Journal of Chromatography B, 878 (2010) 2023–2028

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Separation of fructooligosaccharides using zeolite fixed bed columns

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ARTICLE INFO

Article history:
Received 24 February 2010
Accepted 25 May 2010
Available online 2 June 2010

Keywords:
Fixed bed column
Fructooligosaccharides
Zeolites
Separation
Experimental design
Adsorption

ABSTRACT

Recent studies have shown that the chromatographic separation of mixtures of monosaccharides and disaccharides may be improved by employing Y zeolites, a procedure which holds promise in the separation of oligosaccharides. In the present study, a column packed with zeolite was employed to study the separation of fructooligosaccharides (FOS). FOS were produced by an enzyme isolated from *Rhodotorula* sp., which produces GF_2 (kestose), GF_3 (nystose) and GF_4 (frutofuranosyl nystose). The identification and quantification of the sugars were carried out by ion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The separation of fructooligosaccharides was carried out using a fixed bed column packed with Ba^{2+}-exchange Y zeolites. The effects of temperature (40–50 °C), injected volume per bed volume (2.55–7.64%), superficial velocity (0.1–0.15 cm min^{-1}) and eluent composition (40–60% ethanol) were investigated using a fractionary factorial design with separation efficiency as the response. The results showed that the most favorable conditions for the separation of the oligosaccharide–glucose mixture were 60% ethanol as eluent, temperature of 50 °C, superficial velocity of 0.1 cm min^{-1} and 2.55% injection volume per bed volume of injection mixture, using two columns in series. The values for separation efficiency were 0.60 for oligosaccharide–glucose, 1.00 for oligosaccharide–fructose, 0.22 for oligosaccharide–sucrose, 0.43 for glucose–fructose, 0.82 for glucose–sucrose and 1.23 for fructose–sucrose.

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1. Introduction

Fructooligosaccharides (FOS) such as 1-kestose (GF_2), nystose (GF_3), and 1^F-fructofuranosyl nystose (GF_4) are naturally occurring sugars that represent a major class of fructan oligosaccharides [1]. They are also known as neo-sugars and have numerous beneficial, favorable functional properties, including improvement of the intestinal microflora [2], non-cariogenicity, being diabetic-friendly products, there are clinical studies showing that in some situations they can decrease the serum levels of cholesterol, phospholipids and triglycerides [3,4] and of relieving constipation [5]. FOS have been widely used in bio-industries as sweeteners because of their favorable functional properties [6].

Fructooligosaccharides may be produced by invertases (β-fructofuranosidase) or transferases (β-D-fructosyltransferase) [7], and many studies have reported the production of FOS by fungi such as *Aspergillus niger* [8] and *Aspergillus japonicus* [9]. However, only a few studies have described the production of these oligosaccharides using extracellular enzymes, such as those using *Kluyveromyces* sp. [10], and *Rhodotorula* sp. [11]. The use of two mixed enzyme systems for the production of very pure FOS has been reported in the literature, the first using glucose oxidase and fructosyltransferase [12] and the second using glucose oxidase, catalase and β-fructofuranosidase [13]. After using either of these systems, no further purification is necessary since the FOS produced will already be very pure (about 90%). Purification by nanofiltration is another alternative that has been used in the purification of high molecular weight oligosaccharides [14–16]. FOS marketed actually is obtained from the enzymatic hydrolysis of inulin by inulinase [17] and consists of linear units of fructosyl, this product is commercially known as “Raftilose”, produced by Beneo-Orafti (Belgium) or as “Frutafit”, produced by Sensus (Netherlands).

Purification of the FOS by removing the glucose and sucrose present in the mixture would increase its market value, leading to the use of FOS as an ingredient in products aimed at diabetics [18].

Zeolites are naturally occurring hydrated aluminosilicate minerals. They are a large group consisting of some 40–50 different natural minerals and numerous synthetic forms [19]. The zeolites have high exchange capacity, selectivity and specificity, and good resistance to radiation. The synthetic zeolites are divided mainly into zeolites A, X and Y, being that X and Y zeolites have advantages compared with others zeolites, the largest pore size, about

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doi:10.1016/j.jchromb.2010.05.039

7–8 Å. They also show advantages for immobilization when compared with organic ion exchangers [20]. The zeolites have been used in several applications including the removal of ammonia [21] and of color in the treatment of effluents [22]. The purification of FOS on zeolite fixed bed columns represents little used methodology, but can be applied in the food industry since the zeolites have already been applied in other sectors for the purification, adsorption and separation of compounds and in the chemical industry. Zeolites are already in use in the food industry. Zeolites saturated with CO_2 provide instantaneous carbonation to aqueous preparation. Beer is stabilized with NaA and LiX zeolites, which adsorb the proteins responsible for further degradation. The dealcoholization of beer is done with the help of the dealuminated zeolite Y. The fatty acids of comestible oil are eliminated on zeolite X [23]. Zeolites show promise in column separation procedures, since they can be regenerated, making them financially viable.

In recent years, much research regarding enzymatic processes for obtaining pure sugars has been carried out in the DEA Bioprocess Engineering Laboratory (FEA-UNICAMP-BRAZIL), including the production of pure fructose from a glucose mixture on fixed bed zeolite columns [24] and the purification of dextran, glucose and sucrose using Y zeolite fixed bed columns [25].

Papers can also be found in the literature describing the separation of sugars, especially the separation of glucose and fructose using zeolites exchanged with different ions (Na^+, Ba^{2+}, K^+, Mg^{2+}) [26], and using dealuminate beta zeolites in the separation of isomaltose and glucose [27]. However, no reports have been published on the separation of oligosaccharides, particularly fructooligosaccharides, using zeolite fixed bed columns. Thus the discussion presented in the present paper will be limited to the results found in this study.

The goal of this work was to determine the most suitable conditions for the operating variables of a fixed bed column used to separate FOS from a mixture of sugars containing glucose, fructose and sucrose in addition to the FOS.

2. Materials and methods

2.1. Microorganism and culture conditions

The *Rhodotorula* sp. (LEB-V10: Laboratory of Bioprocess Engineering-UNICAMP) was maintained on agar slants: (2% glucose, 0.5% yeast extract, 1% malt extract, 0.2% Na_2HPO_4, 2% agar) at 5 °C. Cultivation of the strain for enzyme production was carried out in liquid culture: (2% glucose, 2% peptone, 1% yeast extract and 0.5% K_2HPO_4, pH 4.5) at 30 °C for 40 h. For enzyme production, 20 ml of inoculum were transferred to a 500 ml flask containing 200 ml of production medium, and cultured at 30 °C for 36 h, shaken at 250 rpm. The production medium consisted of (per litre): 65 g sugar cane molasses and 90 g corn steep liquor. After cultivation, the cells were separated by centrifugation (5 °C) (7500 rpm, 10 min) and the enzyme in the supernatant recovered as follows: ethanol was added to the supernatant solution at −20 °C up to a final concentration of 70% (v/v) in a stirred reactor at 2 °C under mild agitation. The precipitate was centrifuged, recovered and re-dissolved in sodium acetate buffer (0.05 M, pH 4.5). The enzyme activity was determined according to Kuhn and Maugeri [28].

2.2. Chemicals

The kestose (GF_2), nystose (GF_3) and fructofuranosyl nystose (GF_4) obtained from Wako Pure Chemical Industries (Osaka, Japan), and the sucrose, glucose and fructose obtained from Sigma, were all of analytical grade.

2.3. Analysis of the sugars

The identification and quantification of the sugars (sucrose, glucose, fructose, and FOS) were carried out by ion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The chromatography was performed on a Carbopac PA100 (4 mm × 250 mm) column with a PA100 (4 mm × 50 mm) guard column at 22–24 °C, using a GP50 gradient pump, ED40 electrochemical detector and the software PEAKNET, all from Dionex (USA). The sugars were eluted in 50 mM sodium hydroxide with a linear gradient of sodium acetate (0–500 mM), at a flow rate of 1.0 ml min^{-1}. Before injection, the samples were filtered through 0.45 μm filters and diluted with water when necessary.

2.4. Fructooligosaccharides synthesis

The 50% sucrose solution (w/v) and the enzyme (5 U ml^{-1}) were incubated in sodium acetate buffer (pH 4.5) at 50 °C [11]. Samples were taken and the enzyme inactivated by heating in boiling water for 5 min. The sugars present were analyzed using HPAEC-PAD.

3. Experimental design

A fractional experimental design was employed to check which variables had significant effects on the efficiency of sugar separation. The parameters examined were temperature, (%) injection volume per bed volume, superficial velocity and eluent composition. The levels and variables are shown in Table 1.

3.1. Zeolites

The zeolite NaY (Bayer S.A.) was employed in the Ba^{2+} form composed of: Na_2O (10.57%), Al_2O_3 (16.97%), SiO_2 (58.93%). The moisture content of the zeolite was first established in a muffle furnace at 700 °C. The amount of ions to be exchanged for the equivalent amount of grams of Na_2O present in the zeolite, was then calculated (10.57% in the original zeolite). The amount of zeolite, water and saline were also calculated aiming at a final concentration of 15% solids, which is actually equivalent to the dry zeolite present within the ionic exchange reactor. The amount of zeolite calculated was suspended in water and the pH calibrated to between 5 and 6 with 10% hydrochloric acid. A 35% barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) solution was then added, according to the stoichiometrics required for exchange. The final suspension was poured into centrifuge tubes and placed under constant, mild agitation for 24 h. The temperature for exchange was 75 °C, adjusted by means of a temperature controlled water bath. At the end of 24 h, the suspension was filtered through a Buchner funnel and washed twice. It was first washed with a 35% solution of $BaCl_2 \cdot 2H_2O$, using the same amount used in the exchange. The second wash was

Table 1
Definitions and levels of the independent variables in the 2^{4-1} fractional factorial design.

Independent variables	Symbol	Coded and real levels (in parentheses)		
Temperature (°C)	X_1	−1 (40)	0 (45)	+1 (50)
Superficial velocity (cm min^{-1})	X_2	−1 (0.10)	0 (0.13)	+1 (0.15)
Injection volume/bed volume (%)	X_3	−1 (2.55)	0 (5.1)	+1 (7.64)
Ethanol (%)	X_4	−1 (40)	0 (50)	+1 (60)

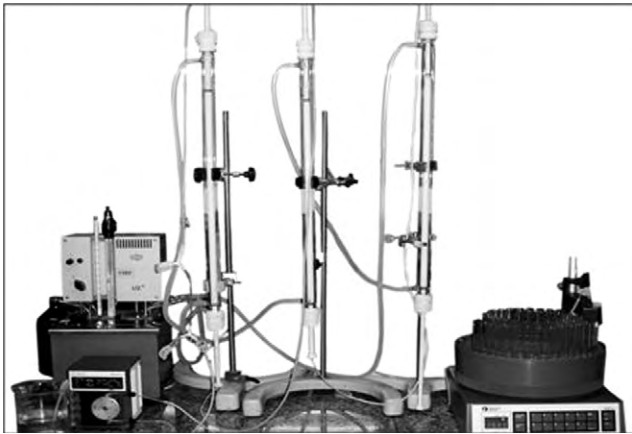

Fig. 1. Experimental set-up for the zeolite fixed bed column system.

carried out with deionized water, using a volume twice that employed in the exchange.

4. Experimental system

The experimental system consisted of three identical water-jacketed columns, each 50 cm × 1 cm i.d., packed with BaY zeolite (Fig. 1). The eluent and feed flow rate were controlled by metered pumps. The temperature within the system was maintained by circulating hot water at a constant temperature through the jackets of all the columns. Samples were taken and the sugars analyzed using HPAEC-PAD.

A statistical factor is defined to characterize the efficiency of separation [29]. For components 1 and 2, $\Delta t_{1,2}$ is defined as:

$$\Delta t_{1,2} = |t_1 - t_2| \tag{1}$$

where

$$\bar{t}_i = \frac{\int_0^{\infty} t_i \omega_i \phi \, dt}{\int_0^{\infty} \omega_i \phi \, dt} \tag{2}$$

where ω_i is the wt% of component i in the effluent and ϕ is the mass flow rate of the mixture. The efficiency of separation is then defined as:

$$(\mathrm{ES})_{12} = \frac{\Delta t_{12}}{\delta_{12}} \tag{3}$$

where

$$\delta_{12} = (\delta_1 \delta_2)^{1/2} \tag{4}$$

$$\delta_i^2 = \frac{\int_0^{\infty} (t_i - \bar{t}_l) \omega_i \phi \, dt}{\int_0^{\infty} \omega_i \phi \, dt} \quad i = 1, 2 \tag{5}$$

Obviously, the largest the factor $(\mathrm{ES})_{12}$ represents the greater efficiency of separation.

5. Results and discussion

5.1. Fractional experimental design

In the present study the BaY zeolite was used on account of the studies carried out by Burkert [25], who studied different ion exchangers (Ba-Y, Ca-Y, Sr-Y, KY) and eventually selected the zeolite BAY for the separation of fructose, glucose and dextran. Zeolite Y was used since the sugar mixture of interest in the separation contained glucose and fructose amongst other sugars, and, according to Burkert [25], NaY zeolite exchanged with barium shows affinity for fructose and glucose.

Table 2 shows the separation efficiency obtained using the 2^{4-1} fractional experimental design. The efficiency varied according to the temperature (°C) (X_1), superficial velocity (cm min^{-1}) (X_2), (%) injection volume/bed volume (X_3) and eluent concentration (%) (X_4).

The fractional experimental design mainly analyzed the separation efficiency between the oligosaccharides and glucose, since these sugars were produced in higher concentrations.

Table 3 shows that only the superficial velocity had a significant effect ($p < 0.1$) on the separation efficiency between oligosaccharides and glucose. The data for separation efficiency between the oligosaccharides and fructose showed that the variables temperature, superficial velocity and eluent concentration had significant effects ($p < 0.1$). The superficial velocity and the temperature had negative effects, showing that the lower the superficial velocity and temperature, the better the separation efficiency. The eluent concentration showed a positive effect, the higher the eluent concentration, the better the separation efficiency. On analyzing the separation efficiency between the oligosaccharides and sucrose, none of the variables studied presented a significant effect on the response as can be seen in Table 3. However sucrose was found at relatively low concentrations in the mixture and can be reduced by means of an optimized synthesis aiming at maximum-conversion, resulting in a minimal content of this carbohydrate.

On analyzing the separation efficiency between glucose and fructose, one can see that the variables of temperature, superficial velocity and ethanol concentration presented effect at the 10% significance level, as seen in Table 3, the ethanol concentration showing a positive effect on the response and the temperature and superficial velocity negative effects.

Table 3 shows, that the variables of superficial velocity and ethanol concentration presented an expressive effect at the 10% significance level on the separation efficiency between glucose and sucrose, the ethanol concentration presenting a positive effect and the superficial velocity a negative effect. On analyzing the separa-

Table 2
Data for the separation efficiencies obtained using the 2^{4-1} factorial design.

Trial	Coded levels				Separation efficiencies (ES)					
	X_1	X_2	X_3	X_4	$ES_{olig-gluc}$	$ES_{olig-fruct}$	$ES_{olig-suc}$	$ES_{gluc-fruct}$	$E_{gluc-suc}$	$ES_{ftruct-suc}$
1	−1	−1	−1	−1	0.30	0.70	0.10	0.42	0.41	0.82
2	+1	−1	−1	+1	0.37	0.84	0.12	0.50	0.50	0.96
3	−1	+1	−1	+1	0.13	0.48	0.09	0.37	0.23	0.58
4	+1	+1	−1	−1	0.012	0.19	0.06	0.18	0.07	0.25
5	−1	−1	+1	+1	0.39	0.95	0.10	0.57	0.50	1.09
6	+1	−1	+1	−1	0.09	0.09	0.11	0.19	0.02	0.21
7	−1	+1	+1	−1	0.04	0.29	0.07	0.27	0.11	0.37
8	+1	+1	+1	+1	0.05	0.21	0.15	0.26	0.20	0.08
9	0	0	0	0	0.36	0.87	0.11	0.52	0.48	1.00
10	0	0	0	0	0.32	0.73	0.07	0.43	0.39	0.81
11	0	0	0	0	0.46	0.89	0.04	0.44	0.51	0.96

Table 3
Effect of the variables on the separation efficiencies between sugars at a 90% level of confidence ($p < 0.1$).

	Temperature (°C)			Superficial velocity (cm min^{-1})			Injection volume/bed volume (%)			Ethanol		
	Effect	Error	*p*-Value	Effect	Error	*p*-Value	Effect	Error	*p*-Value	Effect	Error	*p*-Value
$ES_{olig-gluc}$	−0.085	0.098	0.4225	−0.230	0.098	0.0580'*	−0.061	0.098	0.5604	0.125	0.098	0.2518
$ES_{olig-fruct}$	−0.273	0.062	0.0476*	−0.353	0.062	0.292	−0.168	0.062	0.1130	0.303	0.062	0.0391*
$ES_{olig-suc}$	0.020	0.023	0.4098	−0.015	0.023	0.5312	0.015	0.023	0.5312	0.030	0.023	0.2323
$ES_{gluc-fruct}$	−0.125	0.064	0.0980*	−0.150	0.064	0.0571*	−0.045	0.064	0.5073	0.160	0.064	0.0461*
$ES_{gluc-suc}$	−0.115	0.044	0.1212	−0.205	0.044	0.0434*	−0.095	0.044	0.1644	0.205	0.044	0.0434*
$ES_{fruct-suc}$	−0.340	0.195	0.1316	−0.450	0.195	0.0603*	−0.215	0.195	0.3121	0.265	0.195	0.2226

Table 4
Separation efficiencies using 50 and 60% ethanol eluents, with a column temperature of 50 °C and injected volume of 1 ml.

Superficial velocity (cm min^{-1})	$ES_{olig-gluc}$		$ES_{olig-fruct}$		$ES_{olig-suc}$		$ES_{gluc-fruct}$		$E_{gluc-suc}$		$ES_{fruct-suc}$	
	50%	60%	50%	60%	50%	60%	50%	60%	50%	60%	50%	60%
0.08	0.19	0.26	0.43	0.56	0.10	0.19	0.26	0.30	0.29	0.46	0.53	0.76
0.10	0.13	0.31	0.46	0.76	0.11	0.18	0.27	0.38	0.23	0.49	0.59	0.98
0.13	0.04	0.07	0.33	0.49	0.13	0.19	0.31	0.46	0.17	0.26	0.47	0.70

tion efficiency between fructose and sucrose (Table 3), one can see that only the superficial velocity presented a significant effect on the response, which was negative, meaning that lower superficial velocities improved the separation efficiency of these sugars.

Using the fractional experimental design, it was shown that the variable of superficial velocity was significant for all the responses studied, with the exception of the response for the separation efficiency between oligosaccharides and sucrose. Therefore triple assays were carried out where the superficial velocity was varied from 0.08 to 0.13 cm min^{-1} and all the other variables were maintained fixed. In order to define which standard values for the design to fix for the other variables, the results of the fractional design were considered, and a smaller amount of sugars injected onto the columns was shown to improve separation between the sugars. Therefore a value of 2.55% (injected volume per bed volume) was fixed for injection on to the column. The ethanol concentration was fixed at 50% (v/v), since this variable had a positive effect on all the responses studied, indicating that the higher the concentration of ethanol in the eluent, the better the separation efficiency. Some studies have reported that columns filled with celite/active charcoal (1:1) were used with 95/5 and 90/10 (v/v) water/ethanol solutions to remove the mono and disaccharides, and with 50/50 water/ethanol (v/v) solutions to recover the oligosaccharides [30]. In the present study an experimental design was used due to the significant number of variables, in order to analyze the column efficiency and behavior.

A fixed temperature of 50 °C was chosen since, according to results found in the literature, higher temperatures had a positive effect on the separation of sugars, separation only being hindered by temperatures above 60 °C, which darkened the sample due to caramelization effects [6].

Table 4 shows no significant differences in efficiency when comparing the effects of superficial velocities of 0.08 and 0.1 cm min^{-1}. Then the superficial velocity of 0.1 cm min^{-1} was chosen to continue the experiments because lower flow rates are not interesting were of no interest, since they make the separation process slower.

Table 4 presents the results using 50 and 60% (v/v) ethanol eluent, showing increased efficiency for all the responses studied using 60% ethanol (v/v) in comparison with the results presented for 50% (v/v) ethanol eluent.

Fig. 2 illustrates the concentration of sugars at the column outlet, where it was shown that fructose and glucose had greater affinities for zeolite and that FOS and sucrose showed lower retention times on the column. This occurred because the FOS have larger structures which are not adsorbed into the inner zeolite cavities (micropores), and therefore tend to leave the column first.

The zeolites present a microporous structure, the Y zeolite pores being approximately 7–8 Å [31], making it impossible for the FOS to penetrate inside the zeolite, so they have a lower residence time on the column. The difference in selectivity of Y zeolite could be caused either by the well-defined, repeating crystalline micropore structure of the zeolite, which the macroporous ion exchange resins lack, or possibly because the zeolite uses other adsorption interactions besides ionic interactions [32]. In the aluminosilicate zeolite matrix, Al^{3+} ions are located at the Si^{4+} sites [33,34], resulting in a negatively charged framework. Positive counter ions outside the framework (in the micropores) are required to balance the charge. The amount of counter ions (or acidic sites in the case of H^+) is determined by the amount of Al^{3+} present in the zeolite [32]. The cations can exert influence on the sorbate due to their ion size and force fields resulting from the interaction with the zeolite framework, in this study the Ba^{2+} ion exchange was used because this cation has better affinity for glucose and fructose.

5.2. Separation on fixed bed columns in series

Considering that the separation efficiency of the sugars was relatively low using a single column, additional assays were performed using two and three identical columns in series. Table 5 shows the

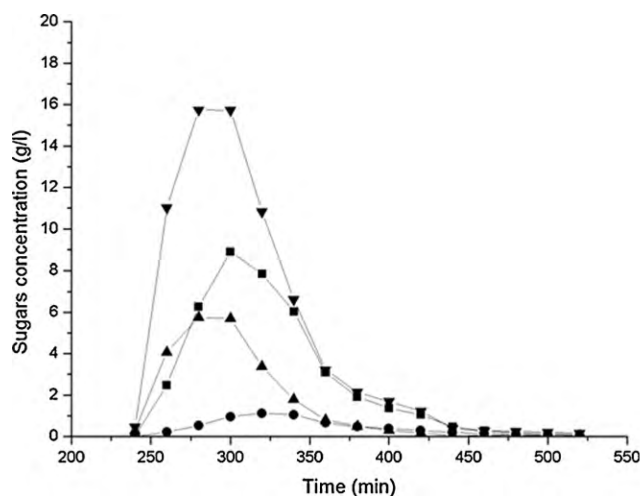

Fig. 2. Time course for the sugars leaving the fixed bed column, with a superficial velocity = 0.1 cm min^{-1}, 60% ethanol as the eluent, at 50 °C: (▼) fructooligosaccharides, (●) fructose, (■) glucose, and (▲) sucrose.

Table 5
Separation efficiencies between sugars when using 60% ethanol eluent, at 50 °C.

Assays	Superficial velocity (cm min^{-1})	$ES_{olig-gluc}$	$ES_{olig-fruct}$	$ES_{olig-suc}$	$ES_{gluc-fruct}$	$ES_{gluc-suc}$	$ES_{fruct-suc}$
One column	0.08	0.53	1.01	0.26	0.52	0.83	1.32
One column	0.10	0.55	1.00	0.16	0.49	0.73	1.20
Two columns	0.10	0.60 ± 0.04	1.00 ± 0.01	0.19 ± 0.03	0.43 ± 0.05	0.82 ± 0.08	1.23 ± 0.03
Three columns	0.10	0.44 ± 0.015	0.90 ± 0.18	0.28 ± 0.08	0.47 ± 0.15	0.75 ± 0.09	1.23 ± 0.29

Table 6
Improvement of the separation efficiency throughout the process, using an ethanol solution as eluent (*E*), at 50 °C and injected volume of 1 ml.

	$ES_{olig-gluc}$	$ES_{olig-fruct}$	$ES_{olig-suc}$	$ES_{gluc-fruct}$	$ES_{gluc-suc}$	$ES_{fruct-suc}$
Column 1 (E = 50%)	0.13	0.46	0.11	0.27	0.23	0.59
Column 2 (E = 60%)	0.31	0.76	0.18	0.38	0.49	0.98
Two columns (E = 60%)	0.60	1.00	0.19	0.43	0.82	1.23
Three columns (E = 60%)	0.44	0.90	0.28	0.47	0.75	1.23

results of the assays with two columns, which were carried out with the superficial velocities that provided the best separation efficiency in the previous assays (0.08 and 0.1 cm min^{-1}), using ethanol concentration of 60% (v/v), at 50 °C. The smaller amount of sugars injected onto the columns was shown to improve separation between the sugars. Therefore a value of 2.55% (injected volume per bed volume) was fixed for injection in all experiments.

Table 5 shows that the separation efficiencies for the superficial velocities of 0.08 and 0.1 cm min^{-1} were again similar, as occurred in the assay with one column. The addition of one more column resulted in an improvement in separation efficiency in practically all the cases, and in the particular case of separating oligosaccharides and glucose, it increased by about 93%.

Since the difference between the efficiencies for velocities of 0.08 and 0.1 cm min^{-1} was meaningless, a new set of experiments with two and three columns was performed in triplicate, using a superficial velocity of 0.1 cm min^{-1} and maintaining all the other parameters as above, in order to compare the separation performances in these cases.

Table 5 shows the results for two and three columns and it can be seen that, contrary to expectations, the separation efficiency did not increase with the addition of a third column. This was probably due to the pressure drop over the columns, which increased substantially with the addition of the third column, creating preferential channels that affected the performance.

Fig. 3 shows the time course for the sugar concentrations at the outlet of the two columns in series, where it was clear that the residence time had doubled as compared to the one-column assays, so there was practically no pressure drop. In addition the separation efficiency coefficients improved in general, as can be seen in Table 6 and Fig. 4. On the other hand, this was not observed in the tests with three columns, where a high pressure drop definitely hindered the process. Regarding the other parameters studied, an increase in the ethanol concentration from 50 to 60% (v/v) led to a positive effect, allowing for better separation.

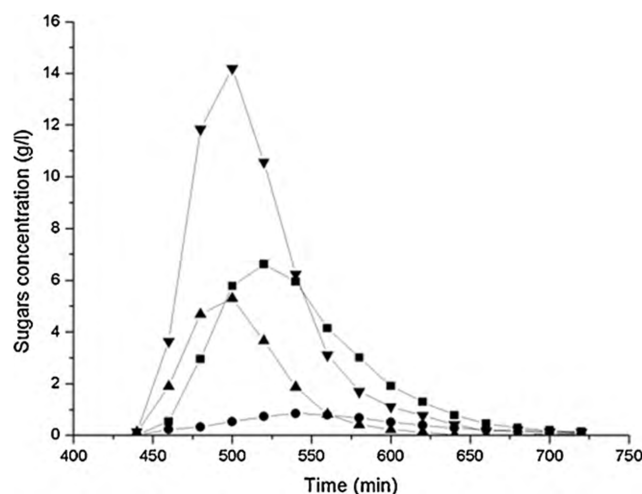

Fig. 3. Time course for the sugars leaving the two serial fixed bed columns, with superficial velocity 0.1 cm min^{-1}, 60% ethanol as the eluent and a temperature of 50 °C: (▼) fructooligosaccharides, (●) fructose, (■) glucose, and (▲) sucrose.

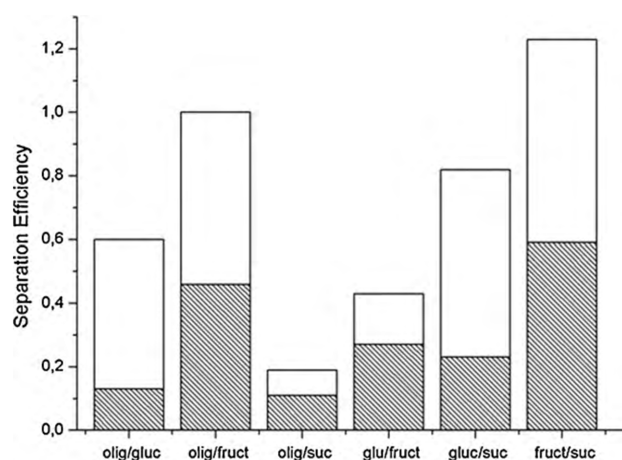

Fig. 4. Comparison of the separation efficiencies for one (hatched) and two (open) columns in series.

Nowadays in industry the process using the fixed bed columns have been used in purification processes of wastewaters and clarification sugars processes with the application of activated carbon columns. Comparing with the current industrial process the separation of sugars using fixed bed columns with zeolites has the advantage of a low-cost process due to the lower cost of application of zeolites as adsorbents in process compared with other chromatography applied (resins), and also for being a technology already well known by the industry for its application in other fields or even to other compounds. A disadvantage would be the little amount of product to be purified, as in processes such as nanofiltration large volumes can be treated.

6. Conclusion

During the preliminary stage of this study, an analysis of the results obtained using a 2^{4-1} fractional experimental design showed that, of all the variables studied, only the superficial velocity showed expressive significance at the 10% level with respect to the response of separation efficiency between the oligosaccharides and glucose. Thus basically only this response was considered in the

analysis of the results, since these sugars were present in greater concentrations. With respect to the study using columns in series, an improvement in the separation process was achieved using two columns in series, especially for the separation of oligosaccharides and glucose. When the column operational conditions were evaluated, it was observed that higher ethanol concentrations promoted better separation.

Additional work should be carried out to consider different particle diameters, so that longer column lengths would be possible. The results from this work showed that fructooligosaccharides could be separated from other sugars using zeolite columns so long as an appropriate number of parameters were correctly fitted, such as column length, particle diameter and superficial velocity.

Acknowledgement

The authors would like to thank FAPESP for their financial support.

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