employed for xylitol production either using chemical or biotechnological techniques.

Works dealing with obtaining and detoxification of hemicellulosic hydrolyzates as well as xylose-to xylitol bioconversion by fermentation were already reviewed (4-6), while Aliakbarian and co-workers (7) have recently provided an overview of xylitol recovery methods such as chromatographic techniques, membrane separation, and crystallization applied either to xylitol solutions obtained by chemical means or to fermented media.

Based on this background, after a brief presentation of xylitol physical and chemical properties, this paper reports an updated literature review of crystallization processes for xylitol recovery from solutions obtained by both ways, and a final discussion of future prospects.

PHYSICAL AND CHEMICAL PROPERTIES OF XYLITOL

Storing xylitol syrup in an icebox for a long period, Wolfrom and Kohn (8) were the first researchers who prepared crystalline xylitol in a metastable, hygroscopic, monoclinic form, with a melting point (MP) of 61-61.5°C. Recrystallization either using methanol or ethanol yielded stable colorless orthorhombic crystals with MP of 93-94.5°C (9), while Kim and Jeffrey (10) obtained two different orthorhombic morphologies (MP = 92-94°C), i.e., elongated needles from tetrahydrofuran or well-developed prisms from ethanol. An approximate melting heat of 38 $kJmol^{-1}$ was reported by several authors (11-13), with the lowest (36.1 $kJmol^{-1}$) and highest (39 $kJmol^{-1}$) values being reported by Nurmi and co-workers (14) and by Martínez and co-workers (15), respectively, while Carpentier and co-workers (12) and Tong and co-workers (16) reported melting entropy value for xylitol of 102 and 90.12 J mol^{-1} K^{-1}, respectively (Table 1). Tong and co-workers (16) reported the only values of combustion ($\Delta_c H°_m$ = -2463.2 ± 1.2 $kJmol^{-1}$) and formation ($\Delta_f H°_m$ -1219.3 ± 0.3 kJmol-1) standards molar enthalpy at 25°C. In addition, these authors reported a solid-liquid phase transition in the temperature range from 87 to 102°C with a peak heat capacity (26128 $Jmol^{-1}K^{-1}$) at 96°C.

Xylitol solubility depends on both the temperature and composition of the solvent mixture; solubility in water did in fact decrease from 85 to 50 g/100 g of solution when temperature was reduced from 60 to 0°C (333 to 273 K), and from 85 to 20 g/100 g of solution at 60°C (333 K) when ethanol concentration in the solvent mixture was increased from 0 to 95.3% (17). Similarly, all the refractive index, density, and viscosity decreased as

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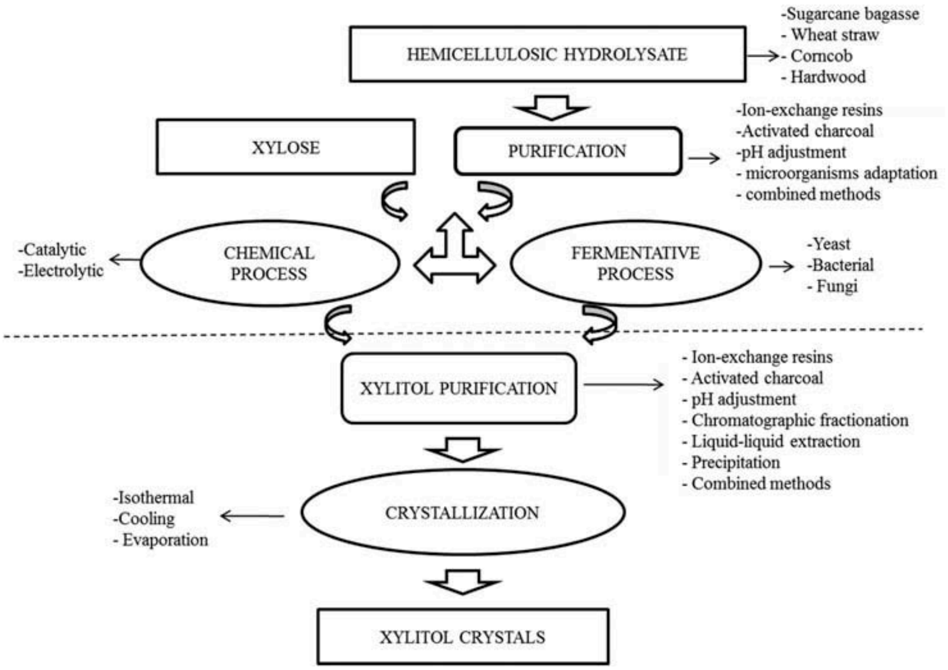

FIG. 1. Flow chart of processes employed for xylitol production either using chemical or biotechnological techniques.

TABLE 1
Thermal characteristics reported for melting of xylitol crystalline powders

Tm (°C)	ΔHm (kJ mol^{-1})	ΔSm (J mol^{-1} K^{-1})	References
61.0-61.5 (metastable) 93.0-94.5 (stable)	ND	ND	Wolfrom and Kohn (8); Carson and co-workers (9)
92-94	ND	ND	Kim and Jeffrey (10)
93-94	38.00	ND	Reamy and Scheweizer (11)
93.70	36.10	ND	Nurmi and co-workers (14)
94.84	37.70	102.00	Carpentier and co-workers (12)
96.07	33.26±0.17	90.12±0.45	Tong and co-workers (16)
93.60	39.49	ND	Martínez and co-workers (15)
91.6-92.3 (different additives)	37.42-38.18±0.76 (different additives)	ND	Seppälä and co-workers (13)

*Tm = melting temperature; ΔHm = melting heat; ΔSm = melting entropy. ND = not determined.

ethanol concentration increased from 0 to 95.3 g/100 g and saturation temperature decreased from 60 to 0°C.

CRYSTALLIZATION OF XYLITOL PRODUCED BY CHEMICAL ROUTES

Crystallization is widely used for xylitol recovery because it allows obtaining such a polyol in a pure form and solid consistency from relatively impure solutions by a single step. In energy terms, crystallization requires less energy than distillation or other commonly used purification methods.

Very soluble compounds like xylitol can be crystallized by many different alternative methods, among which are solvent evaporation that increases concentration, cooling that decreases solubility (18, 19), precipitation, or salting-out, whose choice is suggested by solubility and saturation patterns versus temperature. First, there is a metastable zone where excess solute at the equilibrium concentration is deposited on existing crystals, but no new crystal nucleus is formed. Secondly, there is an intermediate zone where both old and new crystals grow, and nucleation occurs simultaneously. Finally, there is a labile zone where nuclei are formed spontaneously from a clear solution. These three zones are controlled not only by equilibrium, but also by process parameters like agitation, temperature, solution purity, and cooling rate.

In order to produce pure crystalline solids in an efficient way, the cooling rate must be controlled so as to obtain a supersaturated solution and maintain it inside the metastable zone, thus minimizing the formation of new crystals (20), and to control the appearance of the crystal nuclei and crystal growth (21).

Treatment of birch wood hydrolyzate containing in 100 g of solution: 73.0 g. xylose, 6.1 g. arabinose, 9.0 g mannose, 5.1 g galactose, and 6.8 g glucose, using Raney-nickel as a catalyst at 135°C and 40 atm of hydrogen pressure for 2.5 h, allowed for hydrogenation of xylose and other carbohydrates to their respective polyols, and approximately 60% of the original xylan material was recovered as xylitol (22). Subsequent crystallization and separation of the uncrystallized fraction by liquid chromatography in a cross-linked sulfonated polystyrene cation exchange resin, allowed obtaining eluted fractions containing 0.1-1.95 g arabitol, 0.45-1.70 g mannitol, 0.1-1.0 g galactitol, 0.2-0.85 g sorbitol, and 0.85-14.3 g xylitol. The fractions with very high xylitol concentrations were recrystallized, and xylitol was recovered with a maximum yield of 60% and purity higher than 99.5%. Munir and Schiweck (23) proposed the use of a highly acidic, weakly cross-linked divinylbenzene cation exchange resin in calcium form to separate xylitol from centrifuged mother liquor. According to their patent, xylitol readily crystallized and was easily separated with a wire basket, allowing for the recovery of more than 34% of xylitol present in the original mother liquor.

Vourinen (24) patented a three steps-chemical production of xylitol from D-glucose, and mixtures of D-glucose with D-fructose or D-galactose. D-glucose (1050 g) underwent preliminary oxidative cleavage with pressurized oxygen in autoclave at 85°C with an aqueous solution containing sodium hydroxide (18 g), water (264 g), methanol (100 g), and sodium arabinonate (16 g). After a conversion degree of 98% glucose, formed sodium arabinonate crystals (741 g) were recovered by centrifugation, dissolved in water, and passed through an acid cation exchange resin. The resulting arabinonic acid was transformed into γ lactone by concentrating in vacuum the solution when butanol is added and crystallized to successively give crystals of D-arabino-1,4-lactone, which was then reduced to arabinitol by a ruthenium-on-carbon catalyst with a 90% molar yield. After crystallization, a second reaction of hydrogenation allowed the arabinitol crystals to be converted to xylitol (90% molar yield) using the same catalyst. However, the procedure appears to be too costly for large-scale production.

Xylitol crystallization yield and crystal purity using synthetic media in the main studies published since 1990 are reported in Table 2.

Because crystallization of xylitol from saturated solutions is moisture sensitive, chewing gum processing, in which xylitol is routinely used as a sweetener, is difficult to manage. DuRoss (25) used moisture-resistant melt-crystallized xylitol to produce an agglomerated crystal structure with 99.5% (w/w) xylitol. For this purpose, a 70 g xylitol/100 g solution was heated to 170°C, cooled to 90°C under agitation, seeded with 1 g of xylitol crystals, mixed until a noticeable increase in viscosity due to crystal formation (50%) and allowed to crystallize on a tray in the dark.

Attempts were also made to prepare xylitol from xylan-containing material by hydrolyzing it to a mixture of monosaccharides, including xylose, which was then reduced to xylitol using catalysts. For instance, Heikkilä and co-workers (26) reported xylitol crystallization from solutions containing 97 g (11.4 g/100 g), 170 g (19.5 g/100 g), and 185 g (20.9 g/100 g) of xylitol, which were previously prepared by hydrogenation of a material containing per 100 g of solution: 94.2 g xylonic acid and 1.1 g xylose using Ru/carbon (110°C, 13,000 kPa for 3 h), Raney nickel (122°C, 6,500 kPa for 18 h), and Rh/2%Mo/Al_2O_3 (140°C, 6,500 kPa for 18 h), respectively. After concentration and cooling, feed solutions containing 91.3-92.2 g/100 g solution were submitted to a 3°C temperature increase, centrifugation, seeding with 0.05-0.06 g xylitol crystals at 56.5-60°C and cooling to 30°C for 49-69 h, hence obtaining xylitol purity of the mass among 56.5 and 77%. After separation a yield of dried crystals among 30 and 55 g, and crystal purity among 68 and 93.3% were obtained.

Fernandez and co-workers (27), investigating xylitol crystallization from hydro-alcoholic solutions containing arabitol and adonitol, observed that the presence of these conformational isomers in small amounts neither affected xylitol solubility (0.5 to 0.45 kg xylitol kg^{-1} of solvent) in 25-75% (w/w) water/ethanol solution in the temperature range 10-30°C nor enhanced agglomeration. On the other hand, the growth rate of xylitol crystals after addition of adonitol to the system was lower than with arabitol, and this effect was intensified by an increase in the content of the admixtures and somewhat reduced by a temperature raise.

Xylitol can also be crystallized effectively from high viscosity solutions by nucleation when sugars and sugar alcohols such as mannose, xylose, xylitol, mannitol, lactose, lactitol, sucrose, glucose, and fructose are present, and there is high xylitol super saturation. Nurmi and co-workers (28) reported that effective stirring enhances nucleation, enabling it to occur spontaneously, preventing solidification of a crystallization mass and allowing nuclei to develop crystal shapes and to grow until crystal growth is blocked, when the highest yields are found. Samples containing 93.8-94.4 g of xylitol in 100 g of solution were seeded with 10 g ground xylitol and cooled from 50-56°C to 25-20.5°C at different rates (0.042-0.019°C min^{-1}), thus obtaining crystallization masses with 3.0-3.6 supersaturation, whose viscosity (59.5-198 Pa.s) after vigorous stirring was shown to increase as supersaturation level increased and temperature decreased. Final xylitol separation by a pressure filter ensured xylitol yields in the range of 57-68%.

TABLE 2
Xylitol crystallization yield and crystal purity from synthetic media

Starting material	Conditions	Results	References
Melt-crystallized xylitol	70% xyl solution, heated to 170°C and cooled to 90°C under agitation in water bath (80°C). Seeded with 1 g of xyl crystal.	Agglomerated crystal structure with 99.5 % (w/w)	DuRoss (25)
Xylan-containing material	91.4% xyl; 0.06 g xyl crystals as seed at 60 °C; CR = 0.010°C min^{-1}	77.0% xyl purity, 30.72% xyl yield and 81.2% crystal purity	Heikkilä and co-workers (26)
	91.4% xyl; 0.05 g xyl crystals as seed at 60 °C; CR = 0.012°C min^{-1}	64.3% xyl purity, 31.74% xyl yield and 93.3% crystal purity	
	92.2% solution, 0.05 g xyl crystals as seed at 60°C; CR = 0.007°C min^{-1}	56.5% xyl (purity), 29.73% xyl yield and 68% xyl purity	
Hydroalcoholic solutions containing xylitol, arabitol, and adonitol	0.5 to 0.45 kg xyl/kg of solvent, 25/75% water/ethanol mixture from 10.86 to 29.86°C	Agglomeration phenomenon not enhanced; more pronounced reduction of xyl growth rate	Fernandez and co-workers (27)
Solution containing sugars and sugar alcohols such as mannose, xylose, mannitol, lactose, lactitol, sucrose, glucose, fructose	12.3 kg of the CM, 93.8 g of xyl/100 g solution, 10 g ground xyl as seed, 50 °C, CR = 0.042°C min^{-1}	67.0% xyl yield	Nurmi and co-workers (28)
	13.58 kg of the CM, 94.1 g of xyl/100 g solution, 10 g ground xyl as seed, 50°C, CR = 0.030°C min^{-1}	57.0% xyl yield	
	13.52 kg of the crystallization mass containing 94.4 g of xyl/100 g solution, 10 g ground xyl as seed, 56°C, CR = 0.019°C min^{-1}	68.0% xyl yield	
Synthetic solutions	65.6% (w/w) xyl solution; purity over 99% d.s.; 27.4 kg/h; 64±2°C; from 140 to 150 bar; particulate xyl. suspended in a gas (spray nozzle at 60 kg/h at 104-107°C); 4 min	Microcrystals with 0.1% moisture and conditioning it into an agglomerated product (1 to 2 cm)	Heikkilä and co-workers (29)
	66.6 and 72% xyl solution and 55, 60 and 70 bar of feed pressure	0.1 to 1.8% moisture	
Water/ethanol solutions	Isothermal conditions at 5, 25 and 40°C	Xyl crystallization was 11 times faster in 90% ethanol that in 60% ethanol at 25°C; the rate of xyl nucleation increased 7 times at 5°C with 60% ethanol and 30 times at 40°C with 90% ethanol	Vyglazov (30)
Synthetic solutions	728 g/l xyl concentration; -6.0°C crystallization temperature	54% crystal yield; 97.0% xyl purity	De Faveri and co-workers (32)
Water/methanol solutions	0-66.7% methanol content and temperature from 20-55°C	0.02 to 5.10^{-9} m.s^{-1}growth rates and 0.01 to $9.7.10^{9}$ m^{-3}s^{-1} nucleation rates	Hao and co-workers (31)

(*Continued*)

TABLE 2
(Continued)

Starting material	Conditions	Results	References
Hydroalcoholic solution	40, 50 and 60°C saturation temperature and CR = 0.10, 0.25, 0.50°C min^{-1}	g = 2.44; n = 2.44; m = 3.44; $B_N = 4.76.10^{-18}$ kg kg^{-1} of solvent	Martínez and co-workers (36)
Synthetic solutions	Effect of additives	7.3±0.4 mm h^{-1} crystallization front speed, 22 °C, methanol; speeds 33 times higher in vertical tubes and 170 times higher in horizontal tubes than with pure xyl (0.7±0.1 mm/h).	Seppälä and co-workers (13)

*CR = cooling rate; xyl = xylitol; CM = crystallization mass; g = order of crystal growth kinetics; n = order of nucleation kinetics; m = apparent order of nucleation kinetics; B_N = system kinetic constant.

Heikkilä and co-workers (29) crystallized xylitol with purity over 99% d.s. from a 65.6% (w/w) xylitol solution (2 kg) supplied by a spray nozzle at a rate of 27.4 kg h^{-1}, temperature of 64 ± 2°C and feed pressure in the range 140-150 bar. Small particles of dried product were fed into the microcrystallization equipment at a rate of 60 kg h^{-1} for 4 min, while air temperature was maintained at 104-107°C. An agglomerated or porous powder xylitol layer about 1-2 cm thick formed on the screen, where it was left for 60 min, while temperature was reduced from 62 to 45°C, and the resulting xylitol microcrystals (2.6 kg) with 0.1% moisture content were collected, milled, and sieved. When the same protocol was applied varying xylitol concentration (65.6-72 g/100 g solution), temperature (64-65°C), feed pressure (55-70 bar), and air drying temperature (101-108°C) xylitol microcrystals moisture content ranged from 0.1 to 1.8%.

To accelerate crystallization, several additives were tested as seeds (15, 19) using different organic solvents such as methanol, acetone, ethanol, methanol-water, and ethanol-water mixtures (13, 15, 30, 31), and their effects on latent heat, melting temperature, and long-term durability of the supercooled state assessed (13, 15).

For instance, Vyglazov (30) investigated the kinetics of xylitol crystallization from ethanol/water solutions as function of the degree of supersaturation of the initial solution under isothermal conditions in the temperature range 5-40°C. An increase in ethanol concentration from 60 to 90% (v/v) accelerated xylitol crystallization by a factor of 11 at 25°C, while a simultaneous increase in temperature from 5 to 40°C led to a 7-10-fold increase in the rate of xylitol nucleation, especially at liquid-to-solid ratios greater than 3. Changes in the structure of the mixed solvent likely hindered the diffusion of xylitol molecules to the surface of growing crystals, thus limiting xylitol crystallization. As xylitol crystallization rate at 90% (v/v) ethanol concentration was only thrice that at 60% (v/v), it is likely that crystal size depends on ethanol concentration and temperature, with larger crystals formed at lower ethanol levels.

By a 3^2 full-factorial design combined with response surface methodology, De Faveri and co-workers (32) found that an initial xylitol supersaturation value of 728 g l^{-1} in synthetic solutions and cooling temperature of -6.0°C were the operating conditions under which xylitol purity (97%) and crystallization yield (54%) were simultaneously optimized. However, purity close to 100%, which is needed to meet industrial standards, was predicted for conditions (583 g l^{-1} and -2.4°C) leading to unsatisfactory crystallization yield (25%). As suggested by the use in the industrial crystallization practice of supersaturation levels in the range 1.12-1.40, a non-excessive value of this parameter is needed to make the control of crystallization easier (33).

The traditional approach to the optimization problem using the one variable-at-a-time method (34) is the most common one in xylitol crystallization literature. Hao and co-workers (31) determined the solubility and crystallization kinetics of xylitol in a methanol/water system. In general, xylitol solubility, crystal growth, and nucleation rate decreased with increasing methanol content, likely due to a decrease in the collision probability of xylitol molecules in solution, while they increased with temperature. These authors reported growth and nucleation rates in the ranges 0.02-5 x 10^{-9} $m.s^{-1}$ and 0.01-9.7 x 10^{9} $m^{-3}s^{-1}$, while scanning electron microscopy revealed that the final shape of xylitol crystals was rounded and that there was negligible breakage and agglomeration. Based on the theory of population balance, xylitol nucleation and growth rates in methanol/water solutions were assessed using batch data for xylitol crystallization and the nonlinear least-squares method. At constant temperature, the nucleation and growth rate of xylitol decreased with the increase in methanol content.

Liavoga and co-workers (35) obtained xylitol with 95% purity and 10% yield from wheat straw acid hydrolyzate after reduction with sodium borohydride followed by fractional crystallization. The mixture of four alditols (xylitol, L-arabitol, D-sorbitol, and galactitol) was purified by open-column chromatography using a strongly basic anion-exchange resin obtaining 7% xylitol crystals with 99% purity.

Martínez and co-workers (36) studied the combined effects of saturation temperature (40, 50 and 60°C) and cooling rate (0.10, 0.25, and 0.50°C min^{-1}) on kinetics of commercial xylitol crystallization in an ethanol/water (50:50% w/w) solution according to the Nývlt and co-workers (20) method. Crystallization occurred very quickly (900 to 16,440 s), and an increase in the cooling rate from 0.10 to 0.50°C min^{-1} led to a higher supersaturation state and decreased the nucleation temperature by 9, 8 and 3°C. On the other hand, the maximum solution undercooling increased by 9, 7 and 3°C for saturation temperatures of 40, 50, and 60°C. The best correlation between nucleation and growth rates (in log terms) was obtained when the value of the mass concentration exponent (c) in secondary nucleation kinetics of the solids concentration was 0. The nucleation model explained 73% of the total variation in the response as a function of the apparent growth rate, and the regression constant or intercept was 14.88. Estimated kinetic parameters such as orders of growth (g = 2.44) and nucleation kinetics (n = 2.44), apparent order of nucleation kinetics (m = 3.44), and system kinetic constant ($B_N = 4.76 \times 10^{-18}$ kg.kg$_{solv}$$^{-1}$) and the mean crystal size calculated were satisfactorily compared with the experimental one (11.3% mean deviation).

Seppälä and co-workers (13) reported the effect of different additives on the speed of xylitol crystallization front at various supercooling levels. At 22°C, this speed (7.3 ± 0.4 mm h^{-1}) was about 33 times higher than that of pure xylitol (0.7 ± 0.1 mm h^{-1}) in vertical tubes and 170 times higher in horizontal tubes. Methanol was the best additive, whose effect was mainly the result of flow currents generated by its separation during crystallization.

Cheng and co-workers (37) studied xylitol crystallization by seeding a solution containing 800 g l^{-1} at 65°C and cooling the solution at a rate of 1°C min^{-1} up to 15°C with periodic mixing. After separation of xylitol crystals by centrifugation, the mother liquor was further concentrated to 800 g l^{-1} by rotary vacuum evaporation at 65°C and then subject to recrystallization under the same conditions, giving xylitol crystals with 99.5% purity.

CRYSTALLIZATION OF XYLITOL OBTAINED BY BIOTECHNOLOGICAL TECHNIQUES

Biotechnological techniques depend on the quality of the desired product along with the composition of the fermented liquor, which contains yeast, yeast debris, and other contaminants such as ingredients of the cultivation medium, residual substrates, and fermentation by-products. The fermented broth is centrifuged to remove biomass and concentrated in order to increase xylitol concentration (38). Xylitol characteristics such as molecule size and impurities found in fermented broths such as sugar and sugar alcohols, inorganic salts, and polypeptides are critical factors for the selection of a recovery method (19, 39, 40 - 42).

Different strategies such as the use of activated charcoal (43 - 46), pH adjustment (38), ion-exchange resins (15, 37, 43, 47), chromatographic methods (22, 23, 26, 48, 49), membrane separation (52), liquid-liquid extraction, and precipitation (46), or combined methods (19, 50, 51) have been successfully used to clarify the media (Table 3).

Crystallization of products obtained by fermentation can only be performed after the fermented broth has been purified (7, 15, 53). Xylitol crystallization has drawn more attention in the past two decades and is believed to be the final step to obtain xylitol in a highly purified form; however, several impurities coming from the hydrolysis of the lignocellulosic matrix or nutrients added for medium preparation are recognized as prejudicial to the formation of xylitol crystals (7). Owing to the complexity of the fermentation broth, the purity of xylitol obtained by direct crystallization is not acceptable; therefore, the fermented broth must be purified prior to crystallization to produce pure xylitol crystals (19, 54).

Xylitol crystallization yield and crystal purity from xylitol-rich solutions obtained by biotechnological techniques are summarized on Table 4.

Heikkilä and co-workers (48, 49) patented a simple method to produce xylitol from hemicellulosic hydrolyzates that makes use of *Debaryomyces hansenii* and *Candida tropicalis* to convert xylose to xylitol and most hexoses to ethanol. The xylitol-rich solution (86.5 g/100 g solution) was recovered by chromatographic separation from the fermented broth and evaporated at 65°C up to 92 g/100 g solution. Then, 2.2 kg of the evaporated solution was seeded with 0.03% xylitol crystals (0.04 mm size), and the crystallization solution was cooled to 45°C for 55 h. After centrifugation (5 min, 2000 *g*) and water washing, the recovered crystals had a mean size of 0.37 mm and 99.4% purity.

The simplest protocols used to recover biotechnologically-produced xylitol are obviously those employed in fermented synthetic media, because of the nearly negligible amounts of contaminants. As underlined by Misra and co-workers (46), the treatment with activated charcoal followed by vacuum concentration and crystallization is in fact as a cost effective, easy, and environmentally friendly route for xylitol recovery, providing a sound basis for large scale industrial production and purification of such a highly valuable sugar alcohol.

Investigating xylitol adsorption by charcoal from synthetic broths fermented by *D. hansenii* with xylitol concentration in the range 5-200 g l^{-1}, at 25 and 50°C, pH 6.0, under magnetic stirring for 1 h, Sampaio and co-workers (44) obtained the best clarification results using 20 g l^{-1} activated charcoal at 25°C, which allowed removing nearly 79% of colored contaminants, 94% of amino acids, and 69% of total proteins, and recovering xylitol almost completely. In an alternative approach using A-505 anionic-exchange and C-504 cationic-exchange resins, Martínez and co-workers (15) were able to recover nearly 85% of xylitol initially present in the medium.

TABLE 3
Strategies used for the purification of xylitol (other than crystallization)

Method	Fermentation media and microorganism	Best conditions	Results	References
Activated charcoal	Sugarcane hemicellulosic hydrolyzate by *Candida guilliermondii*	25 g/100 ml solution, 80°C, 100 rpm, 1 h, pH 6.0	19.1% xyl loss	Gurgel and co-workers (43)
	Synthetic media by *Debaryomyces hansenii*	20 g/l solution, 25°C, magnetic agitation, 1 h, pH 6.0	≈ 0 xyl loss	Sampaio and co-workers (44)
	Corn fiber hydrolyzate by *C. tropicalis*	2.5 g/100 ml solution, pH 6.0, 80°C, 1h, 100 rpm	69.0% protein removal	Affleck (52)
			79.5% of the UV absorbing material adsorbed about 25-50% xyl	
Ion-exchange resins	Mother liquor	Weakly cross-linked divinylbenzene cation-exchange resin in Ca form	More than 34% of xyl recovered	Munir and Schiweck, (23)
	Sugarcane hemicellulosic hydrolyzate by *C. guilliermondii*	Amberlite 200C cation-exchange and Amberlite 94S anion-exchange resins	21-57% xyl loss	Gurgel and co-workers (43)
	Wheat straw hemicellulosic hydrolyzate by *C. guilliermondii*	A-860S strong base anion-exchange and A-500PS strong base anion-exchange resins	21.1% xyl loss	Canilha and co-workers (47)
	Synthetic media by *C. guilliermondii*	A-505 anion-exchange and C-504 cation-exchange resins	97.5% removal of soluble byproducts, 99.5% removal of coloration medium15.23% xyl loss	Martínez and co-workers (19)
pH adjustment	Sugarcane hemicellulosic hydrolyzate by *C. guilliermondii*	pH 7.0 with NaOH	26.4% removal of proteins	Martínez and Santos, (38)
Liquid-liquid extraction	Synthetic xylose and corn cob hemicellulosic hydrolyzate	1:5 (volume/volume) of fermentation broth and ethyl acetate	21.72 g/l xyl extracted	Misra and co-workers (46)
Membrane separation	Corn fiber hydrolyzate by *Candida tropicalis*	10,000 nominal molecular weight cutoff polysulfone membrane	82.2 – 90.3% of xyl passed through; purity up to 90.3%	Affleck (52)
Precipitation	Synthetic xylose and corn cob hemicellulosic hydrolyzate	1:1 ratio (v/v) fermented broth and acetone	67.44% xyl recovery in aqueous phase	Misra and co-workers (46)

(*Continued*)

TABLE 3
(Continued)

Method	Fermentation media and microorganism	Best conditions	Results	References
Combined methods	Sugarcane bagasse by electrolytic reduction from xylose to xyl	Activated carbon	Xyl granules	Manalo and co-workers (50)
	Sugarcane hemicellulosic hydrolyzate by *C. guilliermondii*	IR 120 cation-exchange and IRA 410 anion-exchange resins	60% crystallization yield and 33% total recovery	Mussatto and co-workers (54)
	Sugarcane hemicellulosic hydrolyzate by *C. guilliermondii*	The fractions were combined, 6.5-fold concentrated at 50°C, maintained at 4° C for 24 h and supplemented with 400 mg of finely ground standard xyl pH 7.0 with NaOHA-505 anion-exchange, C-504 cation-exchange resins	8.15% xyl loss	Martínez and co-workers (19)

*CR = cooling rate; xyl = xylitol.

Gurgel and co-workers (43) used activated charcoal and ion-exchange resins (Amberlite 200C strong cation-exchange resin and Amberlite 94S weak anion-exchange resin) to clarify xylitol-containing sugarcane bagasse hydrolyzate fermented by *Candida guilliermondii*. Optimal clarification with acceptable xylitol loss (19.1%) was obtained with 250 g l^{-1} activated carbon at 80°C and pH 6.0, under agitation at 100 rpm for 1 h. As no xylitol crystal was observed by the naked eye in clarified solution containing 20.15 g l^{-1} xylitol, even after filtration and concentration for 5 weeks at -15°C, it was seeded with 1 g l^{-1} commercial finely ground xylitol, thereby leading, after one week at -15°C, to the formation of crystals with uniform color and needle shape. Even lower xylitol loss (8.15%) was reported by Martínez and co-workers (19) for the treatment of sugarcane hemicellulosic hydrolyzate fermented by *C. guilliermondii* with A-505 anion-exchange and C-504 cation-exchange resins, after pH adjustment to 7.0 and centrifugation. Martinez and Santos (38), who investigated the effect of pH (5.0-9.0) on precipitation of impurities from the same medium, observed maximum precipitation rate and proteins removal (26.4%) at pH 7.0. On the other hand, no precipitates were formed from rice bran derived impurities, and protein removal was not influenced by pH adjustment in the range 5.0-6.0.

Affleck (52) proposed membrane separation as an alternative method for the recovery of xylitol from fermented broth, because it has the potential for energy savings and higher purity. Xylitol produced by *C. tropicalis* from corn fiber hydrolyzate with a xylitol yield factor of 0.6 g g^{-1} of xylose was optimally separated from the impurities with a 10,000 nominal molecular weight cutoff polysulfone membrane, allowing 82.2-90.3% of xylitol contained in the fermentation broth to pass through, while retaining 49.2-53.6% of oligopeptides and peptides. Permeate crystallization led to xylitol crystals with purity up to 90.3%. On the other hand, the activated carbon treatment for 1 h (2.5 g per 100 g of solution, pH 6.0, 80°C, and 100 rpm) was able to more effectively remove colored contaminants (up to 79.5%), but adsorbed about 25-50% of xylitol. Taking into account the common inability of membrane treatment to remove impurities, these results confirm that this kind of polysulfone should only be used in a pretreatment for further purification steps in the presence of colored, viscous mother liquors (52).

Rivas and co-workers (45) reported on the purification of xylitol obtained from corn cob hydrolyzates fermented by *D. hansenii* using charcoal at solid-to-liquid ratios varying from 1:125 to 1:5. According to these authors, the use of 1 kg of charcoal/15 kg of liquor resulted in an increase in xylitol content of up to 0.6873 kg/kg of nonvolatile components, almost complete decolorization (98.8% removal of ICUMSA 4 color), 81.9% removal of proteins, and 66.7% removal of other nonvolatile compounds, while keeping xylitol loss to an acceptable level (only 3.2% of the initial amount). To purify and crystallize xylitol from corncob hydrolyzates fermented by *C. tropicalis*, Wei and co-workers (51) tested a multistep purification protocol consisting of a) decolorization with activated charcoal (1%, 60°C, 165 rpm), b) desalting with a combination of two ion-exchange resins (732 and D301), c) separation with UBK-555(Ca^{2+}), d) vacuum-concentration of the fermentation broth up to supersaturation (750 g l^{-1} xylitol), e) seeding with

TABLE 4
Xylitol crystallization yield and crystal purity from solutions obtained by biotechnological routes

Fermented media and microorganism	Conditions	Results	References
Biomass hemicellulosic hydrolyzates	92% xyl concentration, 0.03% 0.04 mm xyl seed crystals, CR = 0.006 °C min^{-1}	0.37 mm mean size and 99.4% pure crystals	Heikkilä and co-workers (48, 49)
Synthetic media by *D. hansenii* and *C. tropicalis*	20.15 g/l xyl, 1 g/l xyl seed, 1 week at -15°C	Uniform needle shape and crystal color	Gurgel and co-workers (43)
Fermented sugarcane bagasse hydrolyzate by *C. guilliermondii*	582 - 730 g/l xyl; -10, -5 and 0°C; 1 g/l xyl seed	56% crystallization yield or almost 100% purity at 730 g l^{-1} at -5°C	De Faveri and co-workers (55)
Synthetic solution	470 g/l xyl, -10°C, 1 g/l xylitol seed	29% crystallization yield or 92% purity	
Fermented hardwood hemicellulose hydrolyzate by *D. hansenii*			
Synthetic broth by *Debaryomyces hansenii*	675 to 911g/l xyl, -10°C	96 to 97.8% purity	Sampaio and co-workers (44)
Sugarcane hemicellulosic hydrolyzate by *C. guilliermondii*	First crystallization: 935.4 g xyl/l and 13.1 g arabinose/l; 0.5°C min^{-1} CR; ST = 50 °C	85% purity; -147.81 J g^{-1} melting heat; 84.67°C melting point; 3% moisture	Martínez and co-workers (19)
	Second crystallization with 0.1% xyl seed: 42.63 g/100 g solution at CR = 0.1°C min^{-1} and ST = 30°C 52.98 g/100 g solution at CR = 0.25 C min^{-1} and ST = 40°C 63.59 g/100 g solution at CR = 0.50°C min^{-1} and ST = 50°C	91.20-94.85% purity; melting point 91.8- 94.1°C; melting heat -174.18 and -214.62 J g^{-1}; and 1.55 and 2.77% moisture	
Wheat straw hemicellulosic hydrolyzate by *C. guilliermondii*	726.5g/l xyl, 4.3 g/l xylose, 3.2 g/l arabinose, 16.5 g/l glycerol and 12.2 g/l lignin derivatives mixed with commercial xyl solution (100, 70, and 50%) at CR = 0.2-0.4°C min^{-1} and ST = 50°C	95.3- 99.9% purity	Canilha and co-workers (47)
Semisynthetic medium by *C. guilliermondii*	First crystallization: 745.3 g xyl/l, CR = 0.5°C min^{-1} and ST = 50°C	95% purity, -206.71 J g^{-1} melting heat, 91.5°C melting point and 3.08% humidity	Martínez and co-workers (15)
	Second crystallization with 0.1% xyl seed: 42.63 g/100 g solution at CR = 0.10°C min^{-1} and ST = 30°C ST; 52.98 g/100 g solution at CR = 0.25°C/min and ST = at 40°C 63.59 g/100 g solution at CR = 0.50°C min^{-1} and ST = 50°C	98.5-99.2% purity, -236.8 to -244.95 J g^{-1} melting heat, and 93.9-94.1 melting point	

*CR = cooling rate; ST = saturation temperature; xyl = xylitol.

1% xylitol crystals, and f) cooling to -20°C for 48 h. Crystalline xylitol with regular tetrahedral shape, 95% purity, and 60.2% crystallization yield was obtained.

Misra and co-workers (46) proposed a new multistep protocol for xylitol purification from either synthetic xylose solutions or corn cob hemicellulosic hydrolyzates fermented by adapted *C. tropicalis* cells, which consisted of liquid-liquid extraction with 1:5 (v/v) ethyl acetate (21.72 g l^{-1} xylitol extracted), xylitol precipitation (67.44% recovery yield), activated charcoal treatment, vacuum concentration, and crystallization. Under optimal conditions (15.0 g l^{-1} of charcoal concentration at 30°C for 1 h, with 10-fold supersaturation cycles of initial concentration, and initial crystallization temperature of -20°C in order to initiate crystal formation and after shifted to 8°C) to increase the crystallization yield (43.97%). After 4 crystallization cycles, xylitol crystallization yields of 76.2 and 68.1% were obtained in 50 mL and 5.0 l of synthetic broth, respectively. Recovered xylitol had prismatic or orthorhombic needle crystalline structure when ethanol or tetrahydrofuran was used as solvent, and their purity, checked by ^{13}C and ^{1}H nuclear magnetic resonance, mass spectroscopy, and optical rotation, was shown to be almost 99%.

Mussatto and co-workers (54) recovered xylitol from sugarcane bagasse hemicellulosic hydrolyzate fermented by the *C. guilliermondii* yeast by silica gel adsorption and crystallization. In the first step, different ratios of fermented broth volume per gram of silica were employed to pack a stationary phase bed column, and different solvent mixtures of ethyl acetate, ethanol, and acetone were used as eluents. The purified broth was then submitted to different crystallization procedures. The best results (60% crystallization yield and 33% xylitol yield) were obtained when the fractions were combined, concentrated 6.5-fold in rotavapor at 50°C, maintained at 4°C for 24 h, and finally seeded with 400 mg of finely ground xylitol.

De Faveri and co-workers (55) studied xylitol recovery from hardwood hemicellulose hydrolyzate fermented by *D. hansenii* containing 75 g l^{-1} xylitol and 24 g l^{-1} xylose using broth evaporation at 30-50°C, 1.6 x 10^4 Pa and 45-50 rpm until supersaturation was achieved. After addition of 1 g l^{-1} xylitol to favor nucleation, crystallization was carried out by cooling at -10, -5, or 0°C, centrifuging and filtering the solutions, and the results were compared with those obtained with mixed synthetic xylitol/xylose solutions. Promising results both in terms of crystallization yield (56%) and purity degree (close to 100%) were obtained from very concentrated synthetic xylitol solutions (730 g l^{-1}) at relatively high temperature (-5°C), whereas the fermented hydrolyzate gave poorer results (29% crystallization yield and 92% purity) even at lower concentration (470 g l^{-1}) and temperature (-10°C), because of very quick impurity precipitation.

Sampaio and co-workers (44), in a similar study performed on synthetic broth fermented by *D. hansenii*, reported that xylitol crystals with purity in the range 96-97.8% were produced using xylitol concentrations ranging from 675 to 911 g l^{-1} at -10°C, and that an increase in xylitol concentration accelerated xylitol separation by a factor of 14-15. The lower the temperature, the higher the crystallization yield but the lower the crystals purity. The presence of residual xylose, although reducing xylitol percentage in the crystals from 97.7 to 85.3%, increased the crystallization yield from 27 to 42% and accelerated crystal growth, thereby permitting operation at higher crystallization temperature and lower xylitol concentration.

Rivas and co-workers (45) studied xylitol crystallization from concentrated corncob hydrolyzates previously detoxified with charcoal, fermented by *D. hansenii*, and then subjected to sequential stages of adsorption, concentration, precipitation with hydroalcoholic solutions, and further concentration. At -5°C an increase in ethanol percentage from 40 to 60% (v/v) resulted in a progressive rise in the crystallization yield from 15.6 to 47.1%, while at -10°C xylitol content of mother liquors was almost the same, but the crystallization yield was remarkably improved in media treated with 40 and 50% ethanol, because of both a reduction of viscosity and an acceleration of crystallization. Under optimal conditions, crystallization led to regularly shaped, well-formed, homogeneous crystals containing 98.9% (w/w) xylitol.

Martínez and co-workers (19) studied the downstream process for xylitol produced from sugarcane bagasse hemicellulosic hydrolyzate. Preliminary treatment of the hydrolyzate with ion-exchange resin provided a xylose-rich liquor containing 177.8% xylose and 2.10 g l^{-1} glucose, which was fermented by *C. guilliermondii* in a 15 l fermenter with 0.48 g l^{-1} h^{-1} xylitol volumetric productivity, 0.75 g g^{-1} yield, and 81.8% fermentation efficiency after 132 h. A preliminary crystallization step at 0.5°C min^{-1} cooling rate and 50°C saturation temperature allowed obtaining a syrup containing 935.4 g l^{-1} xylitol and 13.1 g l^{-1} arabinose and crystals with worse properties (85% purity, -147.81 J g^{-1} melting heat, 84.67°C melting point, and 3% moisture content) compared with commercial xylitol (99.8% purity, -259.63 J g^{-1} melting heat, 93.61°C melting point, and 0.05% moisture content). A second crystallization step in 50-50% (w/w) water/ethanol solutions at different proportions (42.63, 52.98 and 63.59 g 100 mL^{-1}), saturation temperatures (30, 40, and 50°C) and cooling rates (0.1, 0.25, and 0.50°C min^{-1}), increased crystals purity to 91.20-94.85%, melting point to 91.8-94.1°C and melting heat to -174.18/-214.62 J g^{-1} (as an absolute value), and reduced moisture content to 1.55-2.77%. Scanning electron microscopy revealed the occurrence of irregular agglomerates because the random clusters of crystals adhered and interpenetrated one another after growing together.

Canilha and co-workers (47) purified wheat straw hemicellulosic hydrolyzate fermented by *C. guilliermondii* using sequential adsorption on two strongly basic anion-exchange resins (A-860S and A-500PS), which considerably reduced the content of soluble by-products (by up to 97.7%) and medium color (by 99.5%), but simultaneously increased xylitol loss, even though up to a still acceptable level (21.1%). After medium clarification with resins, vacuum concentration in ethanol solution at 50°C saturation temperature and 0.2-0.4°C min^{-1} cooling rate, and mixing the concentrated medium (0, 30, and 50%) with a commercial xylitol solution (100, 70, and 50%), these authors were successful obtaining xylitol crystals with up to 99.9% purity without any medium concentration at 100% xylitol solution and 95.3-95.9% purity at 30-50% medium concentration and 50-70% xylitol solution. Crystals presented a much more defined geometry than those obtained from mixtures containing wheat straw-derived medium and pure xylitol.

Martínez and co-workers (15) proposed a two-step protocol for xylitol crystallization from a semisynthetic medium fermented by *C. guilliermondii* (61.3 g l^{-1} xylitol), centrifuged and concentrated to produce a syrup with 745.3 g l^{-1} xylitol. A preliminary crystallization step, carried out at 1.17 supersaturation, saturation temperature of 50°C, and a 0.5°C min^{-1} cooling rate, was followed by a second step of seeding with 0.1% xylitol crystals (95% purity, -206.71 J g^{-1} melting heat, 91.5°C melting point, and 3.08% moisture content) at 0.25°C min^{-1} and different saturation temperatures (30-50°C). The lowest saturation temperature (30°C) ensured the highest nucleation rate (4.58×10^{7} m^{-3} s^{-1}), but the crystallization lasted the longest (3,982.8 s), whereas the highest crystal growth rate (5.15×10^{-8} m s^{-1}) and the largest mean size (93.975×10^{-4} m) were obtained at 40°C. At the end of the process, agglomerates and hexagonal xylitol crystals with 98.5-99.2% purity, -236.8/-244.95 J g^{-1} melting heat, and 93.9-94.1°C melting point were obtained.

FUTURE PERSPECTIVES

The increase in demand for xylitol in the food and pharmaceutical market has intensified research for the development of a low cost xylitol production process. Different biomasses can be used as abundant and cheap feedstocks for the production of xylitol from xylose by fermentation. Crystallization is a purification method that leads to crystals with high purity and uniform size; however, optimal protocols to perform this operation are strongly dependent on the raw material and appear to be a rather long-term goal that will require more effort.

CONCLUSIONS

Conversion of lignocellulosic materials into high value products using acid or enzymatic hydrolysis and subsequent fermentation are economically viable and ecologically friendly usages. Biotechnological production of xylitol is a very promising alternative to the present chemical technique because it is simpler, uses less energy, and is less polluting. However, the conversion of biomass to fermentable sugars is quite troublesome. More research needs to be done to develop efficient pretreatment methods to find enzymes that perform hemicellulose conversion, to genetically improve strains able to ferment hemicellulosic sugars, and to find protocols for effective xylitol recovery from fermented broths. This overview on xylitol recovery showed that residual sugar in fermented hydrolyzates influences the purity of crystals and the crystallization yield and that xylitol yield can be substantially improved by processing of mother liquors through successive crystallization steps. The cooling profile, initial supersaturation level, final temperature, crystallization time, and seeding protocol all play important roles in the process; therefore, the search for optimal crystallization conditions is fundamental to maximize the efficiency of the overall process. Of the different attempts reported in the literature to recover xylitol, the treatment of fermented broth with activated charcoal followed by vacuum concentration and crystallization by cooling was undoubtedly the best protocol to produce xylitol crystals from synthetic xylose solutions. As far as the structure of xylitol crystals is concerned, electron microscopic studies revealed hexagonal shape in the absence of solvents or irregular forms, while in organic solvents the shape of xylitol crystals varies according to the solvent, ethanol leading to a prismatic structure and tetrahydrofuran to orthorhombic needles.

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