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RESEARCH ARTICLE

Purification and crystallization of xylitol from fermentation broth of corncob hydrolysates

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Abstract Xylitol, a five-carbon sugar alcohol, is a valuable sugar substitute, and widely used in the pharmaceutical, odontological and food industry due to its interesting properties. In the past decades, the xylitol industry has grown rapidly and more attention has been focused on xylitol purification, which possesses an important proportion of the whole industry. In our paper, the purification and crystallization of xylitol fermentation broth by biotechnology using corncob hydrolysates as substance were studied. An initial xylitol fermentation broth was decolored with activated carbon (1% M-1, 60°C, 165 rpm), desalted with a combination of two ion-exchange resins (732 and D301), and residual sugars were separated with UBK-555(Ca^{2+}). Then the solution was vacuum-concentrated up to supersaturation (750 g/L xylitol). After adding 1% xylitol crystal seeds, the supersaturated solution was cooled to −20°C for 48 h. The crystalline xylitol of a regular tetrahedral shape with purity 95% and crystallization yield 60.2% was obtained from the clarified xylitol fermentation broth. An intact, economical and environmental-friendly route of purification and crystallization of xylitol from fermentation of corncob hydrolysates was obtained, and its experimental procedure and data provided a sound basis for large-scale industrial production.

Keywords xylitol, corncob, activated carbon, ion-exchange resin, purification, crystallization

1 Introduction

Xylitol is not only a sugar-free sweetener, but also has unique properties that make it widely used in the pharmaceutical, healthcare and food industries [1]. Xylitol is used as an alternative sweetener to sucrose for diabetic patients because of its equivalent sweetening power but fewer calories [2], and is possibly even anticarcinogenic because it inhibits the growth of oral bacteria [3]. Due to these properties, xylitol is used as a sweetener in food products such as chewing gum, candy, soft drinks, and personal health products such as mouth wash and tooth paste.

As xylitol is a value-added product with a growing market potential, it is important and necessary to study and optimize its production technology. Xylitol can be obtained by various technologies including the extraction from some fruits and vegetables such as strawberries, raspberries, plums and pears [4], but its small quantities in fruits and vegetables make extraction difficult and uneconomical. Traditionally, large-scale commercial production of xylitol is by chemical reduction of D-xylose in the presence of a nickel catalyst at high temperatures. However, the high temperature (80°C–140°C) and pressure (up to 50 atm) requirements, extensive separation and purification procedures, along with the usage of catalyst makes the chemical route expensive [5]. Therefore, it is worthwhile to explore methods for the effective production of xylitol using microorganisms to reduce manufacturing costs [6,7] .

In this work, xylitol obtained by biotechnological methods was based on fermentation of corncob hydrolysates. Compared with the traditional chemical process, the bioconversion from corncob hydrolysates to xylitol is superior because of its procedural simplicity, low energy consumption and low pollution [8].

Xylitol bioproduction from corncob includes the following steps (Fig. 1): hydrolysis of the hemicelluloses [9], bioconversion of the xylose into xylitol by yeast [10], purification and crystallization of xylitol from the fermented hydrolysates [11–13]. To improve the purity and yield of xylitol crystals, the present study was focused on the purification process by the ion-exchange resins and optimization of the crystallization conditions. Four types of combination between different cation and anion exchange

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resins were studied, which aimed to get the optimum linked operation to remove the inhibitors of the fermentation broth, especially salts. Furthermore, selective adsorption on strong cation-exchange resins was investigated to separate residual sugars which were seldom involved in previous literature.

In the past few years, crystallization of xylitol has drawn more attention. Faveri [12] used response surface methodology to obtain the optimal crystallization conditions of xylitol, under which purity degree 97% and xylitol crystallization yield 54% were optimized. Martíinez [14] investigated the values of saturation temperatures and linear cooling rates, after two crystallization stages, and an increase of the crystal purity from 85% to 91.20%–94.85% was observed. However, the purity and the yield of crystallization did not reach a high level simultaneously in one run. In our study, based on that previously reported, xylitol initial concentration and solvent system as the major factors affecting the purity and yield of crystallization were studied to optimize the crystallization conditions. The research work developed an environmental-friendly, economical bio-based xylitol production process, exhibiting broad and promising prospects for xylitol industrial application.

2 Materials and methods

Figure 1 presents the flow diagram of the bench-scale process used for xylitol bioproduction from corncob. The corncob hemicellulose was hydrolyzed with dilute sulfuric acid, and the hydrolysates were concentrated by vacuum concentration. The inhibiting compounds were adsorbed by activated carbon and ion-exchange resins, whose contents were minimized through detoxification by pH alteration. After nutritional supplementation, the detoxified hydrolysates were used as the source of xylose for xylitol bioproduction, which were fermented by *C.tropicalis As* 2.1776 [15,16]. At the end of the fermentation, the yeast cells were recovered by centrifugation and the fermentation broth was clarified with activated carbon and ion-exchange resins [17]. The xylitol concentration of the clarified fermentation broth was increased by vacuum concentration, and the concentrated fermentation broth was finally subjected to cooling in the presence of ethanol for xylitol crystallization[1)] [18,19].

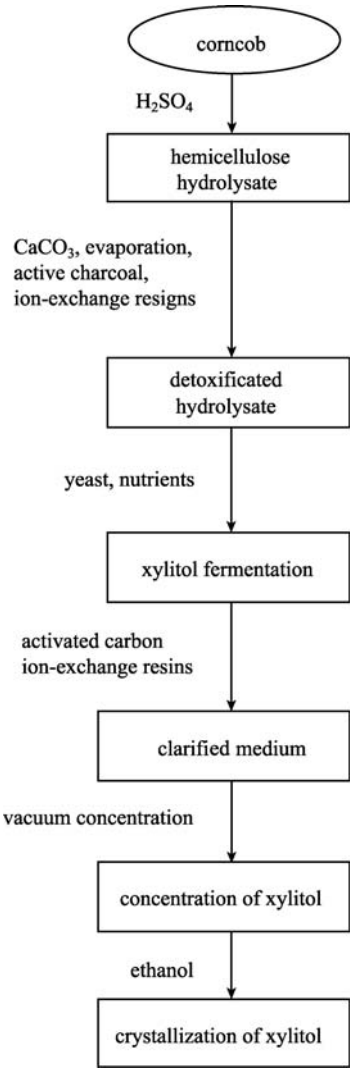

Fig. 1 Flow diagram of the bench-scale process used for xylitol biproduction from corncob

2.1 Preparation of hemicellulosic hydrolysates from corncob for xylitol bioconversion

Hydrolysis of corncob (mass of raw material-to-water ratio was 1∶10) was carried out with dilute sulfuric acid (volume of sulfuric acid-to-water ratio was 1.5∶100) for 2 hours at 100°C. Then the hydrolysate (liquid part) was neutralized by calcium hydroxide. After separating the corncob residue and calcium sulfate precipitate by filtration, the hydrolysate was concentrated at a certain extent with a vacuum vaporizer. Finally, the hydrolysate was treated with activated carbon to remove part of the potential inhibitory substances for xylitol bioconversion using *C. tropicalis As* 2.1776 yeast. After fermentation, the broth was obtained by vacuum concentration.

2.2 Purification of the fermentation broth of corncob hydrolysates

2.2.1 Activated carbon treatment

Activated carbon treatment is an efficient and economic method of reducing the impurities of fermentation broth

1) Fernandes C, Avelino A, Farelo F F. Crystallization of xylitol from hydroalcoholic solutions containing arabitol and adonitol. In: Proceedings of the 14th International Symposium on Industrial Crystallization. September 12–16, 1999, Cambridge, UK

including colorant, phenolic compounds, acetic acid, aromatic compounds, furfural and hydroxymethylfurfural. In our studies, activated carbon was mainly applied to discolor xylitol fermentation broth. In this way, the effects of initial pH values (4.0, 5.0, 6.0 and 7.0), temperatures (30°C, 40°C, 50°C and 60°C), contact time (30 min, 40 min, 50 min and 60 min) and carbon concentrations (1 g, 2 g, 3 g, 4 g and 5 g added to 100 mL fermented broth) were evaluated.

2.2.2 Ion-exchange resins treatment

To reduce the inhibitors of the fermentation broth, including inorganic salt, acetic acid, furfural and hydroxymethylfurfural [20,21], it is necessary to remove them with ion-exchange resins [22]. As shown in Tables 1 and 2, certain parameters (the decolorization ratio, the desalination ratio, the loss ratio of xylitol and the processing capability) of five macroporous ion-exchange resins including 732, D201, D301, D261, D113 were compared in the dynamic adsorption tests. Two glass columns with the internal diameter of 2.5 cm and the length of 50 cm were loaded with cation-exchange resins and anion-exchange resins, respectively. The clarified xylitol fermentation broth was desalted with the combination of two ion-exchange resins. Anion-exchange resin is mainly used for the removal of colored compounds from the fermentation broth, while cation-exchange resin is mainly used for desalination and removal of positively charged organics.

2.2.3 Separation of residual sugars by selective adsorption on strong cation-exchange resins chelated Ca^{2+}

A 50 cm glass column having an inside diameter of 2.5 cm was loaded with 30 mL strong cation-exchange resin chelated Ca^{2+}. Deionized water was then pumped through the column and a flow rate of 0.5 mL/min was maintained. For a period of 50 minutes, the feed was switched to the purified fermentation broth, and then switched back to water at a flow rate of 0.5 mL/min. 90 mL fractions were absorbed in the resins, then washed with deionized water, fractions 1–7 tubes (20 mL) containing xylitol, xylose and arabinose, and fractions 7–27 tubes (60 mL) containing pure xylitol were combined. Residual sugars was separated to get xylitol-rich fractions. The pure xylitol fractions were combined and concentrated under vacuum to crystalize.

Separation between residual sugars (xylose and arabinose) and xylitol becomes difficult due to their similar structures. Xylose and arabinose are both more than 5% in the xylitol fermentation broth. They can improve the viscosity of the fermentation broth, thereby influencing the purity and the crystal shape of xylitol. A selective adsorption process applied strong cation-exchange resins chelated Ca^{2+} for the separation of xylitol was established, because it has been discovered that strong cation-exchange resins chelated Ca^{2+} adsorbs xylitol more strongly than residual sugars. After adsorption equilibrium, residual sugars were washed away by desorption solution first, then xylitol was washed away following residual sugars. Therefore, xylitol was separated from residual sugars in the fermentation broth and the purity of xylitol was increased. The adsorption and desorption characteristics of FLC-10(Ca^{2+}) and UBK-555(Ca^{2+}) for separating residual sugars from the fermentation broth were compared.

2.3 Xylitol crystallization

Crystallization and precipitation from solutions are responsible for 70% of all solid materials produced by the chemical industry, which is an important separation and

Table 1 Physical properties of the macroporous ion-exchange resins used

type of resins	particle diameter/mm	moisture content/%	exchange capability /(mM·g-resin^{-1})	density/(g·mL^{-1})	surface functional group
D201	0.3–1.25	65–75	3.7	1.05–1.12	$-N^{+}(CH_3)_3$
D261	0.315–1.25	50–60	3.6	1.06–1.13	$-N^{+}(CH_3)_3$
D301	0.315–1.25	50–60	4.8	1.03–1.07	$-N(CH_3)_2$
732	0.315–1.25	46–52	4.5	1.24–1.28	$-SO_3H$
D113	0.315–1.25	45–52	10.8	1.14–1.20	$-SO_3H$
UBK-555	0.19–0.24	42–46	> 2.0	1.07–1.12	$-SO^{-}_{3}\cdot 1/2Ca^{2+}$
FLC-10	0.25–0.335	55–65	4.5	1.20–1.30	$-SO^{-}_{3}\cdot 1/2Ca^{2+}$

Table 2 Results of ion-exchange resins used to determine the conditions to be used during clarification of the fermentation broth

T_p [a)]	Y_c [b)]/%	Y_s [c)]/%	V_c [d)]	Y_{xyl} [e)]/%
732n + D261	93.78	44.28	2.5	8.5
732 + D201	97.64	62.76	3.0	7.4
732 + D301	98.48	72.76	5.5	5.2
D113 + D301	99.34	34.32	1.5	5.3

a) T_p: the types of the resins; b) Y_c: the decolorazition ratio; c) Y_s: the desaltification ratio; d) V_c: the capacity of the resins(5BV); e) Y_{xyl} : the loss ratio of xylitol

purification process in a wide variety of industries, because low energy consumption and yield with high purification are operated through crystallization. The crystallization steps consisted of concentration of the fermentation broth up to supersaturation, cooling of the supersaturated solutions, ethanol precipitation, separation of crystals by centrifugation and final filtration.

2.3.1 Concentration and ethanol precipitation of the clarified liquors

Following the liquid phase from the above treatments, the pre-purified fermentation broth was concentrated by vacuum evaporation at 60°C to reach xylitol super-concentration. The resulting solution was mixed with ethanol at different volume ratios, stirred and kept for 48 h at −20°C. The precipitate was separated by centrifugation (4500 rpm, 15 min), weighed and dissolved in water to analyze the purity degree and crystallization yield.

2.3.2 Optimization of crystallization conditions

The combined effects of initial xylitol supersaturation value and solvent system (water-ethanol) on xylitol crystallization were investigated. Batch crystallization tests were carried out to optimize the operating conditions on the crystallization procedure. After crystallization, the solutions were subjected to centrifugation at 4500 rpm for 15 min to separate the xylitol crystals. Finally, the precipitated crystals were separated by vacuum filtration through filters with 0.45 μm membrane and dissolved in water to be analyzed.

Mother liquors from crystallization and xylitol crystals were analyzed using the methods described below. The crystals were filtered or centrifuged, and the mother liquor containing uncrystallized xylitol was recycled.

2.4 Analytical methods

2.4.1 HPLC analysis

The xylose, arabinose and xylitol concentrations were determined by high performance liquid chromatography (HPLC) (Waters, USA), equipped with a refractive index detector (Shimadzu L-7490, Kyoto, Japan). A sugar-pak™ column (6.5×300 mm, Waters, Milford, USA) was used for the analysis. The column was operated in an oven (Shimadzu L-7300, Kyoto, Japan) at 80°C and deionized water was used for the mobile phase. The flow rate was increased linearly from 0.1 mL/min for 100 min until the flow rate was equal to 0.5 mL/min. Sugars and xylitol were detected and quantified by comparing their retention times to authentic standards. Samples were prepared for analysis by diluting 40-fold. The diluted samples were filtered using 0.45 μm membrane and 10 μL was injected and analyzed. The loss ratio of xylitol was calculated using Eq. (1).

$$\alpha\% = \frac{C_0V_0 - C_1V_1}{C_0V_0} \times 100\%, \quad (1)$$

α, the loss ratio of xylitol; V_0, the volume of fermentation broth before decoloration or desalination (mL); V_1, the volume of the fermentation broth after decoloration or desalination (mL); C_0, the concentration of the fermentation broth before decoloration or desalination (g/L); C_1, the concentration of the fermentation broth after decoloration or desalination (g/L).

The xylitol purity was calculated using Eq. (2).

$$PD\% = \frac{CV}{m} \times 100\%, \quad (2)$$

PD, the purity degree of xylitol; m, the mass of xylitol crystal (g); V, the volume of dissolving xylitol crystals (L); C, the concentration of dissolving xylitol crystals (g/L).

2.4.2 Determination of colorant of xylitol fermentation broth

The colorant was determined by measuring the optical density (OD) at 420 nm using an UV-vis spectrophotometer UV-2000 (Unic, Shanghai, China). The decolorization ratio was calculated using Eq. (3).

$$\eta\% = \frac{A_0 - A}{A_0} \times 100\%, \quad (3)$$

η, the decolorization ratio; A_0, the absorbance of the fermentation broth before decoloration in 420 nm; A, the absorbance of the fermentation broth after decoloration in 420 nm.

2.4.3 Determination of ion content of fermentation broth

A conductivity instrument (DDS-12A, Shanghai, China) is used to measure the electrical conductivity to determine ion content of a fermentation broth. Under certain conditions, the ion content is directly proportional to the conductivity. The desalination ratio was calculated using Eq. (4).

$$\beta\% = \frac{G_0V_0 - G_1V_1}{G_0V_0} \times 100\%, \quad (4)$$

β: the desalination ratio; G_0: the conductivity of the fermentation broth before desalination (μS/cm); G_1: the conductivity of the fermentation broth after desalination (μS/cm); V_0: the volume of the fermentation broth before desalination (mL); V_1: the volume of the fermentation broth after desalination (mL).

3 Results and discussion

3.1 Characterization of the fermentation broth of corncob hydrolysates

In previous work, the hydrolysates were detoxified, concentrated, supplemented with nutrients and fermented by the yeast *C. tropicalis As* 2.1776. The maximum yield was 0.8 g of xylitol per gram of xylose consumed. Then the desired fermentation broth of xylitol was obtained. The fermentation broth was filtered to remove biomass, giving a solution containing volatile compounds in the chemical processing of the raw material and nonvolatile compounds (xylitol and unfermented sugars). The composition of the fermentation broth and their concentrations are presented in Table 3.

Table 3 Composition of the mother fermentation broth

component	concentration /(g·L^{-1})
xylitol	92.84
xylose	7.59
arabinose	5.54
furfural	2.39
acetic acid	0.11

3.2 Activated carbon treatment

Results shown in Fig. 2 demonstrate that powdered types of activated carbon (M1, GH-95L, Lh) were better than granular types of activated carbon (LH, WL, W-2) by the high decolorization ratio and low xylitol loss ratio. After the single factor analysis of variance, the optimal conditions of decolorization were obtained: 4% M1 was added to pH 5 xylitol fermented broth at 60°C, and stirred at 165 rpm for 50 min. The decolorization ratio could reach more than 96%, while the xylitol loss ratio was less than 5%.

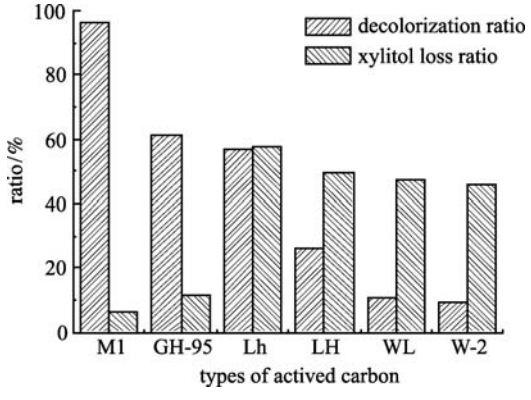

Fig. 2 Results of decolorizaton ratio and xylitol ratio of different types of activated carbon

3.3 Ion-exchange treatment

As can be seen in Table 2, a series composed of the anion-exchange resins in OH^- form and cation-exchange resins in H^+ form were chosen to clarify the bulk fermentation broth. The 732 and D301 showed the best desalination and the highest processing capability. Figure 3 shows that most of the salts were removed from the xylitol fermentation broth by the combined 732 and D301 resins at the flow rate of 0.5 mL/min. The 70% salts were desalted and the decolored ratio reached 98%, and 5.5 BV of the sample was treated. The loss ratio of xylitol could be kept at an acceptable level using the two resins in series, which allowed by-products removal.

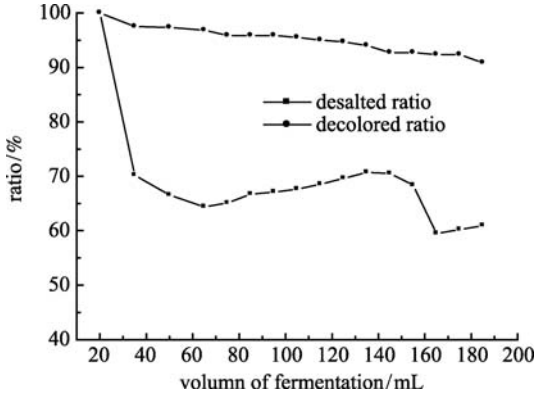

Fig. 3 Desalted ratio and decolored ratio curves of 732 linked with D301

3.4 Separation of residual sugars

The results shown in Figs. 4–7 indicated that the BK-555 (Ca^{2+}) resin offered the better adsorption and desorption capacity for xylitol than the other. Thus, xylitol and residual sugars were successfully separated by UBK-555 (Ca^{2+}).

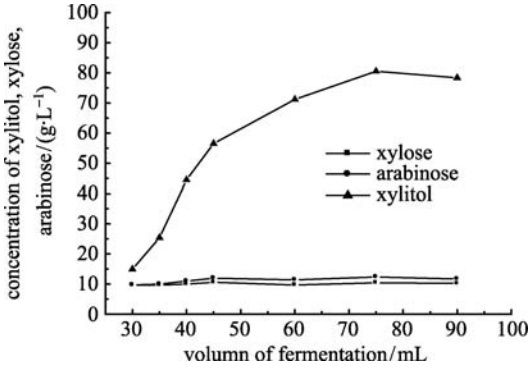

Fig. 4 Dynamic adsorption curve of xylose, arabinose and xylitol on column packed with FLC-10(Ca^{2+}) resin at 0.5 mL/min flow rate

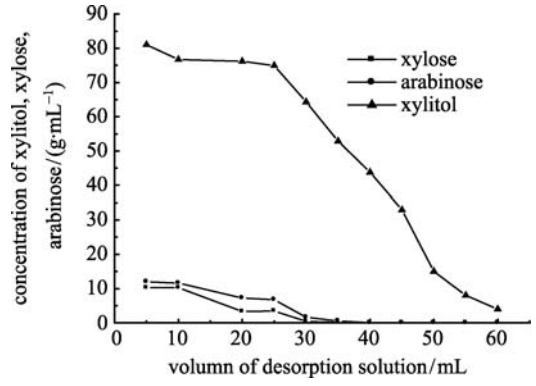

Fig. 5 Dynamic desorption curve of xylose, arabinose and xylitol on column packed with FLC-10(Ca^{2+}) resin at 0.5 mL/min flow rate

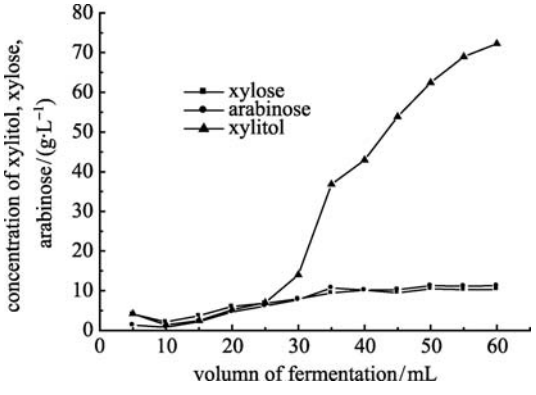

Fig. 6 Dynamic adsorption curve of xylose, arabinose and xylitol on column packed with UBK-555(Ca^{2+}) resin at 0.5 mL/min flow rate

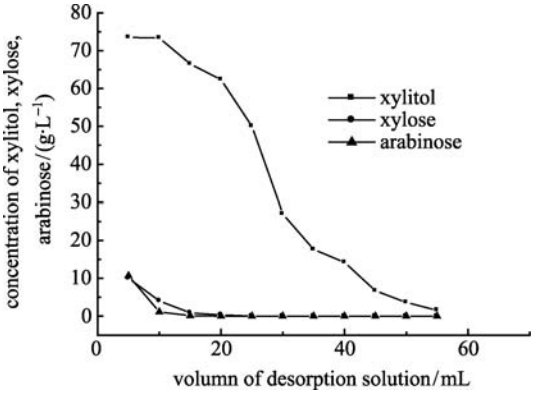

Fig. 7 Dynamic desorption curve of xylose, arabinose and xylitol on column packed with UBK-555(Ca^{2+}) resin at 0.5 mL/min flow rate

3.5 Xylitol crystallization

The crystal study was divided into two parts. First, to analyze the key factors that affect xylitol crystallization, a series of crystallization tests were performed on fermented commercial xylose and fermented hemicellulose hydrolysates. Second, crystallization tests were carried out at different starting xylitol concentrations and solvent systems to optimize crystallization conditions. The xylitol crystallization yield (Y) was expressed as the mass ratio of recovered xylitol to starting xylitol, and the purity degree (PD) was calculated as the mass ratio of recovered xylitol to total precipitate.

3.5.1 The foremost factors affecting xylitol crystallization

The dark-yellow xylitol fermentation broth, besides xylitol, was composed of several main soluble by-products, including xylose, arabinose, colorant and inorganic salts, which affect xylitol crystallization. To analyze the key factors that affect xylitol crystallization, a series of crystallization tests were performed on fermented commercial xylose (test 1) and fermented hemicellulose hydrolysates (test 2–4). Compared with the results of tests (from 1 to 4), with the concentration of residual sugars increasing, the xylitol purity and yield were decreased. The possible reason is that the residual sugar was crystallized together with xylitol. Meanwhile, the xylitol crystal of test 1 is white granular crystal, but the crystal shapes of test 2–4 are pale-yellow and sticking, owing to the relatively large viscosity of the residual sugar. The existence of the residual sugar does not only affect the purity and yield, but also affects the crystalline form. Then a set of comparative experiments to analyze the impact of pigment and inorganic salts on the xylitol crystallization was performed. Without decoloring and desalting the xylitol fermentation of test 1 and 2, the results were that the purity and yield were decreased. However, the change was not significant, compared to the impact of the residual sugar. Further, pigment and inorganic salts have little impact on the crystal shape. The experimental data (Table 4) demonstrated that the residual sugars (arabinose and xylose) were the foremost factors affecting the yield and purity of xylitol. The data in Table 4 represented the negative effect of the presence of residual sugars that decreased the purity degree of xylitol crystallization. However, two ways could be used to get crystalline xylitol of high purity: the fermented conditions optimized to get the fermentation broth containing low-concentration residual sugars, and the residual sugars separated from xylitol using strong cation-exchange resin chelated Ca^{2+}.

Table 4 The effect of residual sugars on purity degree

test	Yr[a)]/%	PD[b)]/%
1	2.2	95.84
2	13.12	82.33
3	28.38	70.77
4	38.56	60.44

a) Yr: the content of residual sugars; b) PD: purity degree

3.5.2 Optimization of crystallization conditions

The influences of xylitol concentration and solvent system (ethanol-water) on xylitol crystallization were investigated. The tests were carried out at atmospheric pressure and a constant, selected temperature (T = – 20°C) to ensure effective crystallization during every experiment. Sets of crystallization tests were performed on purified fermentation broths which were concentrated to xylitol concentrations ranging from 630 to 910 g/L. Through observing of the phenomenon, it was evident that an increase in xylitol concentration accelerated xylitol into the solid phase. As Table 5 shows, with the initial xylitol concentration increasing, the xylitol crystallization yield was improved, but purity was decreased. In particular, crystallization took place more quickly at 908.27 g/L xylitol than 630–750 g/L, but purity was the lowest. In the view of purity and xylitol crystallization yield, the optimum initial xylitol concentration of the crystallization media was about 750 g/L. By adding 1% xylitol crystal seeds, crystallization time was shortened.

Table 5 Effects of initial xylitol concentration on xylitol crystallization

test	crystal seed/%	Xyt_0[a)]/(g·L^{-1})	Xyt_e[b)]/(g·L^{-1})	Y_c[c)]/%	PD[d)]/%
1	1	65.86	632.33	45.32	98.47
2	1	73.73	691.98	52.43	97.32
3	1	79.99	750.00	60.02	95.00
4	1	73.73	908.27	74.74	80.77

a) Xyt_0: starting xylitol concentration; b) Xyt_e: xylitol concentration after evaporation; c) Yc: total crystallization yield; d) PD: purity degree

Table 6 lists the results of tests performed wherein the higher the ratio of ethanol and water, the lower the purity degree was; meanwhile, the crystallization yield showed a completely opposite behavior, since the solubility of xylitol in water is decreased by the presence of ethanol. The strategy employed in the present study to promote xylitol crystallization yield consisted of cooling the concentrated medium in the presence of ethanol. It was possible to increase the crystallization yield in the ethanol water mixture, with the purity degree lowering.

Table 6 Effects of the ethanol/water volume ratio on the xylitol crystallization

test	ethanol:water (V:V)	Y_{24}[a)]/%	Y_{48}[b)]/%	PD[c)]/%
1	1:3	87.33	95.35	63.48
2	1:2	83.44	90.66	66.52
3	1:1	52.67	56.67	69.82
4	0:1	23.53	37.78	97.57

a) Y_{24}: crystallization ratio after 24 h; b) Y_{48}: crystallization ratio after 48 h; c) PD: purity degree

4 Conclusions

This research work developed an environmental-friendly, economical bio-based xylitol production process, exhibiting broad and promising prospects for xylitol industrial application. After decolorization by activated carbon, desalination by ion-exchange resins and separation from residual sugars, the solution was concentrated up to supersaturation (750 g/L xylitol). After adding 1% xylitol crystal seeds to shorten the crystallization time, the supersaturated solution was cooled to –20°C for 48 h; both purity (95%) and xylitol crystallization yield (60.2%) were simultaneously optimized. The high-purity crystalline xylitol of a regular tetrahedral shape was obtained from the clarified xylitol fermentation broth. According to the present results, it can be concluded that xylitol was separated by crystallization from fermented hemicellulose hydrolysates highly efficiently.

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