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Xylitol recovery by crystallization from synthetic solutions and fermented hemicellulose hydrolyzates

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Abstract

Preliminary xylitol separation tests were carried out using solutions with relativity high concentrations of xylose and xylitol to simulate the actual composition of hemicellulose hydrolyzates. Xylitol was recovered by a crystallization methodology consisting of dilute solution evaporation up to supersaturation, supersaturated solutions cooling, separation of crystals by centrifugation, and final filtration. Two sets of tests were performed on xylitol–xylose synthetic solutions and an additional one on fermented hardwood hemicellulose hydrolyzate. The best results in terms either of crystallization yield (0.56) or purity degree (1.00) were obtained with quite concentrated solutions (730 g/l) at relatively high temperature (−5 °C). Besides, xylitol solubility limits in the solution, which are very important for future scale-up of the process, were estimated at different crystallization temperatures. Product yields and crystal purity were calculated and crystallization kinetics were investigated.

Keywords: Xylitol recovery; Crystallization; Synthetic solution; Fermented hemicellulose hydrolyzate

1. Introduction

Xylitol is a polyol with some interesting properties which make it an important product for the food industry. It has similar sweetness as sucrose, is non-cariogenic, tolerated by diabetics, recommended for obese people and, because of its negative heat of dissolution, used as a part of the coating of pharmaceutical products [1,2]. Nowadays, xylitol is synthesized by hydrogenation of xylose present in lignocellulosic hydrolyzates. But the solution produced by this process requires expensive purification and separation steps to obtain pure xylitol.

It can alternatively be produced by biotechnological methods based on fermentation of agro-industrial residues, which could potentially compete with the traditional chemical way. *Pachysolen tannophilus*, *Debaryomyces hansenii* and *Candida guillermondii* showed good performances as xylitol producers [3]. Xylitol production by these yeasts is regulated by oxygen, pH, temperature, starting xylose level, presence of other sugars and inoculum level [4–6]. Besides, the fermentation of hydrolyzates is hindered by inhibiting compounds such as furfural, acetic acid, lignin degradation products (phenolic compounds) and metal cations [4,7,8].

Xylitol recovery is the hardest step of the whole fermentation process because of the low product concentration as well as the complex composition of the fermentation broth. The literature on polyol recovery from fermented broths is quite poor owing to the innovating characteristics of the process. The work of Gurgel et al. [9] suggests very long process time (about 1 week) but did not report completely the operative conditions.

The present work aims at setting up a xylitol recovery methodology from fermented and purified broths. For this purpose, the best conditions to separate xylitol were determined combining low pressure evaporation and cooling. Preliminary experiments were carried out under conditions able to prevent the crystallization of xylose, which is present at relatively high concentrations in fermented solutions. Tests were performed on synthetic solutions to optimize the recovery efficiency and on hardwood hemicellulose hydrolyzate to point out the possible influence of compounds dissolved in the fermented broths.

2. Materials and methods

2.1. Crystallization procedure

The experiments were performed in a bench-scale system (Fig. 1) composed of (a) a rotavapor (Büchi, R-114, Flawil, Switzerland) used as low pressure–concentration unit, provided with temperature and pressure control system and a vacuum pump, (b) a crystallization unit working at

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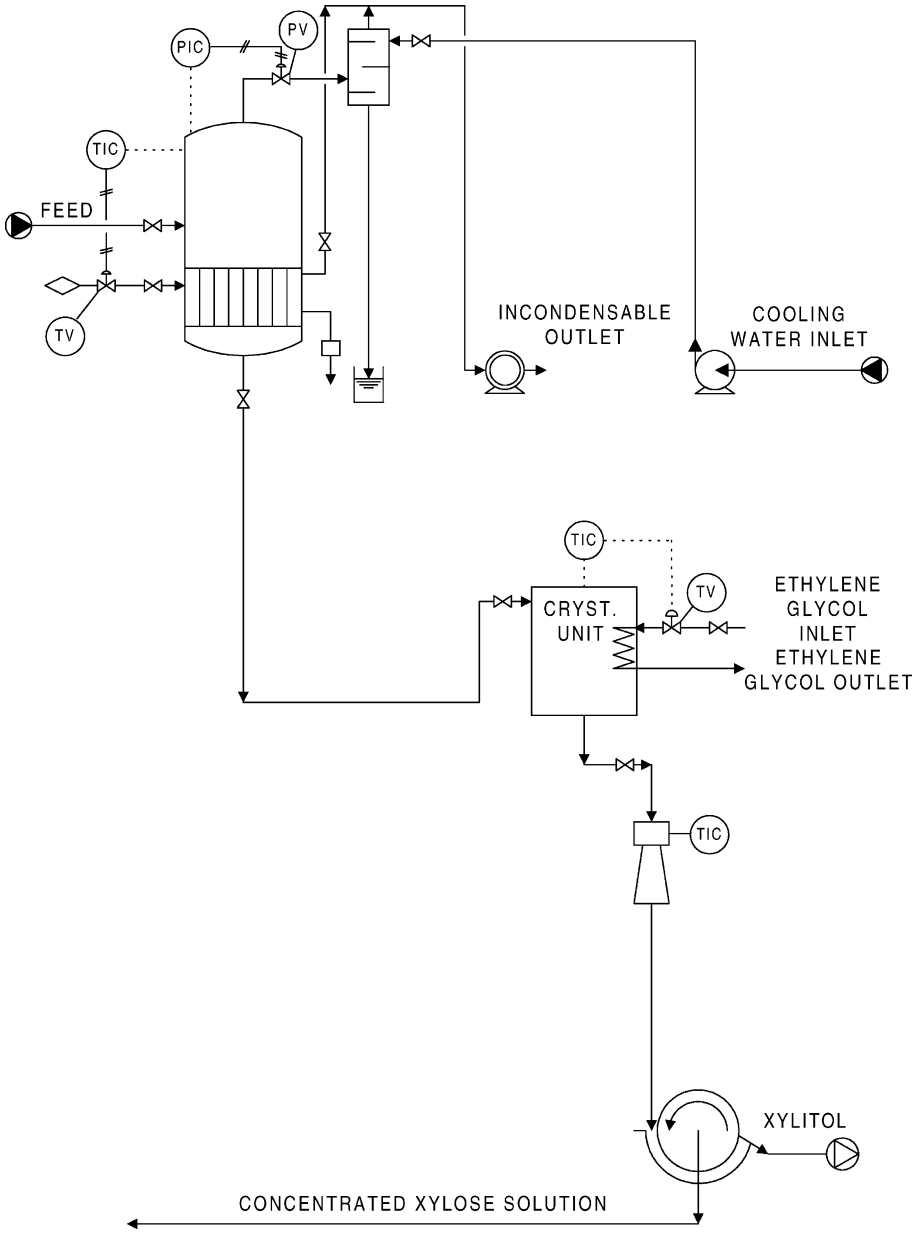

Fig. 1. Flow-sheet of the bench-scale system used for xylitol crystallization tests.

atmospheric pressure, (c) a centrifuge equipped with process parameters control system (ALC International, ALC4237R, Milan), and (d) a vacuum filtration system. Crystallization assays were done using either xylitol–xylose synthetic solutions or fermented and centrifuged hardwood hemicellulose hydrolyzate solutions with variable concentrations.

D. hansenii (NRRL Y 7426) was used for preliminary fermentations of hemicellulose hydrolyzate, which were performed in a 3 l working volume fermentor (Applikon, Z61103CT04, Schiedam, The Netherlands). The composition of the hydrolyzate as well as the conditions and analytical techniques used during these tests were previously reported [3].

To avoid any possible organic compounds degradation and to prevent any liquid loss, at the beginning of each experiment the solution was concentrated in rotavapor at low pressure (1.6×10^4 Pa), low temperature (30–50 °C), and slow rotational speed (45–50 rpm). After concentration, xylitol–xylose solutions were subjected to crystallization in a simple crystallization unit assembled in our laboratory. It consisted of 25 ml glass test-tubes submerged into an ethylene glycol bath (Julabo, F25, Seelbach, Germany) and gently stirred by means of a mechanical system. As soon as the crystallization temperature was reached, finely ground commercial xylitol was added, up to 1.0 g/l, to favor the nucleation of xylitol crystals. After crystallization,

the solutions were subjected to centrifugation at 5000 rpm for 15 min to separate xylitol crystals. Temperature was maintained close to the crystallization temperature (−10, −5, 0 °C) by means of the centrifuge regulation system. Finally, xylitol crystals were separated by vacuum filtration through filters with 0.45 μm pore diameter. The crystals were re-dissolved in water and analyzed by HPLC (Bio-Rad, HRLC 800, Richmond).

2.2. Analytical methods

Xylose and xylitol concentrations either in synthetic solutions or in fermented hemicellulose hydrolyzates were determined by HPLC using an ion-exchange AMINEX (Bio-Rad, HPX-87C, Richmond) column and a refractometer (Bio-Rad, 1770, Richmond). A 30% acetonitrile–water solution was used as mobile phase. Calibration curves were obtained by injecting standards solutions with concentrations ranging from 3.0 to 15 g/l xylose and 9.0 to 45 g/l xylitol. The analyses were carried out at 70 °C and a flow rate of 0.6 ml/min.

3. Results and discussion

The experimental study was subdivided in three subsequent phases. Preliminary evaporation and crystallization tests were carried out at two different starting xylitol (270 and 360 g/l) and xylose (93 and 116 g/l) concentrations and two different temperatures (−10 and −15 °C) to point out the temperature–concentration limit under which the crystallization phenomenon did not occur or a sudden and fast crystal and ice mixture became visible. Crystallization experiments were then carried out using xylitol–xylose solutions at a xylitol concentration beyond the solubility limit, which was determined through preliminary assays. For this purpose, two different starting xylitol concentrations (582 and 730 g/l) were tested at three different temperatures (−10, −5 and 0 °C) to determine the best crystallization conditions and to recover the product as pure as possible. A final set of crystallization tests was performed using hardwood hemicellulose hydrolyzate solutions.

3.1. Evaporation tests

The operative conditions of evaporation tests on both synthetic and fermented solutions are listed in Table 1. These results demonstrate that the low pressure evaporation process allowed to concentrate the solutions, with starting concentration similar to that obtained by fermentation, up to very high levels, thus simplifying the subsequent crystallization process and making it as profitable as possible. To obtain limpid fermented hemicellulose hydrolyzate solutions, necessary for efficient crystallization, the suspension from test no. 9 was finally filtered up to 436 g/l xylitol and 137 g/l xylose concentrations.

Table 1
Operative conditions used in evaporation tests[a]

Test	T_e (°C)	t_e (h)	Xyt_0 (g/l)	Xyt_e (g/l)	Xyl_0 (g/l)	Xyl_e (g/l)
1	30–40	1.5	135	270	45	93
2	30–40	1.5	180	360	60	116
3	30–40	1.5	180	360	60	116
4	40–50	2.0	225	582	75	203
5	40–50	2.0	225	582	75	203
6	40–50	1.0	225	582	75	192
7	40	1.0	390	730	130	223
8	40	1.0	390	730	130	223
9	30	4.0	75	361	24	113
10	30	1.5	75	470	24	147

[a] Synthetic xylose–xylitol solutions: test nos. 1–8; fermented hemicellulose hydrolyzate solutions: test nos. 9–10; T_e: evaporation temperature; t_e: evaporation time; Xyt_0: starting xylitol concentration; Xyt_e: xylitol concentration after evaporation; Xyl_0: starting xylose concentration; Xyl_e: xylose concentration after evaporation.

3.2. Crystallization tests

The tests were carried out at atmospheric pressure and maintaining constant the selected temperature during every experiment. Table 2 shows the values of preliminary tests, carried out on some solutions referred to as in Table 1, under conditions that did not allow any crystallization. The lowest xylitol solubility limits were so determined at the selected temperatures. In fact, because of xylitol solubility reduction due to the simultaneous presence of xylose or by-products in the solutions, previous data referring to temperatures higher than 0 °C and to pure xylitol solutions [10] are of scarce significance for the purposes of the present work.

Test no. 1 ($T = -10$ °C; 270 g/l xylitol) and no. 2 ($T = -10$ °C; 360 g/l xylitol) gave limpid solutions, while the solution used for test no. 3 ($T = -15$ °C; 360 g/l xylitol) froze after about 16 h, so that it was impossible to make any analytical control. Finally, even during test no. 9 ($T = -10$ °C; 436 g/l xylitol), carried out on hemicellulose hydrolyzate, the crystallization phenomenon did not take place. Since the above results demonstrated that solutions needed to be remarkably concentrated to allow xylitol crystallization, xylose and xylitol concentrations were increased proportionally to maintain a constant ratio between their concentrations. The results of crystallization tests performed at different temperatures ($T = -10, -5, 0$ °C) on solutions

Table 2
Preliminary crystallization tests[a]

Test	T_c (°C)	t_c (h)	Xyt_e (g/l)	Xyt_c (g/l)	Xyl_e (g/l)	Xyl_c (g/l)
1	−10	4	270	270	93	93
2	−10	20	360	360	116	116
3	−15	16	360	n.d.	116	n.d.
9	−10	5	436	429	137	136

[a] T_c: crystallization temperature; t_c: crystallization time; Xyt_e: xylitol concentration after evaporation; Xyt_c: xylitol concentration after crystallization; Xyl_e: xylose concentration after evaporation; Xyl_c: xylose concentration after crystallization. Test numbering is the same as in Table 1.

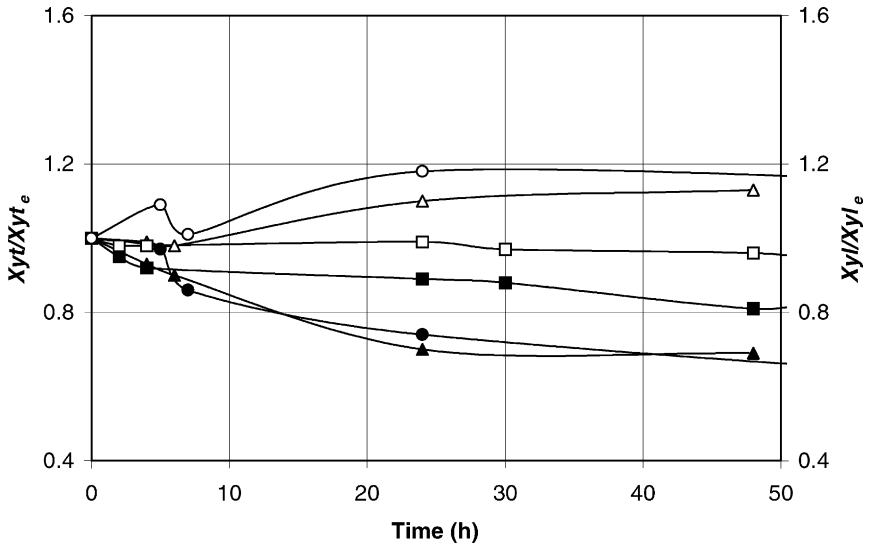

Fig. 2. Crystallization tests at a supersaturation xylitol concentration of 582 g/l. Xylitol: (●) test no. 4, (▲) test no. 5, (■) test no. 6; xylose: (○) test no. 4, (△) test no. 5, (□) test no. 6.

containing 582 and 730 g/l xylitol are shown in Figs. 2 and 3, respectively, while Fig. 4 illustrates the performance on fermented hemicellulose hydrolyzate ($T = -10\,^{\circ}$C; 470 g/l xylitol).

As a general rule, a decrease in temperature and an increase in concentration both favored the crystallization, while the generalized growth in xylose concentration, observed during the starting phases of these tests, was likely due to the volume reduction consequent to xylitol precipitation. Besides, the concentration changes shown after preliminary crystallization should be ascribed to experimental uncertainties rather than to changes in the mass of crystals deposited. As shown in more detail later, the highest crystallization yield (0.56) was obtained at 730 g/l xylitol and $-5\,^{\circ}$C. It should be noticed that this test was performed at a particularly high supersaturation ratio (1.25), with respect to the industrial practice, because of the lack of information in the literature about xylitol solubility in binary mixtures with xylose. However, the aim of this preliminary study was obtaining experimental data of xylitol solubility, at different temperatures, which are necessary for further and more in-depth works. On the contrary, the experiment at $-10\,^{\circ}$C and 730 g/l did not give satisfactory results because at such high concentration and low temperature solid precipitation was too fast. Xylose and xylitol mass balances demonstrated that the crystallization procedure selected in this study was satisfactorily accurate, ensuring a crystal loss never exceeding 10%.

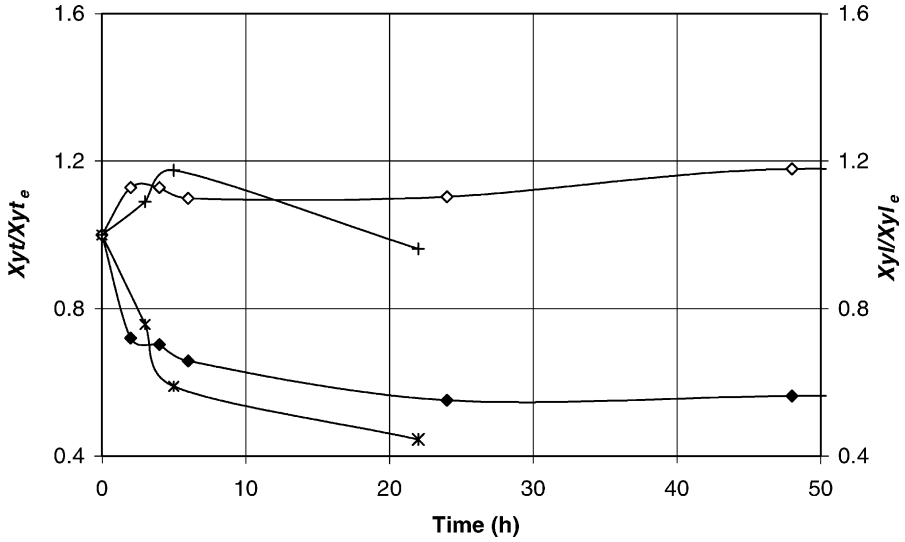

Fig. 3. Crystallization tests at a supersaturation xylitol concentration of 730 g/l. Xylitol: (✱) test no. 7, (◆) test no. 8; xylose: (+) test no. 7, (◇) test no. 8.

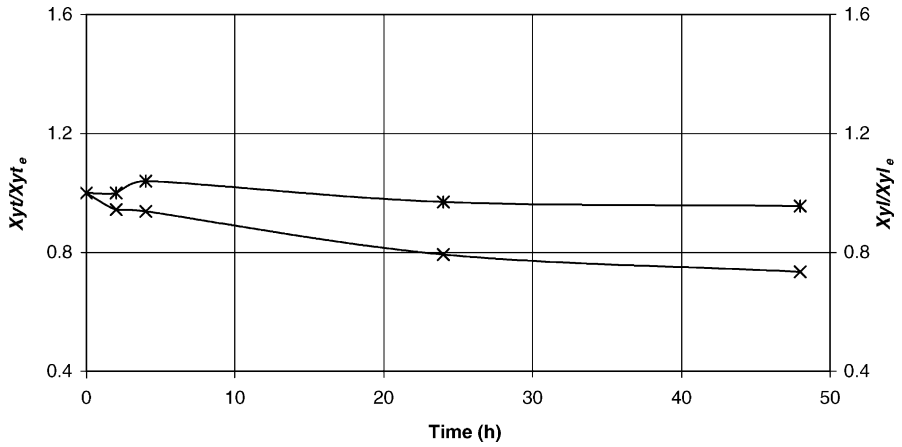

Fig. 4. Crystallization test no. 10 on fermented hemicellulose hydrolyzate: (×) xylitol; (✱) xylose.

Final xylitol concentrations detected at the end of crystallization tests on synthetic solutions were utilized to obtain the xylitol solubility curves at different concentrations shown in Fig. 5. These curves give useful preliminary information to build up a mathematical model that is able to describe the dependency of crystallization efficiency on mixture concentration.

Table 3 lists the values of the total crystallization yield (Y_c), defined as the ratio of the total mass separated by precipitation to the starting mass of xylitol in the solution, of the purity degree (PD), calculated as the ratio of xylitol to the total mass of the recovered precipitate, and of the xylitol yield (Y_{xyt}), expressed as the total crystallization yield multiplied by the PD. These results on the whole show a satisfactory total crystallization yield and excellent PD, close to 1.00. As previously mentioned, the best results were obtained at 730 g/l xylitol and −5 °C: under these conditions most of xylose was kept in the solution, whereas about 50% xylitol was recovered. Finally, despite the presence of additional solutes imposed to perform test no. 10 on hemicellulose hydrolyzate at quite lower xylitol concentration (470 g/l), yields and PD comparable to those on synthetic solutions were obtained.

Table 3
Results of crystallization tests performed under different conditions[a]

Test	T_c (°C)	Xyt_e (g/l)	Y_c	PD	Y_{xyt}
4	−10	582	0.41	0.92	0.38
5	−5	582	0.31	1.00	0.31
6	0	582	0.20	0.98	0.20
7	−5	730	0.56	1.00	0.56
8	0	730	0.48	0.93	0.45
10	−10	470	0.29	0.92	0.27

[a] T_c: crystallization temperature; Xyt_e: xylitol concentration after concentration; Y_c: total crystallization yield; PD: purity degree; Y_{xyt}: xylitol yield.

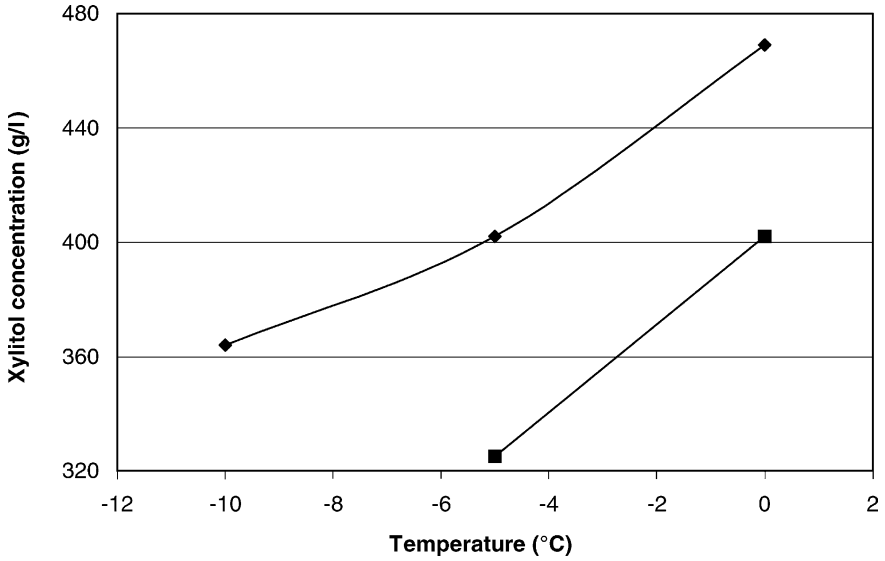

Fig. 5. Xylitol solubility dependence on crystallization temperature in the simultaneous presence of xylose: (◆) Xyt_e = 582 g/l, Xyl_e = 203 g/l; (■) Xyt_e = 730 g/l; Xyl_e = 223 g/l.

3.3. Crystallization kinetics

According to the methodology reported by Bravi and Mazzarotta [11,12], crystallization kinetics, for starting limpid solutions, can be described by a supersaturation function:

$$r = k(X_r - X_r^*)^m \quad (1)$$

where X_r is the actual solution concentration, X_r^* the saturation concentration at a given temperature, k a kinetic constant and m the growth rate order. Eq. (1) may not be applied to ternary systems, since the driving force for the crystal growth cannot be defined properly. However it can be adopted as an assumption of simplification. This rate can also be expressed as

$$r = -b\frac{dX_r}{dt} \quad (2)$$

where b is a proportionality parameter depending on temperature.

Combining Eqs. (1) and (2)

$$\frac{dX_r}{dt} = -k'(X_r - X_r^*)^m \quad (3)$$

where k' is the crystallization kinetic constant.

Since our solutions held two compounds at relatively high concentrations, the actual concentration X_r is given by the sum of the concentrations of both components and the term $(X_r - X_r^*)$ becomes

$$X_r - X_r^* = \mathrm{Xyt} + \mathrm{Xyl} - (\mathrm{Xyt} + \mathrm{Xyl})^* \quad (4)$$

Because xylose concentration was kept almost constant during crystallization, Eq. (4) becomes

$$(\mathrm{Xyt} + \mathrm{Xyl})^* = \mathrm{Xyt}^* + \mathrm{Xyl} \quad (5)$$

Table 4
First and second-order kinetic constants estimated for xylitol crystallization under different conditions

Test	$\mathrm{Xyt_e}$ (g/l)	T_c (°C)	k' $(\mathrm{h^{-1}})_{m=1}$	r^2	k' $(\mathrm{l\,g^{-1}\,h^{-1}})_{m=2}$	r^2
4	582	−10	0.049	0.952	0.396	0.955
5	582	−5	0.065	0.999	0.428	0.999
6	582	0	0.034	0.654	0.465	0.760
7	729	−5	0.250	0.956	1.213	0.851
8	729	0	0.266	0.802	1.650	0.928
10	470	−10	0.064	0.991	1.165	0.991

Combining Eqs. (4) and (5), we obtain

$$X_r - X_r^* = \mathrm{Xyt} - \mathrm{Xyt}^* \quad (6)$$

where only xylitol concentration appears.

According to this model, the experimental results presented in Section 3.2 were correlated with either the first or the second growth rate order ($m = 1$ and 2). Data seemed to satisfy, with better approximation ($0.76 < r^2 < 1.00$), second-order kinetics, according to the following equation:

$$\frac{1}{\mathrm{Xyt} - \mathrm{Xyt_c}} = \frac{1}{\mathrm{Xyt_e} - \mathrm{Xyt_c}} + k't \quad (7)$$

obtained by integrating Eq. (3) and combining with Eq. (6), even if first-order kinetics ($0.65 < r^2 < 1.00$) did not constitute a bad interpretation as well. In fact, as shown in Table 4, only experiment no. 7 seemed to be better correlated with first-order kinetic. This result is in good agreement with those reported in the literature for solutions of different organic compounds, which satisfied crystallization kinetics with order ranging from 1.18 to 2.64 [11].

Plotting in Figs. 6 and 7 the crystallization results, integrating Eq. (3) either with $m = 1$ or 2, the values listed in Table 4 were calculated for the crystallization kinetic constant, k'. The highest values of both first- and second-order

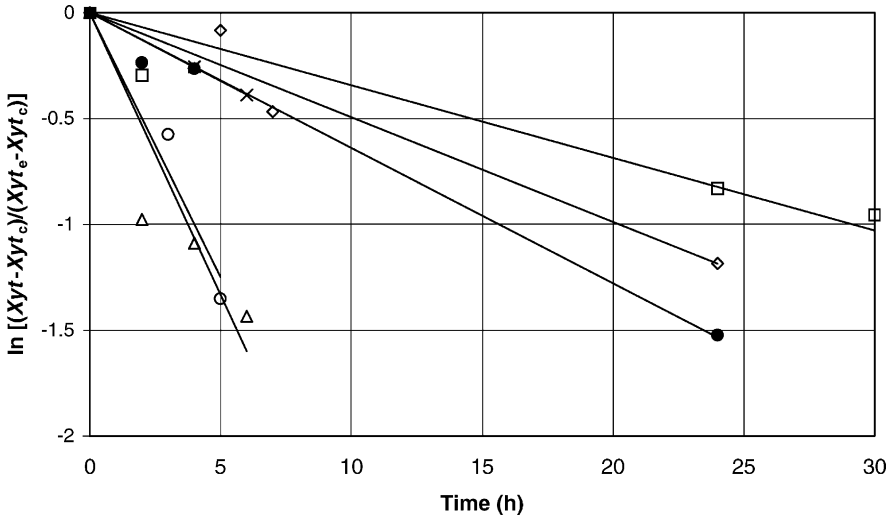

Fig. 6. Results of xylitol crystallization tests plotted according to first-order kinetics: (◇) test no. 4; (×) test no. 5; (□) test no. 6; (○) test no. 7; (△) test no. 8; (●) test no. 10.

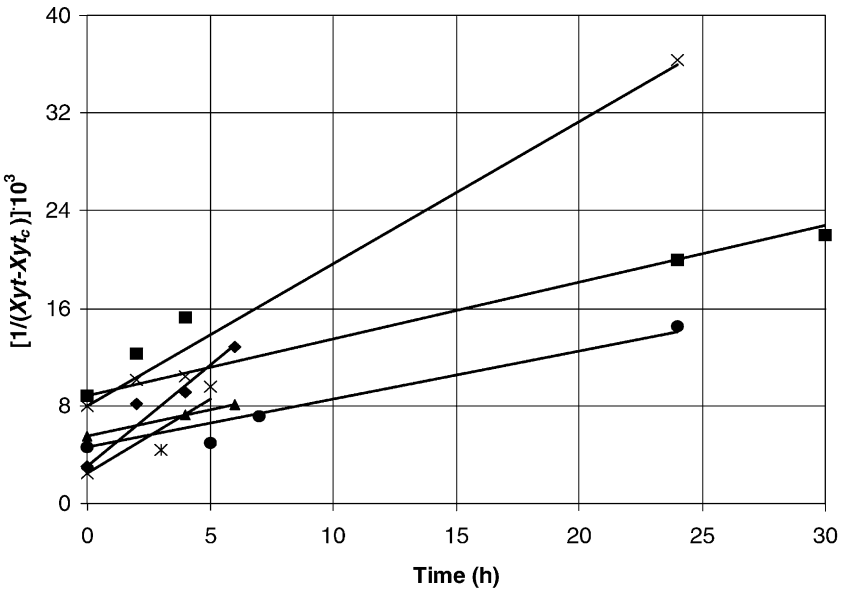

Fig. 7. Results of xylitol crystallization tests plotted according to second-order kinetics: (●) test no. 4; (▲) test no. 5; (■) test no. 6; (✕) test no. 7; (◆) test no. 8; (×) test no. 10.

kinetic constants were obtained with test nos. 7 and 8 because the large amount of dissolved compounds remarkably increased xylitol crystallization rate. In particular, test no. 10, performed on hemicellulose hydrolyzate, gave better results than tests on synthetic solutions with even higher starting xylitol concentrations (test nos. 4–6). In fact, organic compounds present in fermented hemicellulose hydrolyzate (xylose, arabinose, arabitol, mannose, mannitol, etc.) lowered the whole broth solubility, thus aiding xylitol crystallization. Finally, the fact that our crystallization experiments were of short duration than those reported in the literature for an analogous system [9] is noteworthy.

4. Conclusions

The data collected in this work on xylitol crystallization from synthetic solutions and fermented hemicellulose hydrolyzate, in the simultaneous presence of xylose, are quite encouraging: yield even exceeded 0.50 and crystal PD was always >0.90. The best results were obtained with quite concentrated solutions (730 g/l) at relativity high temperature (about −5 °C). The experimental results allowed us to approximately evaluate the crystallization kinetics as a function of supersaturation and total solute concentration. Crystallization tests followed second-order kinetics, even though first-order kinetics also described the process reasonably well.

Further studies are planned to confirm the validity of the proposed kinetic model as well as to clarify the influence of xylose concentration on the general economy of the process. In fact, since total xylitol and xylose concentration must be equal to the solubility threshold and xylose concentration keeps almost constant during crystallization—provided it does not exceed the saturation curve—xylitol concentration decreases as that of xylose increases.

According to the present results, which are far to be exhaustive, it is possible to conclude that xylitol separation by crystallization from fermented hemicellulose hydrolyzate is feasible.

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