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Immobilisation of fructosyltransferase from *Aspergillus aculeatus* on epoxy-activated Sepabeads EC for the synthesis of fructo-oligosaccharides

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Abstract

The transfructosylating activity present in two commercial pectinase preparations (Pectinex Ultra SP-L, from *Aspergillus aculeatus*, and Rapidase TF, from *Aspergillus niger*) was studied. Pectinex Ultra SP-L, which has a high transferase/hydrolase ratio, was covalently immobilised on a polymethacrylate-based polymer (Sepabeads® EC) activated with epoxy groups. The influence of pore volume and average pore size on biocatalyst performance was studied for two of these carriers (Sepabeads EC-EP3 and EC-EP5). Several parameters that affect immobilisation, such as buffer concentration, pH and amount (mg) of protein added per gram of support (varied over the range 30:1–200:1), were analysed. We found that Pectinex Ultra SP-L can be efficiently immobilised on these supports without adding any external salt or buffer. Using Sepabeads EC-EP5 – whose pore volume (1.67 cm^3/g) and pore size (800 nm) are higher than those corresponding to Sepabeads EC-EP3 – the activity towards sucrose reached 25.9 U/g biocatalyst. The immobilised fructosyltransferase was applied to the batch synthesis of fructo-oligosaccharides (FOS) using 630 g/l sucrose to shift activity towards transfructosylation in detriment of hydrolysis. The FOS concentration reached a maximum value of 387 g/l after 36 h (240 g/l 1-kestose, 144 g/l nystose and 3 g/l 1^F-fructofuranosyl-nystose), which corresponds to 61.5% (w/w) of the total carbohydrates in the mixture. The features of these immobilised biocatalysts are very attractive for their application in batch and fixed-bed bioreactors.

Keywords: β-Fructofuranosidase; Transfructosylase; Prebiotics; Polymethacrylate; Pectinex Ultra SP-L

1. Introduction

There is now an array of first-generation oligosaccharides available at industrial scale that are used as food ingredients due to their prebiotic properties [1–3]. As they are selectively fermented by Bifidobacteria, the prebiotics boost the total number of these microorganisms present in the colon, causing a number of beneficial effects on our health [3]. Addition of such molecules to food products may help to prevent illness, control calcium balance and contribute to the reduction of antibiotic consumption [4]. Fructo-oligosaccharides (FOS) constitute one of the most established groups of prebiotic oligosaccharides in the world. FOS are fructose oligomers with a terminal glucose group, in which 2–4 fructosyl moieties are linked via β(1 → 2)-glycosidic bonds. Their structural formula is α-D-glucopyranosyl-(1 → 2)-[β-D-fructofuranosyl-(1 → 2)-]$_n$ (GF_n). Commercial FOS, mainly composed of 1-kestose (GF_2), nystose (GF_3) and 1^F-fructofuranosyl-nystose (GF_4), are industrially produced through fructosyl-transfer

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from sucrose using a fungal enzyme. Other kinds of fructose oligomers, such as levans and the fructans of mixed type, should be distinguished from FOS [5].

The enzymes catalysing the production of FOS are β-fructofuranosidases—also called invertases (EC 3.2.1.26) and fructosyltransferases (EC 2.4.1.9). However, assignation of FOS-producing enzymes as β-fructofuranosidases or fructosyltransferases still remains controversial [6]. These enzymes are produced by many higher plants (asparagus, chicory, onion, Jerusalem artichoke, etc.) and microorganisms, especially fungi (*Aureobasidium pullulans*, *Aspergillus niger*, *Aspergillus oryzae*, etc.) [6–9]. Interestingly, expensive activated substrates (sugar nucleotides or sugar-1-phosphates) are not required as donors for the synthesis in vitro of FOS [10], as these enzymes utilise sucrose as the sole energy source for oligosaccharide synthesis [11].

There are two strategies for producing FOS by the action of β-fructofuranosidases: reverse hydrolysis and transfructosylation. The FOS yield depends on the relative rate of synthesis and hydrolysis reactions. In contrast, the second group of enzymes (fructosyltransferases) show a little affinity towards water as acceptor.

Pectinex Ultra SP-L is a commercial enzyme preparation from *Aspergillus aculeatus* used in the food industry for fruit juice processing to reduce viscosity. It contains different pectinolytic and cellulolytic enzymes (endo-polygalacturonase, endo-pectinylase and pectin esterase) [12], and other activities, such as β-galactosidase [13], chitinase [14] and transgalactosidase [15]. The presence of a transfructosylating activity in Pectinex Ultra SP-L has been also reported [16–18].

For the industrial application of enzymes acting on water-soluble substrates, such as carbohydrates, an effective immobilisation method is required to facilitate the continuous processing and reuse of the biocatalyst [19,20]. In this context, the use of available carriers for covalent immobilisation of enzymes is of great interest. Sepabeads EC are polymethacrylate-based carriers for enzyme immobilisation [21]. The series Sepabeads EC-EP are epoxy-activated, with a high reactive group density. The chemistry for attachment of the enzyme to the support is straightforward. Compared with other epoxy acrylic polymers, Sepabeads EC-EP possess a high mechanical stability and do not swell in water. Furthermore, the raw materials applied for the production of these supports are included in the EU list of resins allowed for the processing of foodstuffs [22].

In this work, the transfructosylating activity present in commercial Pectinex Ultra SP-L was immobilised on Sepabeads EC-EP. The main immobilisation parameters (activity, bound protein, etc.) were analysed in terms of the textural properties of the carriers (pore size, pore volume, surface area, etc.). The immobilised biocatalysts were applied to the FOS batch production, and compared with the soluble enzyme.

2. Experimental procedures

2.1. Materials

Pectinex Ultra SP-L (Batch No. KRN05401) and Rapidase TF (Batch No. R5935) were kindly donated by Novozymes A/S and DSM, respectively. Sepabeads EC-EP3 (Batch No. SY1P8/39) and EC-EP5 (Batch No. E407P094) were kindly provided by Resindion S.R.L. (Mitsubishi Chemical Corporation, Milan, Italy). Glucose and dinitrosalicylic acid were from Sigma. Merck supplied sucrose and fructose. 1-Kestose and nystose were from TCI Europe. A fructo-oligosaccharides standard mixture (Actilight 950P, Beghin-Meiji) was kindly provided by Impex Química (Barcelona, Spain). All other reagents and solvents were of the highest available purity and used as purchased.

2.2. Standard activity assay

The enzymatic activity towards sucrose was measured following the initial rate of reducing sugars production by the dinitrosalicylic acid (DNS) method [23]. The spectrophotometric assay was adapted to a 96-well microplate scale. The reaction mixture (50 μl) contained 10 g/l sucrose in 50 mM sodium acetate buffer (pH 5.6). A calibration curve was obtained with a 2 g/l glucose solution. The enzyme solution (5 μl, conveniently diluted to fit into the calibration curve) was then added, and the microplate (containing the reaction mixture and the standards) was incubated at 60 °C and 200 rpm for 20 min in an orbital shaker (Stuart Scientific). Then, 50 μl of 10 g/l DNS was added to each well. The plate was sealed with a seal plate tape (GeneMate) and incubated for 30 min at 85 °C in a Binder incubator to develop colour. Then, the microplate was cooled, 150 μl of water was added to each well and the absorbance was measured at 540 nm using a microplate reader (model Versamax, Molecular Devices). One unit of activity was defined as that catalysing the formation of 1 μmol reducing sugar per minute under the above conditions. For the determination of activity of the immobilised biocatalysts, approximately 20 mg beads were incubated at 60 °C and 200 rpm for 20 min with 0.5 ml of 10 g/l sucrose in 50 mM sodium acetate buffer (pH 5.6). The sample was then centrifuged 5 min at 6000 rpm, 50 μl of supernatant was transferred to a well plate and the rest of procedure was performed as described above.

2.3. Characterisation of supports

Mercury intrusion porosimetry analysis of the supports was performed using a Fisons Instruments Pascal 140/240 porosimeter. To ensure that the samples were moisture free, they were dried at 100 °C overnight, prior to measurement. The recommended values for the mercury contact angle (141°) and surface tension (484 mN/m) were used to evaluate the pressure/volume data by the Washburn equation assuming a cylindrical pore model [24]. The specific surface

area (S_{BET}) of the supports was determined from analysis of nitrogen adsorption isotherms at −196 °C. The samples were previously degassed at 100 °C for 12 h to a residual vacuum of 5×10^{-3} Torr to remove any loosely held adsorbed species, using a Micromeritics ASAP 2010 device. Water content of the supports was determined using a DL31 Karl–Fisher titrator (Mettler). Scanning electron microscopy (SEM) was performed using an XL3 microscope (Philips) on samples previously metallised with gold.

2.4. Immobilisation of fructosyltransferase

Pectinex Ultra SP-L (5 ml, with or without previous buffer adjustment) and Sepabeads EC-EP3 or EC-EP5 were mixed and incubated for 24 h at room temperature with roller shaking. The ratio protein:support was varied over the range 30–200 mg protein per gram of carrier. The biocatalyst was then filtered using a glass filter (Whatman), washed (3 × 10 ml) with 50 mM sodium acetate buffer (pH 5.6), dried under vacuum and stored at 4 °C.

2.5. Batch production of fructo-oligosaccharides

Soluble or immobilised fructosyltransferase (1.5 U, determined by the standard DNS assay) was added to a solution containing 630 g/l sucrose in 50 mM sodium acetate buffer (pH 5.6). Total reaction volume was 5.0 ml. The mixture was incubated at 60 °C in a thermomixer (Omnilab, Eppendorf) at 1000 rpm. At different times, 40 μl aliquots were extracted from the reaction mixture, incubated 5 min at 90 °C to inactivate the enzyme and diluted with four volumes of water. Samples were centrifuged 5 min at 6000 rpm using an Eppendorf with a 0.45 μm Durapore® membrane (Millipore), and analysed by HPLC.

2.6. HPLC analysis

The concentrations of the different products from the homologous series were analysed by HPLC with a quaternary pump (Delta 600, Waters) coupled to a Lichrosorb-NH2 column (250 mm × 4.6 mm) (Merck, Spain). The mobile phase was acetonitrile:water (75:25, v/v), conditioned with helium and used at a flow rate of 0.7 ml/min. The column temperature was kept constant at 25 °C. A differential refractometer (model 9040, Varian) was used and set to a constant temperature of 30 °C. The data obtained were analysed using the Millennium Software, using external standards for calibration in the range 0–100 g/l.

2.7. Determination of protein

Protein concentration was determined by the method of Bradford (Bio-Rad protein assay) using bovine serum albumin as standard. The protocol was adapted to 96-well microplates. The amount of protein bound to the support was calculated by using the following formula: (amount of protein before immobilisation) − (amount of protein recovered in the filtrate and washings).

3. Results and discussion

3.1. Transfructosylating activity of Pectinex Ultra SP-L and Rapidase TF

The presence of a fructosyl-transfer activity in commercial Pectinex Ultra SP-L from Novozymes A/S (used in juice clarification) was first reported by Hang and Woodams [16]. Rapidase TF is a commercial pectinase from DSM. We assayed both preparations with 630 g/l sucrose at 60 °C in 50 mM sodium acetate buffer (pH 5.6), adding 0.3 U/ml (determined by the standard DNS assay with 10 g/l sucrose). Chromatograms of the reaction mixture obtained with each of these enzymes are illustrated in Fig. 1. Using Pectinex Ultra SP-L, the peak of fructose was very small compared with that of glucose, which indicated the high fructosyl-transfer capacity of the enzyme at this sucrose concentration. Rapidase TF produced a higher proportion of fructose and a lower yield of fructo-oligosaccharides. Interestingly, the appearance of extra peaks (probably regioisomers of 1-kestose and nystose) were observed with Rapidase TF.

The time course of the reaction catalysed by Pectinex Ultra SP-L is depicted in Fig. 2. The maximum concentration of 1-kestose (280 g/l) was reached in 24 h. Then, the produced 1-kestose was converted into nystose, and the latter transformed

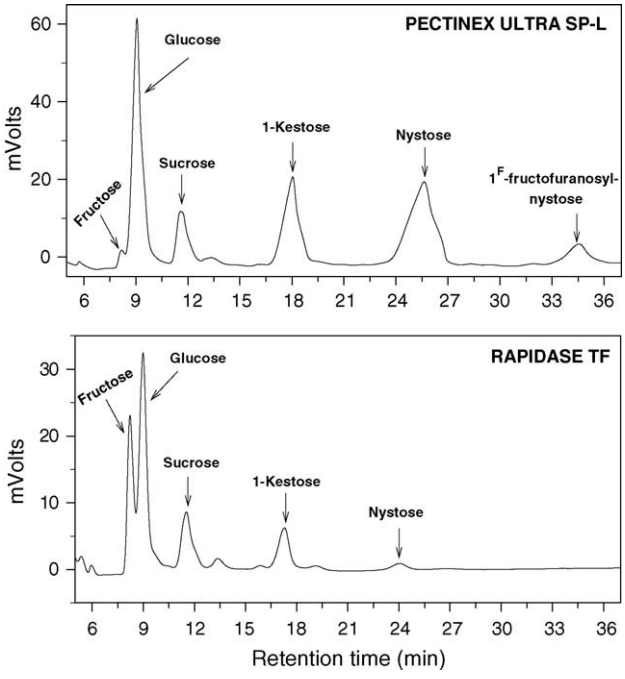

Fig. 1. HPLC chromatograms corresponding to the reaction of sucrose with Pectinex Ultra SP-L and Rapidase TF. *Experimental conditions*: 630 g/l sucrose, 0.3 U/ml (DNS assay), 50 mM sodium acetate buffer (pH 5.6) and 60 °C.

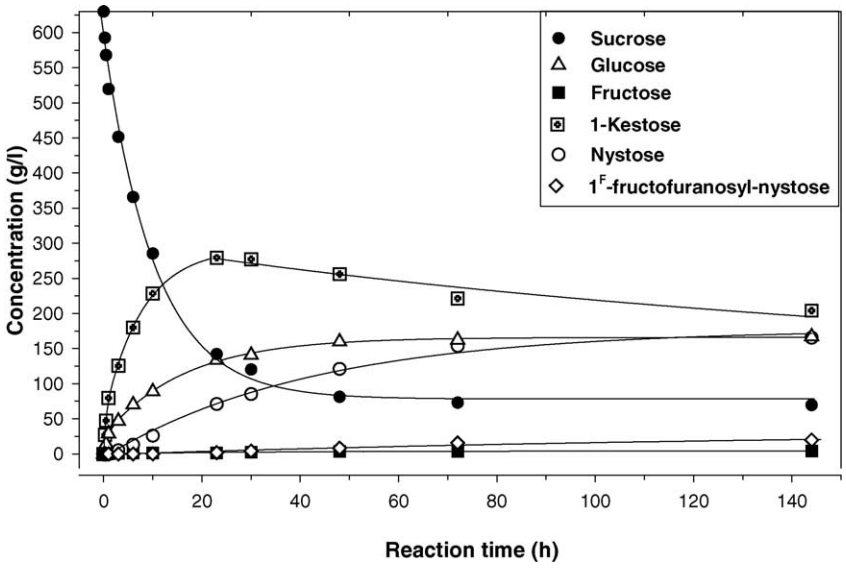

Fig. 2. Time course of the fructo-oligosaccharides production catalysed by soluble Pectinex Ultra SP-L. *Experimental conditions*: 630 g/l sucrose, 0.3 U/ml, 50 mM sodium acetate buffer (pH 5.4) and 60 °C.

into 1^F-fructofuranosyl-nystose. These compounds form a homologous series. The absence of the pentasaccharide in the reaction catalysed by Rapidase TF may be a consequence of the low concentration of nystose (Fig. 1). After 144 h, a position close to equilibrium was found with Pectinex Ultra SP-L; at this time, 62% (w/w) of the total carbohydrates in the reaction mixture were FOS. The other compounds were glucose (26.5%), sucrose (11%) and fructose (0.5%). In order to facilitate the use of Pectinex Ultra SP-L in batch and fixed-bed reactors, the new epoxy-activated Sepabeads EC carriers were tested as matrices for covalent immobilisation of enzymes with fructosyl-transfer activity.

3.2. Properties of Sepabeads EC-EP3 and EC-EP5

Sepabeads® EC are polymethacrylate-based supports that exhibit high mechano-osmotic stability, low compressibility and high resistance to microbial attack. These carriers are particularly suitable for covalent immobilisation of enzymes for industrial applications because of their excellent mechanical properties when used in reactors.

Two epoxy-activated related supports, Sepabeads EC-EP3 and EC-EP5, were assayed. The highly porous polymer matrix of Sepabeads EC is illustrated in the SEM micrographs (see Fig. 3 for Sepabeads EC-EP3). The aggregates shown in picture A are formed during metallisation with gold (the original carrier is composed of individual particles). The shape of the nitrogen adsorption/desorption isotherm, shown in Fig. 4, for Sepabeads EC-EP5 was of type II, typical for materials with no micro (0–2 nm) or mesopores (2–50 nm), as indicated by the absence of a hysteresis loop between the adsorption and desorption branches. However, Sepabeads EC-EP3 contains a low amount of mesopores, as reflected by the increment of the adsorption isotherms in the 0.3–1.0 range of relative pressure. The existence of these mesopores in Sepabeads EC-EP3 is corroborated by Fig. 5, which represents the total pore volume and pore size distribution of both carriers, and was obtained by combination of nitrogen isotherms and mercury porosimetry analyses. As shown, both samples were essentially macroporous (>50 nm) solids, although the total pore volume (1.67 cm^3/g) of Sepabeads EC-EP5 is significantly higher than that corresponding to Sepabeads EC-EP3 (1.19 cm^3/g). The average pore size of both supports is also different; the maximum in the pore size distribution curve was 130 nm for Sepabeads EC-EP3 and 800 nm for Sepabeads EC-EP5.

The textural properties of both supports are summarised in Table 1. The density of epoxide groups was similar for both supports, which may result in a similar number of bonds between the enzyme and the carrier. It is noteworthy that the water retention capacity of both supports was very high, greater than 60%.

3.3. Effect of pH and ionic strength on immobilisation

The coupling of enzymes to epoxy-activated carriers is commonly carried out at high ionic strength, because it has been postulated that, in a first step, a salt-induced association between the macromolecule and the support surface takes place [21,25]. This interaction increases the effective concentration of nucleophilic groups on the protein close to the epoxide reactive sites. However, the salt concentration needed to immobilise an enzyme is highly protein-dependent [25]. The epoxy (oxirane) groups may react with different nucleophiles of the protein as a function of pH. At neutral or slightly alkaline pH, with the thiol groups; at pH > 9, with the amino groups; at pH > 11, with phenolic groups of tyrosines; at slightly acidic pH, with carboxyl groups [26]. Although

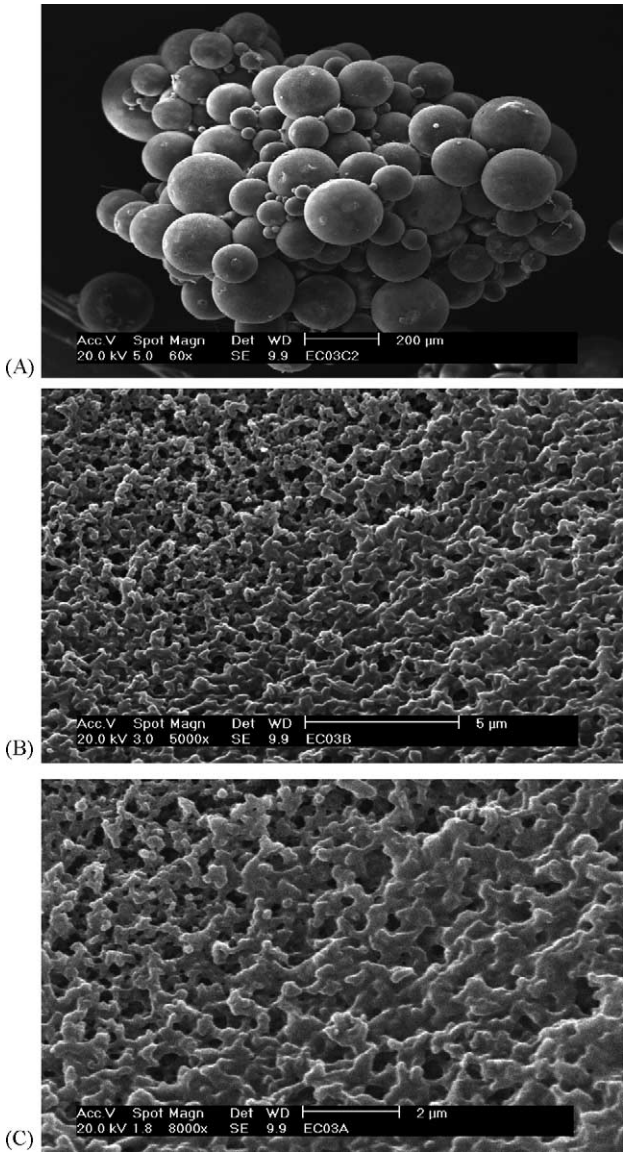

Fig. 3. Scanning electron micrographs of Sepabeads EC-EP3: (A) 60×, (B) 5000× and (C) 8000×.

the type of residues involved may affect the enzyme orientation on the solid support, it has been recently demonstrated by Wilchek and Miron [27] that there is little difference in biocatalyst performance when using random or oriented immobilisation.

For the above reasons, the effect of pH and ionic strength on the immobilisation of the transfructosylating activity present in Pectinex Ultra SP-L was studied. The protein concentration in this commercial preparation was 13.8 mg/l. The activity towards sucrose, using the DNS assay, was 220 U/ml. Probably, the enzyme (or enzymes) responsible for the activity on sucrose constitute only a minor percentage of the proteins present in Pectinex Ultra SP-L. In order to bind the enzyme to the support using different functional groups, the immobilisation was performed at two pH values (5.5 and

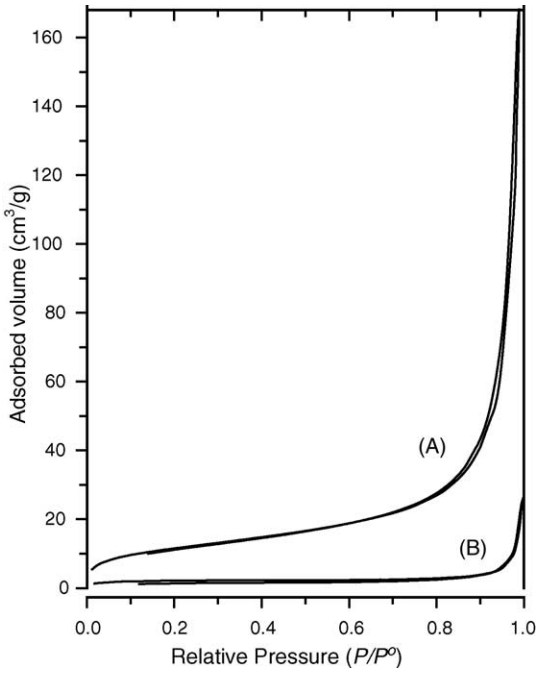

Fig. 4. Nitrogen adsorption–desorption isotherms of: (A) Sepabeads EC-EP3 and (B) Sepabeads EC-EP5.

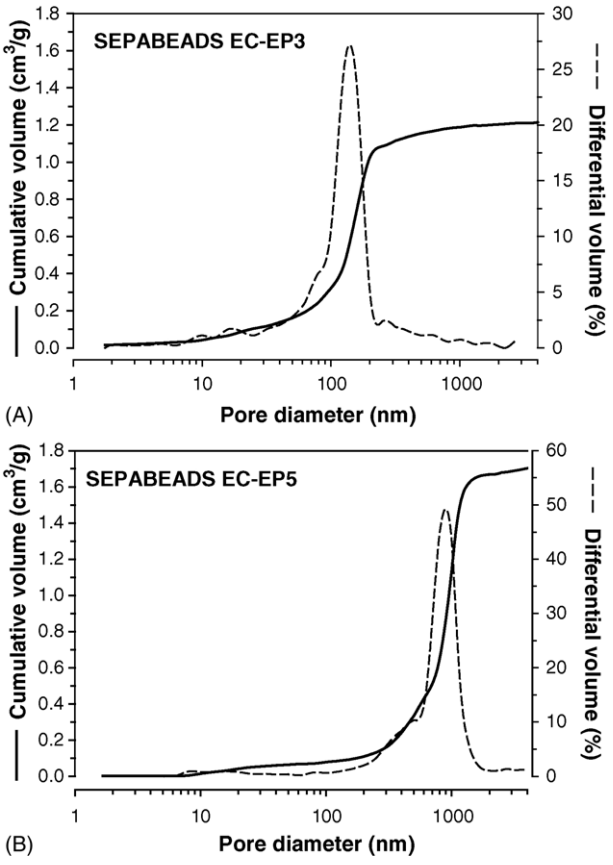

Fig. 5. Total pore volume (solid line) and pore size distribution (dashed line) of: (A) Sepabeads EC-EP3 and (B) Sepabeads EC-EP5.

Table 1
Textural properties of the supports studied

Support	Content of epoxide groups (μmol/g)[a]	Particle size (μm)[b]		BET surface area (m^2/g)[c]	Density of epoxide groups (μmol/m^2)	Total pore volume (cm^3/g)[d]	Average pore size (nm)[d]	Water content (%)[e]
		Range	Average					
Sepabeads EC-EP3	106	7–225	77	43	2.5	1.19	130	60
Sepabeads EC-EP5	23	19–215	139	9	2.4	1.67	800	65

[a] Provided by the supplier.
[b] Determined by Hg porosimetry.
[c] Measured by N_2 adsorption.
[d] By combination of N_2 isotherms and Hg porosimetry.
[e] Determined by Karl–Fisher titration.

Table 2
Effect of buffer and its concentration on the immobilisation of transfructosylating activity in Pectinex Ultra SP-L on Sepabeads EC-EP

Support	Immobilisation buffer		Protein content (mg/g biocatalyst)[a]	Activity (U/g biocatalyst)[b]
	pH	Concentration (M)		
Sepabeads EC-EP3	Sodium carbonate (pH 9.0)	0.3	10.3	0.74
		0.5	8.3	1.20
	Potassium phosphate (pH 5.5)	0.3	2.9	4.20
		0.5	1.0	0.67
Sepabeads EC-EP5	Sodium carbonate (pH 9.0)	0.3	17.5	15.2
		0.5	10.5	0.69
		1.0	10.3	0.79
	Potassium phosphate (pH 5.5)	0.3	6.4	3.20
		1.0	3.6	2.90

Immobilisation conditions: 30 mg protein added per gram of support, 24 h, room temperature and roller shaking.
[a] Total amount of protein bound per gram of biocatalyst.
[b] Determined by the standard DNS assay.

9.0), adjusting the pH of commercial Pectinex Ultra SP-L with potassium phosphate or sodium carbonate, respectively. At pH 5.5, the reactive groups of the protein are the carboxylic heads of the aspartic and glutamic side chains, as well as the C-terminal α-carboxylic group [26]. At pH 9.0, the amino and thiol groups in the protein are able to bind to the support. The buffer concentration was varied over the range 0.2–1.0 M. The amount of total protein "offered" per gram of support was 30 mg.

Table 3
Direct immobilisation of transfructosylating activity in Pectinex Ultra SP-L on Sepabeads EC-EP3 and EC-EP5

Support	Ratio protein: support (mg/g)	Protein content (mg/g biocatalyst)[a]	Activity (U/g biocatalyst)[b]
Sepabeads EC-EP3	60/1	21.1	14.1
	100/1	22.7	16.9
	200/1	31.4	18.8
Sepabeads EC-EP5	60/1	17.3	20.3
	100/1	27.1	23.4
	200/1	54.7	25.9

Experimental conditions: 24 h, room temperature and roller shaking.
[a] Total amount of protein per gram of biocatalyst.
[b] Determined by the standard DNS assay.

We assumed that the other enzymes present in Pectinex Ultra SP-L were also being immobilised in Sepabeads EC-EP. As shown in Table 2, the amount of protein bound to the support was higher at lower ionic strength. A maximum

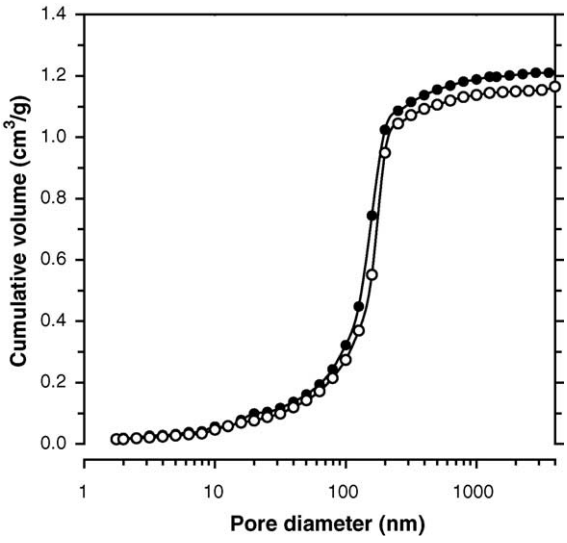

Fig. 6. Total porosity of Sepabeads EC-EP3 before (●) and after (◯) immobilisation of Pectinex Ultra SP-L.

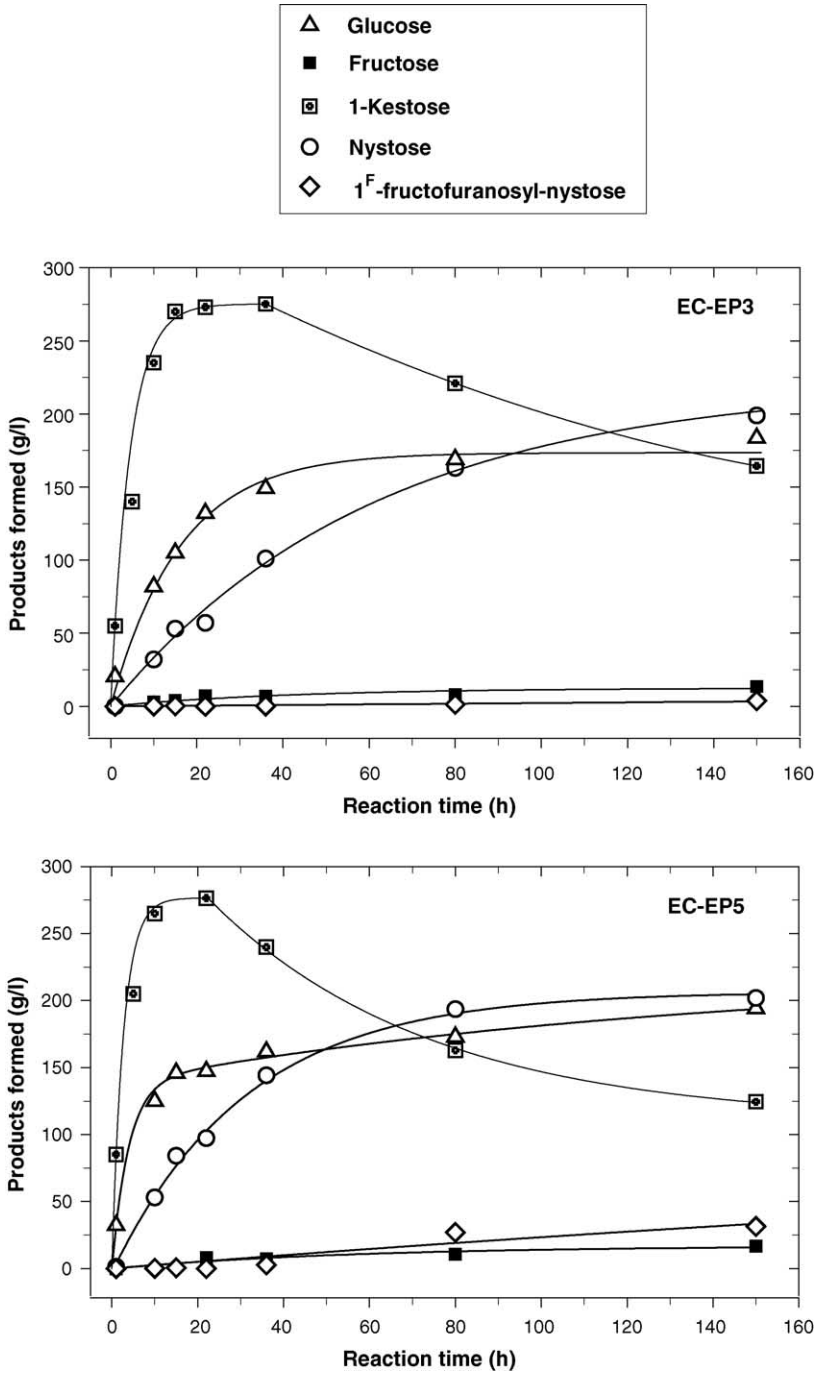

Fig. 7. Batch synthesis of fructo-oligosaccharides catalysed by immobilised Pectinex Ultra SP-L in Sepabeads EC-EP3 (top) and EC-EP5 (bottom). *Experimental conditions*: 630 g/l sucrose, 0.3 U/ml (standard DNS assay), 50 mM sodium acetate buffer (pH 5.4) and 60 °C.

of 34 and 58% of the "offered" protein was bound to Sepabeads EC-EP3 and EC-EP5, respectively. In general, Sepabeads EC-EP5 retained more protein than EC-EP3 under the same experimental conditions, probably as a consequence of its higher porosity. The total bound protein was higher at pH 9.0 than at pH 5.5.

Regarding biocatalyst activity, a low buffer concentration resulted in higher activity with both supports. The highest activity of the biocatalyst (15.2 U/g) was achieved with Sepabeads EC-EP5, using 0.3 M sodium carbonate (pH 9.0). With Sepabeads EC-EP3, slightly acidic pH (5.5) at low buffer concentration (0.3 M) had the highest biocatalyst activity (4.2 U/g). The incubation time was also analysed. We observed that a further increase from 24 to 72 h did not result in a significant increase of biocatalyst activity.

Based on the above results at low ionic strength, the direct immobilisation on Sepabeads EC-EP of Pectinex Ultra SP-L, whose pH is 4.8, without addition of buffer or salt to

control pH and ionic strength of the enzyme preparation was studied. Interestingly, as shown in Table 3, results were very satisfactory. The protein loading (mg) per gram of support was varied over the range 60/1–200/1. The amount of bound protein varied from 21.1 to 31.4 mg protein per gram of support for Sepabeads EC-EP3, and from 17.3 to 54.7 mg/g for Sepabeads EC-EP5. Chiang et al. [28] bound up to 0.8 mg β-fructofuranosidase from *A. niger* per gram of carrier on methacrylamide-based polymeric beads, whereas Hayashi et al. [29] immobilised only 0.07 mg β-fructofuranosidase from *Auereobasidium* sp. ATCC 20524 per gram of porous silica.

Using the direct method, biocatalyst activity reached 18.8 and 25.9 U/g with Sepabeads EC-EP3 and EC-EP5, respectively. The improvement, with respect to buffer addition, was especially significant in the case of Sepabeads EC-EP3. Although fructosyl-transfer enzymes have been immobilised by different techniques, such as adsorption [30], entrapment [31] or covalent attachment [32], the biocatalyst activity per mass unit is not commonly reported. However, Chiang et al. [28] described a maximum activity of 77 U/g for β-fructofuranosidase from *A. niger* covalently attached to methacrylamide-based polymeric beads. More recently, fructosyltransferase from *A. aculeatus* present in Pectinex Ultra SP-L was covalently bound to Eupergit C [33]; although the activity recovery was high, no activity per gram of biocatalyst was reported. We believe that a (semi)purification of the enzyme responsible for fructosyl-transfer in Pectinex Ultra SP-L, combined with the high binding capacity of Sepabeads EC-EP carriers, might result in an immobilised biocatalyst with a considerable volumetric activity.

The effect of enzyme immobilisation on the porosity of the supports was also analysed. Fig. 6 shows that a only a slight decrease (approximately 4%) of pore volume of Sepabeads EC-EP3 was produced as a consequence of enzyme immobilisation, although the shift in the maxima of the pore size distribution from 150 to 162 nm was consistent with the blocking of the narrower pores.

3.4. Application of immobilised biocatalysts to fructo-oligosaccharides synthesis

We tested two immobilised biocatalysts, obtained using the direct method on Sepabeads EC-EP3 and EC-EP5, in the transfuctosylating reaction on sucrose. A high substrate concentration (630 g/l) was assayed to favour transferase activity. For these experiments, the same number of enzyme units (0.3 U/ml) were used (measured by the DNS method with 10 g/l sucrose, in 20 min).

The progress of the reaction is depicted in Fig. 7 where it may be observed that, compared with progress curve of Fig. 2, the batch reaction pattern for FOS formation was not altered by enzyme immobilisation. The profile was quite similar for both immobilised biocatalysts. The reaction was slightly faster with Sepabeads EC-EP5 than with the corresponding EC-EP3, probably due to both its higher pore volume and wider pores leading to improved internal diffusion of substrates and products. Using EC-EP5, the FOS concentration reached a maximum value of 387 g/l after 36 h (240 g/l 1-kestose, 144 g/l nystose and 3 g/l 1^F-fructofuranosyl-nystose). At this reaction time, the percentage of FOS in the solids was 61.5%, referred to total carbohydrates in the mixture. After 150 h, the mass distribution of the carbohydrates was 57–58% FOS, 29–31% glucose, 9.5–10.5% sucrose and 2–3% fructose. The FOS yield is comparable to that produced by other immobilised biocatalysts previously reported [28,33].

4. Conclusions

As shown, the performance of the immobilised biocatalysts described in this work was satisfactory. Their high mechanical stability, absence of swelling in water and approval for foodstuffs processing converts them into very interesting alternatives for fructo-oligosaccharides synthesis, compared with other related carriers. In addition, direct immobilisation of transfructosylating activity present in Pectinex Ultra SP-L on epoxy-activated Sepabeads acrylic polymers is a very simple method that is easily scaleable. The use of the above biocatalysts for the continuous production of fructo-oligosaccharides in a fixed-bed reactor is currently being studied.

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