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Dextranase immobilization on epoxy CIM® disk for the production of isomaltooligosaccharides from dextran

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ABSTRACT

Endodextranase D8144 from *Penicillium* sp. (EC 3.2.1.2.) was immobilized on an epoxy-activated monolithic Convective Interaction Media (CIM®) disk in order to produce isomaltooligosaccharides (IMOS) from Dextran T40 in a continuous IMmobilized Enzymes Reactor (IMER). Enzymatic parameters and structure of IMOS were studied for free and immobilized enzymes. The immobilization efficiency of endodextranase D8144 was about 15.9% (w/w) and the real specific activity was close to 6.5 U mg enz^{-1}. The Km values (4.8 ± 0.2 g L^{-1}) for free and immobilized enzymes were the same, showing the absence of diffusional limitation. Moreover, specific patterns of DPs (Degrees of Polymerization) distributions were observed during the enzymatic hydrolysis by HPAEC-PAD (High Pressure Anion Exchange Chromatography-Pulsed Amperometric Detection). Thus, sought-after sizes of IMOS (DPs 8–10) were generated all over the hydrolysis. Finally, the results showed the high stability of this IMER since a relative enzymatic activity about 78% was measured after 5400 volumes column.

1. Introduction

Oligosaccharides, commonly found in nature as glycoconjugates, play a fundamental part in many biological processes. These macromolecules exhibit structural diversity greatly higher than proteins and nucleic acids (Turnbull & Field, 2007; Varki, 1993). Since last years, oligosaccharides were increasingly employed as functional or therapeutical drugs. Nevertheless, in most cases, the lack of performing and creative industrial oligosaccharides processes is considered as the main scientific bottleneck limiting their exploitation as biological molecules. For this reason, development of oligosaccharides engineering strategies is of primary importance. Natural polysaccharides can be depolymerized by physical (Wolff, Watson, Sloan, & Rist, 1953; Zou et al., 2012), chemical (Zief, Brunner, & Metzendor, 1956) and enzymatic procedures (Delattre, Michaud, Courtois, & Courtois, 2005; Khalikova, Susi, & Korpela, 2005). However the physical and chemical strategies are known for their random and hardly controllable cleavage (Delattre et al., 2005).

Therefore, enzymatic cleavage by using glycoside hydrolases (EC 3.2.1.-) and polysaccharide lyases (EC 4.2.2.-), which are easily available at low cost, are one of the best ways to selectively obtain oligosaccharides from natural polysaccharides. Controlling the degree of polymerization (DP) of oligosaccharides is moreover possible if an endolytic mechanism is involved. The production of oligosaccharides from polysaccharides by highly specific enzymes has been abundantly studied during the last decade notably in nutraceutical, agronomy or agro-industry (Akpinar, Erdogan, & Bostanci, 2009; Laroche & Michaud, 2007; Pierre, Maache-Rezzoug, Sannier, Rezzoug, & Maugard, 2011; Pierre et al., 2013; Wang, 2009). However, few authors have worked on polysaccharides degradations through the use of immobilized enzymes (Aslan & Tanriseven, 2007; Delattre, Michaud, & Vijayalakshmi, 2008; Lali, Manudhane, Motlekar, & Karandikar, 2002; Turecek, Pittner, & Birkner, 1990). Even, immobilized enzymes generally overpass classical limitations described for free enzymes (Sheldon, 2007).

IMmobilized Enzymes Reactors (IMER) possess several advantages such as reusability, high enzyme stability, scale-up reduction

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cost and the possibility to work in automatic continuous flow system for long periods (Markoglou & Wainer, 2003). They also turn out to be economically beneficial for the production of high value molecules (Fu et al., 2012). During the design of IMERs, one of the most important parameters is the ideal choice of the matrix for the enzymes immobilization. This choice is closely dependant on the surface area, the cost, the thermal and chemical stabilities and the mass transfer characteristics (Bartolini, Cavrini, & Andrisano, 2005). In most of cases, the main drawback of porous beads IMERs was the low mass transfer observed by authors (Petro, Svec, & Fréchet, 1996). Consequently, recent polymeric macroporous monolithic supports have been developed to improve (i) the large surface area, (ii) the good mass transfer characteristics and (iii) the low back-pressure (Champagne et al., 2007; Josic, Buchacher, & Jungbauer, 2001). Monolith matrices are well characterized for their low column back pressure and high efficiency in terms of chromatographic parameters. In fact, the diffusion path is lower due to the high convective effect allowing a very good mass transfer (Champagne et al., 2007; Josic et al., 2001). These last years, original monolith matrices have been employed for enzymes immobilization. Convective Interaction Media® (CIM) disks are macroporous poly(glycidyl methacrylate-co-ethylene dimethacrylate) (Fig. 1A) and were already suggested for IMERs design (Delattre & Vijayalakshmi, 2009; Josic et al., 2001; Vodopivec, Podgornik, Berovič, & Štrancar, 2003). In this way, the immobilization of a pectin lyase on an epoxy-activated CIM® disk allowed obtaining large amounts of purified oligogalacturonans within a short delay (Delattre et al., 2008). Tavernier et al. (2008) also showed the possibility to control the degree of polymerization (DP) of oligoglucuronans through the substrate flow rate by using an immobilized glucuronan lyase.

The main goal of this paper is to demonstrate the potential of macroporous epoxy-activated monolithic support for the covalent immobilization of endodextranases to produce isomaltooligosaccharides (IMOS) from dextran. Dextran is an extracellular homopolysaccharide of α-D-glucopyranose (D-Glc*p*) with mainly α-(1,6) linkages and α-(1,2), α-(1,3) and α-(1,4) branching in the backbone chain. It is produced from sucrose by bacteria such as *Leuconostoc*, *Lactobacillus*, *Streptococcus*, which possess glucansucrase activities (E.C. 2.4.1.5) (Badel, Bernardi, & Michaud, 2010). Firstly, IMOS were produced by free endodextranase D8144 from *Penicillium* sp. Enzymatic parameters were calculated in conventional conditions and DPs of IMOS were estimated. Secondly, IMOS were then produced after immobilizing the same enzymes on an epoxy-activated CIM® disk in order to show the potential of this new endodextranase based monolithic IMERs for rapid on-line dextran depolymerization.

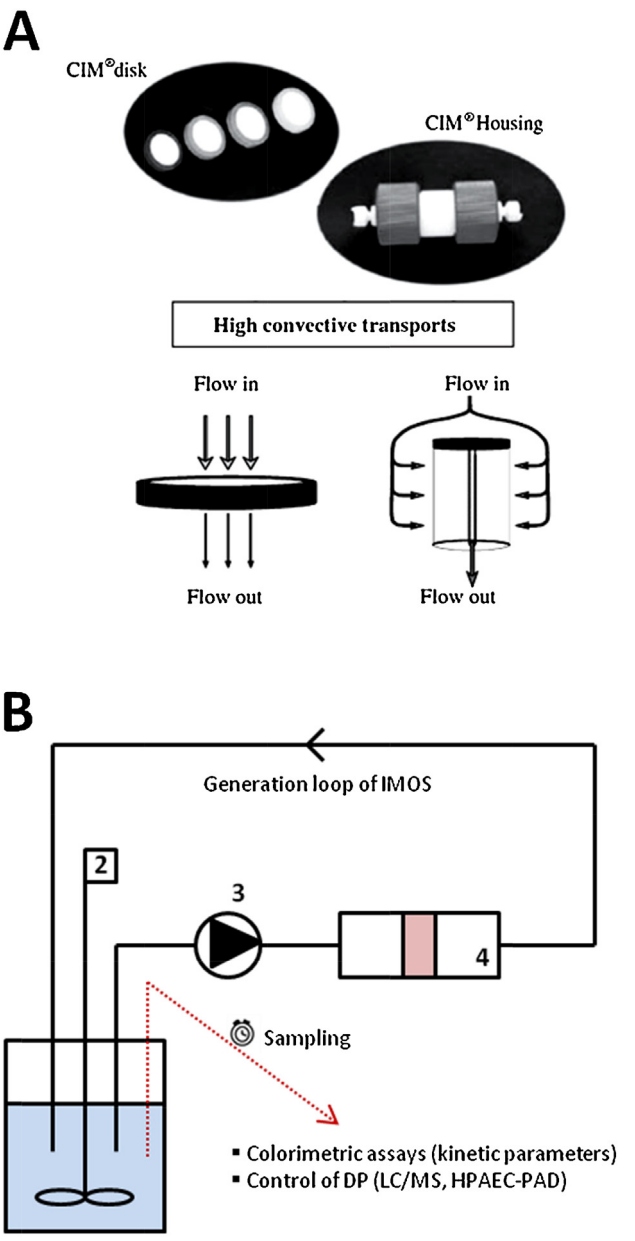

Fig. 1. (A) Schematic conception of Convective Interaction Media (CIM) monolith column described by Champagne et al. (2007) and (B) design of the IMmobilized Endodextranase Reactor (IMER) system: (1) Dextran T40 in phosphate buffer solution (100 mM; pH 6), (2) control of pH, temperature, flow rate and concentration, (3) peristaltic pump, (4) immobilized endodextranases on epoxy-activated CIM® disk.

2. Materials and methods

2.1. Enzymes and reagents

Purified endodextranase D8144 from *Penicillium* sp. was purchased from Sigma–Aldrich (Lyon, France). Epoxy-activated CIM® disks (213.7175) and supports were from BIA separations (Slovenia). Dextran used for the study was Dextran T40 (Dextranum 40 for injection, 40 kDa) from Pharmacosmos (Denmark). It contains 1.2% ramifications corresponding to a particularly low branched dextran, according to previous studies (Jeanes et al., 1954). For the HPAEC-PAD analysis, sodium acetate was purchased from Sigma–Aldrich and sodium hydroxide solution was obtained from Fisher Scientific (Illkirch, France). Acetic acid and acetonitrile solutions used for the HPLC/ESI-MS analyses were purchased from Carlo Erba (Peypin, France). Other chemicals were of analytical purity and purchased from Sigma–Aldrich.

2.2. Protein and reducing sugar analyses

Proteins were measured according to the Lowry protein assay using bovine serum albumin as a standard (Lowry, Rosebrough, Farr, & Randall, 1951). Absorbance at 750 nm was measured with an UV-1700 spectrophotometer (Shimadzu, Duisburg, Germany). Reducing sugars were determined by measuring absorbance at 540 nm (Shimadzu, Duisburg, Germany) according to the 2,2-bicinchoninate assay using isomaltose as standard (Waffenschmidt & Jaenicke, 1987). All the measurements were done in duplicate.

2.3. Production of IMOS by free endodextranase D8144

The time-course of reducing sugar concentrations was performed to determine the free endodextranase activity, using a

Radley Carousel 12 plus (Radley Discovery Technologies, Shire Hill, UK). Briefly, 1.6 U of endodextranase D8144 were added to 20 mL of 1–8% (w/v) Dextran T40 solution in potassium phosphate buffer (100 mM; pH 6) at 37 °C and under stirring (400 rpm). 500 μL were taken at regular time intervals, then boiled for 30 min at 100 °C in a dry bath to inactivate the enzymes and finally stored at −20 °C before reducing sugar analyses. One unit (U) of endodextranase activity was defined as the amount of enzymes necessary to release reducing sugars equivalent to 1 μmol of isomaltose per min from Dextran T40 used as substrate at optimal temperature and pH (37 °C; pH 6) (Aslan & Tanriseven, 2007; Rogalski et al., 1998). Initial velocities (Vi), maximal velocity (Vm) and dissociation constant (Km) were estimated for free endodextranase D8144 by using Michaëlis–Menten and Lineweaver–Burk methods (Johnson & Goody, 2011; Lineweaver & Burk, 1934). All the experiments were done at least in triplicate.

2.4. Production of IMOS by the immobilized endodextranase reactor

2.4.1. The immobilized endodextranase reactor system

The IMER used in this study (Fig. 1B) for both immobilization of endodextranase D8144 and dextran hydrolysis, consisted in a Gilson Minipuls 3 peristaltic pump (Middleton, WI, USA) providing flow rates up to 5.5 mL min^{-1}, an epoxy-activated CIM® disk placed on its support and a 10 mL agitated feed tank. The whole system was placed in an oven to control the temperature.

2.4.2. Immobilization procedure of endodextranase D8144

The CIM® disk was previously equilibrated with a potassium phosphate buffer solution (100 mM; pH 8) during 30 min at 20 °C and under stirring (120 rpm). 444 μg of proteins corresponding to 92 U of endodextranase D8144 were dissolved in 2.8 mL of potassium phosphate buffer solution (100 mM; pH 8) and placed under circulation at 0.3 mL min^{-1} through the epoxy-activated CIM® disk for 20 h at 20 °C. This dynamic immobilization step was then followed by a static one in which the disk was plunged in the same endodextranase D8144 solution for 5 h under gentle stirring (120 rpm). In order to inactivate any residual epoxy sites, the CIM® disk was then flushed for 12 h with 30 mM ethanolamine solution (in 100 mM potassium phosphate buffer solution, pH 8) at 0.3 mL min^{-1} and 20 °C. The immobilization procedure was ended by washing the CIM® disk for one hour with a potassium phosphate buffer solution (100 mM; pH 6) at 0.3 mL min^{-1} and 20 °C. Three different epoxy CIM® disks were used to confirm the robustness of the immobilization procedures.

2.4.3. Immobilized endodextranase activity assays

Before any activity assay, the IMER was placed at 37 °C and flushed during 30 min with potassium phosphate buffer (100 mM; pH 6). As for free enzymes, pH 6 was used since previous authors observed no pH shift after the immobilization of another endodextranase onto an epoxy-activated Eupergit C support (Aslan & Tanriseven, 2007). Four mL of Dextran T40 solutions (1–8%; w/v; 100 mM potassium phosphate buffer, pH 6) were then placed under circulation through the immobilized endodextranases CIM® disk at a flow rate of 0.3 mL min^{-1} for 11 h corresponding to 51 cycles at 37 °C. One cycle was defined as the time necessary to flush the 4 mL of the Dextran T40 solution through the endodextranases CIM® disk. Twenty μL were taken at regular time intervals, then boiled for 30 min at 100 °C in a dry bath to inactivate the enzymes and finally stored at −20 °C before reducing sugar analyses (Section 2.3). Finally, the IMER was flushed for 30 min with 100 mM potassium phosphate buffer (pH 6) at 0.3 mL min^{-1} and 37 °C, then stored in the same buffer at 4 °C before the next utilization.

2.4.4. Operational and storage stability

Operational and storage stability of the IMER were tested for 78 days by determining the relative activity after 10 days of storage at 4 °C in 100 mM potassium phosphate buffer (pH 6). Each 10 days, immobilized endodextranase activity assays were conducted in the same conditions as described in Section 2.4.3. Reactions were performed in triplicate during 11 h (51 cycles) at a flow rate of 0.3 mL min^{-1} using dextran T40 solution at 2% (w/v) in potassium phosphate buffer (100 mM; pH 6).

2.5. Structural analysis of IMOS by analytic chromatography

2.5.1. HPLC/ESI-MS analyses

Electrospray mass spectra in the positive mode were obtained on a HPLC/ESI-MS system from Agilent (1100 LC/MSD Trap mass spectrometer VL) with a differential refractometer (Agilent 1100) and a Modulo-Cart QK UPTISPHERE 6 DIOL column (UP6OH*25QK, 250 mm × 4 mm, Interchrom) at 35 °C and a flow rate of 1 mL min^{-1} (split 1/1 between refractometer and MS), using 67/33% (H_2O-acetic acid 0.1%)/(CH_3CN–acetic acid 0.1%) (v/v) as eluent. The sample volume injection was 20 μL. Nitrogen was used as dried gas at 9 L min^{-1} at 350 °C and at a nebulizer pressure of 45 psi. Scan range was performed between 190 and 2200 *m*/*z* with a target mass of 1200 *m*/*z* and a compound stability of 30%. Data acquisition and processing were carried out using Chemstation for LC 3D systems B.01.03-SR2 (Agilent, UK), LC/MSD Trap software 5.3 (Agilent, UK) and Data analysis for LC/MSD Trap 3.3 (Agilent, UK).

2.5.2. HPAEC-PAD analyses

HPAEC-PAD analyses were carried out on a Dionex ICS3000 ion chromatography system with a CarboPAC™ PA200 (4 mm × 250 mm) analytical column equipped by a CarboPAC™ PA200 (4 mm × 50 mm) guard cartridge. All samples analyzed were filtered at 0.2 μm. The sample volume injection was 25 μL and the system was operated at 25 °C at a flow rate of 0.5 mL min^{-1} using a gradient of eluent A (NaOH 100 mM) and eluent B (sodium acetate 250 mM in NaOH 100 mM). The gradient contained four steps (expressed in percent B in A): 0% during 10 min; 0–100% from 10 to 50 min; 100% from 50 to 70 min; 0% from 70 to 80 min. Glucose (DP 1), isomaltose (DP 2), and isomaltotriose (DP 3) were used as standard for quantification. Carbohydrates were detected on a pulsed amperometric ED50 detector (Dionex Corp., Sunnyvale, CA, USA). Data acquisition and processing were carried out using Chromeleon® software 6.8 (Dionex Corporation, Sunnyvale, CA, USA).

3. Results and discussion

3.1. Kinetic parameters of free and immobilized endodextranase D8144

Convective Interaction Media disk (CIM® disk) was used to immobilize endodextranase D8144. Table 1 presents the yields of immobilized proteins and enzymatic activities obtained after the immobilization of endodextranase D8144 on epoxy-activated CIM® disk. The immobilized proteins content was about 70 μg corresponding to an immobilization yield of 15.9% (w/w) while the

Table 1
Protein contents, enzymatic activities and immobilization yields of endodextranase D8144 on epoxy-activated CIM® disk.

Endodextranase D8144	t_0	t_{end}	Immobilized	Immobilization yield
Protein content (μg)	444	374	70.0	15.9%
Activity (U)	98.5	80	18.3	18.6%

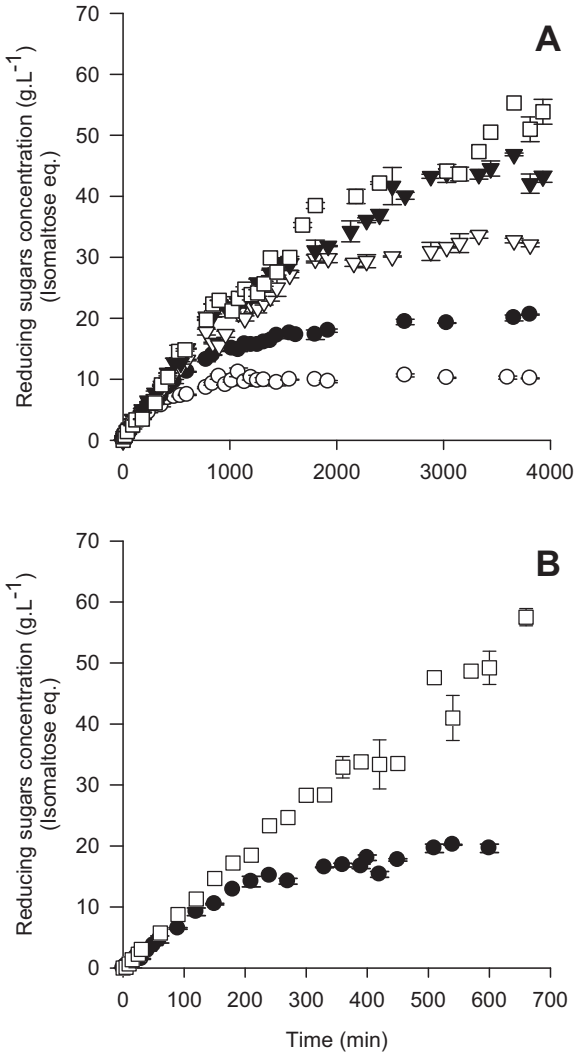

Fig. 2. Time-courses of reducing sugar concentrations during the enzymatic degradation of Dextran T40 using (A) free endodextranase D8144 (1.6 U) and (B) immobilized endodextranase D8144 (0.46 U) on CIM® disk at a flow rate of 0.3 mL min^{-1}. (○) Dextran T40 1% (w/v), (●) 2% (w/v), (▽) 4% (w/v), (▼) 6% (w/v), (□) 8% (w/v).

theoretical activity of immobilized enzymes was equal to 18.3 U (261 U mg enz^{-1}) corresponding to an activity yield close to 18.6% (U/U). These yields were in agreement with other works, *e.g.* Nicoli, Gaud, Stella, Rudaz, and Veuthey (2008) who obtained an immobilized trypsin (EC 3.4.21.4) yield above 18% (w/w). It is noteworthy that a wide range of immobilization yields were described in the literature, varying from 15 to 60% (w/w) (Berruex & Freitag, 2002; Tavernier et al., 2008; Vodopivec et al., 2003). Johansson, Orgen, and Olsson (1983) reported that numerous factors could affect the immobilization efficiency on CIM® disk as the enzyme loading, the molecular weight of the enzyme, the quantity of free amine groups or the hydrodynamic characteristics of monolith supports. Besides, the procedure of immobilization is of crucial importance since long static incubations allow improving the quantity of immobilized proteins by 25% (Benčina, Podgornik, Štrancar, & Benčina, 2004; Tavernier et al., 2008; Vodopivec et al., 2003).

Time-courses of enzymatic activities measured by reducing sugars assays are described in Fig. 2. Accordingly to the literature for free enzymes in conventional media, reducing sugar concentrations raised during the hydrolysis time of increasing substrate concentrations (Fig. 2A). The same tends were observed using the

Table 2
Kinetic parameters for free and immobilized endodextranase D8144.

Endodextranase D8144	Free	Immobilized[a]
Vm (U L^{-1})	80.0 ± 0.9	114.5 ± 6.4
Km (g L^{-1})	4.7 ± 0.2	4.8 ± 0.8
Specific activity (U mg enz^{-1})	390.3 ± 7	6.54 ± 0.37

[a] Experiments were performed with a Dextran T40 flow rate of 0.3 mL min^{-1}.

IMER (Fig. 2B). Note to mention that the enzymatic degradation using immobilized endodextranases D8144 (0.46 U) was five times faster than using free endodextranase D8144 (1.6 U), which was already described by Tavernier et al. (2008). Beside, approximately 1% and 19% of high-weight dextran were not fully degraded after 600 min of hydrolysis with the epoxy CIM® Disk, respectively for 2% and 8% of dextran T40 solutions. Kinetic parameters for both free and immobilized endodextranases are detailed in Table 2. The Km value for immobilized enzyme was 4.8 ± 0.2 g L^{-1} and was closely the same for free enzyme, highlighting the absence of diffusional limitation (Vodopivec et al., 2003). Nevertheless, the specific activity measured for immobilized enzyme (6.5 U mg enz^{-1}) was 40 times lower than the theoretical one (261 U mg enz^{-1}). This result was better than previous studies since Tavernier et al. (2008) measured an enzymatic activity of immobilized glucuronan lyase 1300 times lower than the theoretical immobilized activity. Anyway, this drop was already well described in the literature and was explained by conformational and steric effects involved during random immobilization (Nicoli et al., 2008; Vodopivec et al., 2003). Considering that mass transfer effects were negligible in CIM® disk (Vodopivec et al., 2003), chemical and physical stresses

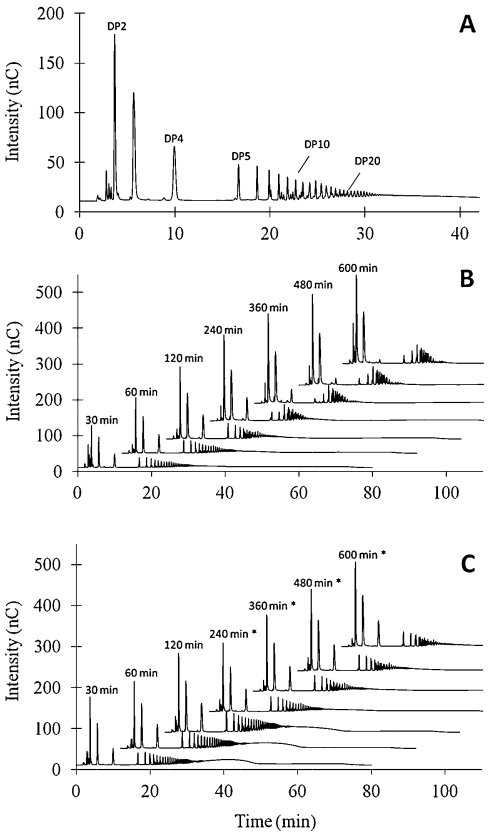

Fig. 3. HPAEC-PAD chromatograms of IMOS generated during the hydrolysis of Dextran T40 (A) 8% (w/v) hydrolyzed for 240 min, (B) 2% (w/v) and (C) 8% (w/v) using the IMER at a flow rate of 0.3 mL min^{-1}. Samples were diluted 100 times or (*) 500 times before analysis.

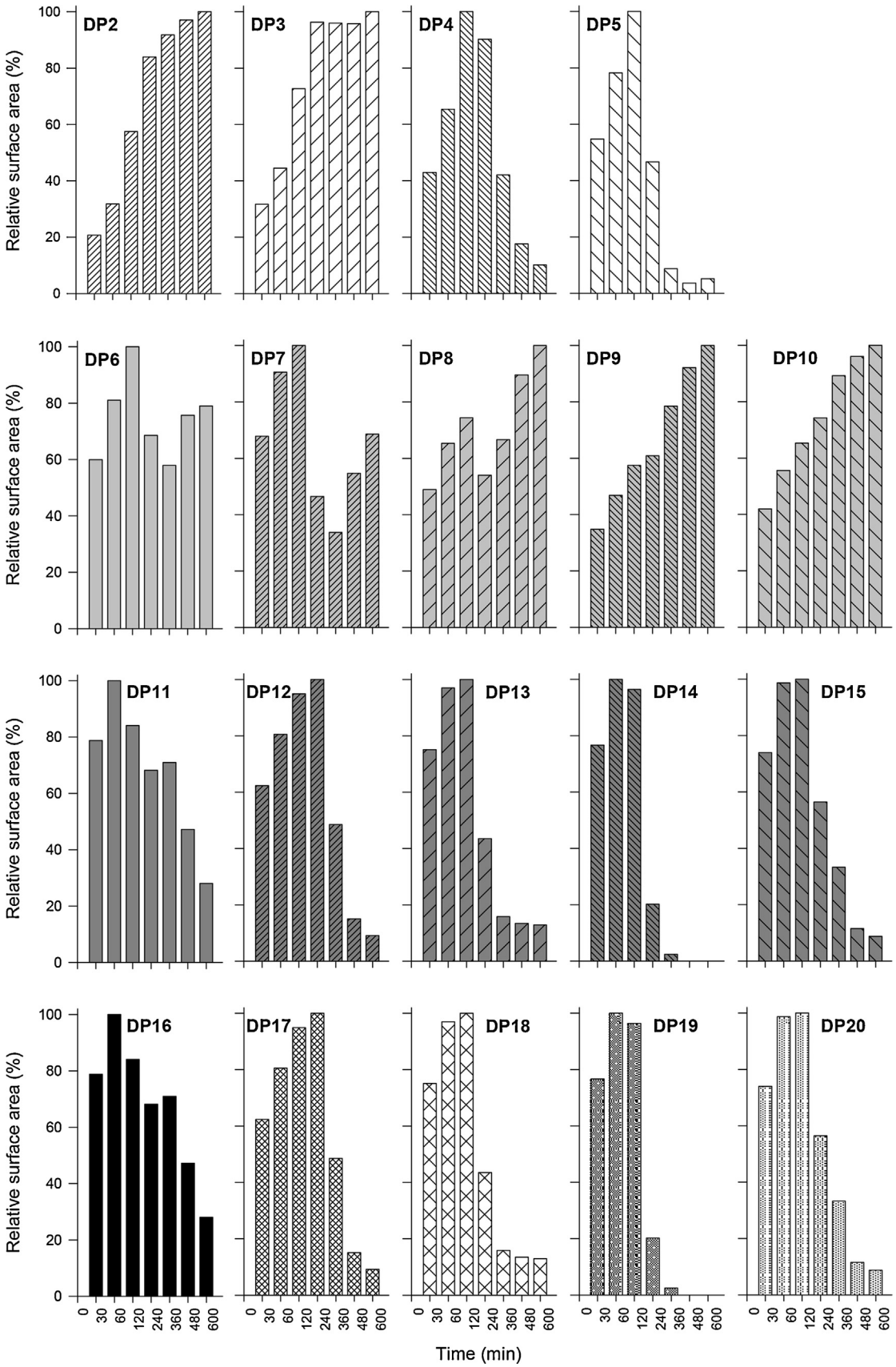

Fig. 4. Changes in relative surface area (%) of the different DPs of IMOS produced during the enzymatic degradation of Dextran T40 2% (w/v) using the IMER at a flow rate of 0.3 mL min^{-1}.

applied on proteins also resulted in modifications of macro- and micro-environments, thus reducing enzymatic activity of immobilized enzymes (Abou-Rebyeh, Körber, Schubert-Rehberg, Reusch, & Josic, 1991; Johansson et al., 1983; Wang, Bhattacharyya, & Bachas, 2001).

3.2. Characterization of IMOS produced by free and immobilized endodextranase D8144

IMOS obtained during the hydrolysis of Dextran T40 2 or 8% (w/v), using the IMER operating at a flow rate of 0.3 mL min^{-1}, were monitored by HPAEC-PAD analyses. LC–MS was used for the calibration of the HPAEC-PAD method and allowed the characterization of IMOS from DP 2 + K$^+$ (m/z = 381), DP 3 + K$^+$ (m/z = 543), DP 4 + K$^+$ (m/z = 705), DP 5 + K$^+$ (m/z = 867) to DP 6 + K$^+$ (m/z = 1029). HPAEC-PAD profiles are represented in Fig. 3.

Firstly, a population of IMOS of DPs ranging from 2 to 20 was observed after the enzymatic degradation of Dextran T40 2 and 8% (w/v) using the IMER. We observed a change of DPs population during the enzymatic hydrolysis. Indeed, the longer the hydrolysis time was, the higher was the quantity of small DPs. Different families of DPs were also produced during the enzymatic hydrolysis of Dextran T40 2% and 8%. In order to determine the distribution of these DPs families, relative surface areas for each DP were calculated from HPAEC-PAD results for the enzymatic degradation of Dextran T40 2% (w/v) using the IMER at 0.3 mL min^{-1} (Fig. 4). DPs 11–20 were generated until 60–120 min then degraded into smaller DPs which probably fed DPs between 6 and 10. DPs 8–10 seemed more saved during the hydrolysis time than DPs 6 or 7 which decreased. In the same way, DPs 4 and 5 were probably used by enzymes for generating the lowest DPs, *i.e.* DP 2 and DP 3 whose distribution increased all over the degradation. The same calculation was performed for the enzymatic degradation of Dextran T40 8% using the IMER. Different results were observed concerning DPs 8–10, which increased until 120 min then largely decreased, as we already observed it in Fig. 4C. This difference of distribution for DPs 8–10 between the enzymatic degradation of Dextran T40 2 and 8% was of primary importance. Indeed, Low Molecular Weight (LMW) IMOS possess interesting and simple structure for chemical modifications (sulfation) and antitumor (Xiao et al., 2011) or nutraceutical activities (Grimoud et al., 2010; Neeser & German, 2004). Thus, these first results showed the possibility to produce high distribution of LMW IMOS (DPs 8–10) all over the enzymatic hydrolysis depending on the concentration of substrate. These observations were already suggested by Tavernier et al. (2008) but not experimentally assessed. Therefore, the present study highlighted the potential of this new IMER for the toll-manufacturing production of LMW IMOS.

3.3. Storage and operating stability

Residual activities of the IMER are represented in Fig. 5. After 5400 volumes column corresponding to 90 h of operation, more than 78% of enzymatic activity were kept, including 78 days of storage at 4 °C. These results were close to those obtained by Benčina, Babič, and Podgornik (2007) since the authors measured a residual activity of 77% after 28 days of storage using an immobilized ribonuclease on Epoxy CIM® disk. It is noteworthy that our immobilized endodextranase activity stayed stable during 50 supplementary days of storage. Some authors suggested that the decrease of enzyme activity might be attributed to the capacity of non-deactivated epoxy groups to react again with the immobilized enzymes (Benčina et al., 2007).

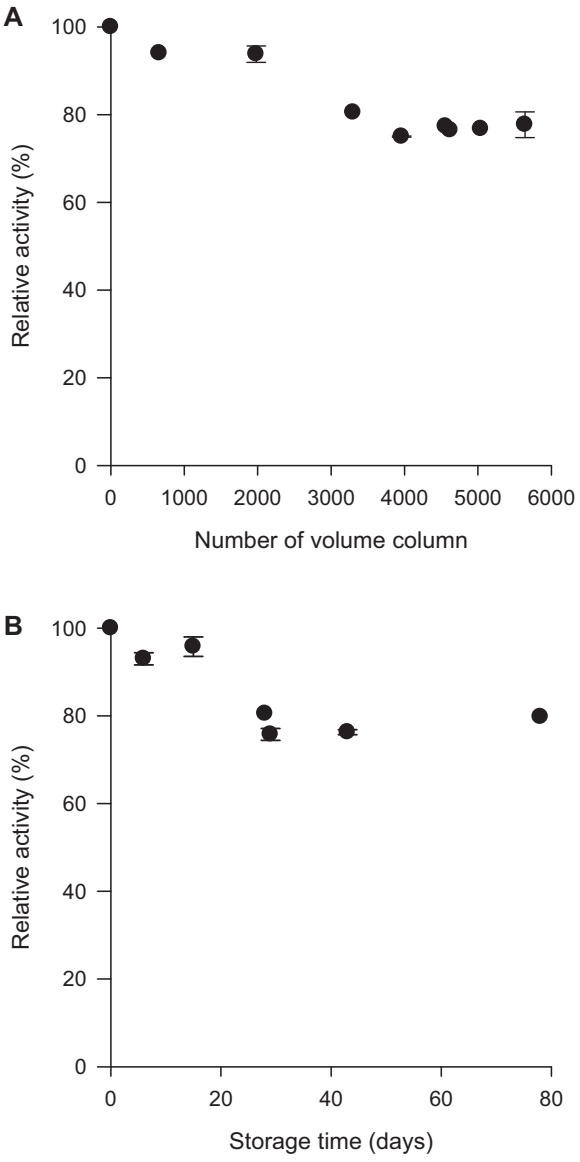

Fig. 5. Operational (A) and storage (B) stability assessments of endodextranase D8144 immobilized on an epoxy-activated CIM® disk.

4. Conclusion

A new endodextranase based monolithic IMER was developed and allowed the rapid on-line Dextran T40 depolymerization. Although the immobilization efficiency of endodextranase D8144 was rather low, *i.e.* about 15.9% (w/w), the real specific activity of immobilized enzymes was better than other works since we obtained an activity close to 6.5 U mg enz^{-1}. The difference between the real enzymatic activity and the theoretical one (261 U mg enz^{-1}) of immobilized enzymes was probably due to conformational and steric effects during the immobilization. The Km values (4.8 ± 0.2 g L^{-1}) for free and immobilized enzymes were closely the same due to the absence of diffusional limitation. DPs of IMOS were investigated and HPAEC-PAD analyses clearly showed its potential as a rapid routine chromatographic method for the detection and quantification of IMOS. Moreover, specific patterns of DPs distributions were observed during the enzymatic hydrolysis. DPs 11–20 were generated during the first 120 min then degraded into smaller DPs. Ratio of DPs 8–10 increased all over the hydrolysis showing the possibility to produce interesting and sought-after

sizes of IMOS in these conditions. Further experiments could be performed to highlight the effect of parameters as the quantity of immobilized enzymes, substrate concentration and flow rate on DPs distribution of IMOS. Besides, the IMER kept more than 77% of its residual activity for 78 days (including storage) and 90 hours of hydrolysis, indicating a high stability of the immobilized enzymes. Finally, the easy implementation of the immobilization procedure and the facility to associate the CIM® disk housing with automatic equipment are two major elements making our immobilized endodextranase on epoxy-activated CIM® disk a suitable and attractive way for production of isomaltooligosaccharides from Dextran by IMER.

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References

Abou-Rebyeh, H., Körber, F., Schubert-Rehberg, K., Reusch, J., & Josic, D. (1991). Carrier membrane as stationary phase for affinity chromatography and kinetic studies of membrane-bound enzymes. *Journal of Chromatography*, *566*, 341–350.

Akpinar, O., Erdogan, K., & Bostanci, S. (2009). Enzymatic production of xylooligosaccharide from selected agricultural wastes. *Food and Bioproducts Processing*, *87*, 145–151.

Aslan, Y., & Tanriseven, A. (2007). Immobilization of *Penicillium lilacinum* dextranase to produce isomaltooligosaccharides from dextran. *Biochemical Engineering Journal*, *34*, 8–12.

Badel, S., Bernardi, T., & Michaud, P. (2010). New perspectives for *Lactobacilli* exopolysaccharides. *Biotechnology Advances*, *29*, 54–66.

Bartolini, M., Cavrini, V., & Andrisano, V. (2005). Choosing the right chromatographic support in making a new acetylcholinesterase-micro-immobilised enzyme reactor for drug discovery. *Journal of Chromatography A*, *1065*, 135–144.

Benčina, K., Podgornik, A., Štrancar, A., & Benčina, M. (2004). Enzyme immobilization on epoxy- and 1,1′-carbonyldiimidazole-activated methacrylate-based monoliths. *Journal of Separation Science*, *27*, 811–818.

Benčina, M., Babič, J., & Podgornik, A. (2007). Preparation and characterization of ribonuclease monolithic bioreactor. *Journal of Chromatography A*, *1144*, 135–142.

Berruex, L. G., & Freitag, R. (2002). Affinity-based interactions on disks for fast analysis, isolation and conversion of biomolecules. In M. N. Gupta (Ed.), *Methods of affinity based separations of enzymes and proteins* (pp. 83–113). Basel: Birkhäuser.

Champagne, J., Delattre, C., Shanthi, C., Satheesh, B., Duverneuil, L., & Vijayalakshmi, M. A. (2007). Pseudoaffinity chromatography using a Convective Interaction Media®-disk monolithic column. *Chromatographia*, *65*, 639–648.

Delattre, C., Michaud, P., Courtois, B., & Courtois, J. (2005). Oligosaccharides engineering from plants and algae – Applications in biotechnology and therapeutic. *Minerva Biotechnologica*, *17*, 107–117.

Delattre, C., Michaud, P., & Vijayalakshmi, M. A. (2008). New monolithic enzymatic micro-reactor for the fast production and purification of oligogalacturonides. *Journal of Chromatography B*, *861*, 203–208.

Delattre, C., & Vijayalakshmi, M. A. (2009). Monolith enzymatic microreactor at the frontier of glycomic toward a new route for the production of bioactive oligosaccharides. *Journal of Molecular Catalysis B: Enzymatic*, *60*, 97–105.

Fu, H., Dencic, I., Tibhe, J., Sanchez Pedraza, C. A., Wang, Q., Noel, T., et al. (2012). Threonine aldolase immobilization on different supports for engineering of productive, cost-efficient enzymatic microreactors. *Chemical Engineering Journal*, *207–208*, 564–576.

Grimoud, J., Durand, H., Courtin, C., Monsan, P., Ouarné, F., Theodorou, V., et al. (2010). In vitro screening of probiotic lactic acid bacteria and prebiotic glucooligosaccharides to select effective synbiotics. *Anaerobe*, *16*, 493–500.

Jeanes, A., Haynes, W. C., Wilham, C. A., Rankin, J. C., Melvin, E. H., Austin, M. J., et al. (1954). Characterization and classification of dextrans from ninety-six strains of bacteria. *Journal of the American Chemical Society*, *76*, 5041–5052.

Johansson, G., Orgen, L., & Olsson, B. (1983). An enzymatic flow injection method for the determination of oxygen. *Analytica Chimica Acta*, *145*, 101–108.

Johnson, K. A., & Goody, R. S. (2011). The original Michaelis constant: Translation of the 1913 Michaelis–Menten paper. *Biochemistry*, *50*, 8264–8269.

Josic, D., Buchacher, A., & Jungbauer, A. (2001). Monoliths as stationary phases for separation of proteins and polynucleotides and enzymatic conversion. *Journal of Chromatography B*, *752*, 191–205.

Khalikova, E., Susi, P., & Korpela, T. (2005). Microbial dextran-hydrolyzing enzymes: Fundamentals and applications. *Microbiology and Molecular Biology Reviews*, *69*, 306–325.

Lali, A., Manudhane, K., Motlekar, N., & Karandikar, P. (2002). Depolymerization of starch and pectin using superporous matrix supported enzymes. *Indian Journal of Biochemistry and Biophysics*, *39*, 253–258.

Laroche, C., & Michaud, P. (2007). New developments and prospective applications for β-(1,3) glucans. *Recent Patents on Biotechnology*, *1*, 59–73.

Lineweaver, H., & Burk, D. (1934). The determination of enzyme dissociation constants. *Journal of the American Chemical Society*, *56*, 658–666.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–275.

Markoglou, N., & Wainer, I. W. (2003). Immobilized enzyme reactors in liquid chromatography: On-line bioreactors for use in synthesis and drug discovery. In I. D. Wilson (Ed.), *Handbook of analytical separations: Volume 4: Bioanalytical separations* (pp. 215–234). Oxford: Elsevier Science Ltd.

Neeser, J. R., & German, J. B. (2004). *Bioprocesses and biotechnology for functional foods and nutraceuticals*. New York: Marcel Dekker Inc.

Nicoli, R., Gaud, N., Stella, C., Rudaz, S., & Veuthey, J. L. (2008). Trypsin immobilization on three monolithic disks for on-line protein digestion. *Journal of Pharmaceutical and Biomedical Analysis*, *48*, 398–407.

Petro, M., Svec, F., & Fréchet, J. M. J. (1996). Immobilization of trypsin onto molded macroporous poly(glycidyl methacrylate-co-ethylene dimethacrylate) rods and use of the conjugates as bioreactors and for affinity chromatography. *Biotechnology and Bioengineering*, *49*, 355–363.

Pierre, G., Maache-Rezzoug, Z., Sannier, F., Rezzoug, S. A., & Maugard, T. (2011). High-performance hydrolysis of wheat straw using cellulase and thermomechanical pretreatment. *Process Biochemistry*, *46*, 2194–2200.

Pierre, G., Salah, R., Gardarin, C., Traikia, M., Petit, E., Delort, A. M., et al. (2013). Enzymatic degradation and bioactivity evaluation of C-6 oxidized chitosan. *International Journal of Biological Macromolecules*, *60*, 383–392.

Rogalski, J., Glowiak, G., Szczodrak, J., Pleszczyńska, M., Szczodrak, Z., & Wiater, A. (1998). Purification and immobilization of dextranase. *Acta Biotechnologica*, *18*, 63–75.

Sheldon, R. A. (2007). Enzyme immobilization: The quest for optimum performance. *Advanced Synthesis & Catalysis*, *349*, 1289–1307.

Tavernier, M. L., Petit, E., Delattre, C., Courtois, B., Courtois, J., Strancar, A., et al. (2008). Production of oligoglucuronans using a monolithic enzymatic microreactor. *Carbohydrate Research*, *343*, 2687–2691.

Turecek, P. L., Pittner, F., & Birkner, F. (1990). Degradation of polysaccharides by immobilized depolymerizing enzymes. *International Journal of Food Science and Technology*, *25*, 1–8.

Turnbull, J. E., & Field, R. A. (2007). Emerging glycomics technologies. *Nature Chemical Biology*, *3*, 74–77.

Varki, A. (1993). Biological roles of oligosaccharides: All of the theories are correct. *Glycobiology*, *3*, 97–130.

Vodopivec, M., Podgornik, A., Berovič, M., & Štrancar, A. (2003). Characterization of CIM monoliths as enzyme reactors. *Journal of Chromatography B*, *795*, 105–113.

Waffenschmidt, S., & Jaenicke, L. (1987). Assay of reducing sugars in the nanomole range with 2,2′-bicinchoninate. *Analytical Biochemistry*, *165*, 337–340.

Wang, J., Bhattacharyya, D., & Bachas, L. G. (2001). Improving the activity of immobilized subtilisin by site-directed attachment through a genetically engineered affinity tag. *Fresenius' Journal of Analytical Chemistry*, *369*, 280–285.

Wang, Y. (2009). Prebiotics: Present and future in food science and technology. *Food Research International*, *42*, 8–12.

Wolff, I. A., Watson, P. R., Sloan, J. W., & Rist, C. E. (1953). Controlled thermal depolymerization of dextran. *Industrial & Engineering Chemistry*, *45*, 755–759.

Xiao, C. L., Tao, Z. H., Guo, L., Li, W. W., Wan, J. L., Sun, H. C., et al. (2011). Isomaltooligosaccharide sulfate inhibits tumor growth and metastasis of hepatocellular carcinoma in nude mice. *BMN Cancer*, *11*, article no. 150.

Zief, M., Brunner, G., & Metzendor, J. (1956). Fractionation of partially hydrolyzed dextran. *Industrial & Engineering Chemistry*, *48*, 119–121.

Zou, Q., Pu, Y., Han, Z., Fu, N., Li, S., Liu, M., et al. (2012). Ultrasonic degradation of aqueous dextran: Effect of initial molecular weight and concentration. *Carbohydrate Polymers*, *90*, 447–451.