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

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14 **Highlights**

15 ▪ Endodextranases were immobilized for the first time on epoxy activated CIM® disk.
16 ▪ Isomaltooligosaccharides were produced by a new IMmobilized Enzymes Reactor
17 (IMER).
18 ▪ Immobilized enzymes were active (70 µg proteins, 6.5 U.mg enz^{-1}, Km=4.8 $g.L^{-1}$).
19 ▪ Specific patterns of DPs distributions were observed during the enzymatic hydrolysis.
20 ▪ The IMER showed good operational (90 h) and storage stability (78 days).
21
22
23

24 **Abstract**

25 Endodextranase D8144 from *Penicillium sp.* (EC 3.2.1.2.) was immobilized on an epoxy-
26 activated monolithic Convective Interaction Media (CIM®) disk in order to produce
27 isomaltooligosaccharides (IMOS) from Dextran T40 in a continuous IMmobilized Enzymes
28 Reactor (IMER). Enzymatic parameters and structure of IMOS were studied for free and
29 immobilized enzymes. The immobilization efficiency of endodextranase D8144 was about
30 15.9 % (w/w) and the real specific activity was close to 6.5 U.mg enz^{-1}. The Km values (4.8 ±
31 0.2 $g.L^{-1}$) for free and immobilized enzymes were the same, showing the absence of
32 diffusional limitation. Moreover, specific patterns of DPs (Degrees of Polymerization)
33 distributions were observed during the enzymatic hydrolysis by HPAEC-PAD (High Pressure
34 Anion Exchange Chromatography-Pulsed Amperometric Detection). Thus, sought-after sizes
35 of IMOS (DPs 8-10) were generated all over the hydrolysis. Finally, the results showed the
36 high stability of this IMER since a relative enzymatic activity about 78 % was measured after
37 5400 volumes column.

38 ***Keywords:*** *CIM® disk, Dextran, Endodextranase, Immobilized enzyme,*
39 *Isomaltooligosaccharides (IMOS).*

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40 **1. Introduction**

41 Oligosaccharides, commonly found in nature as glycoconjugates, play a fundamental part in
42 many biological processes. These macromolecules exhibit structural diversity greatly higher
43 than proteins and nucleic acids (Turnbull & Field, 2007; Varki, 1993). Since last years,
44 oligosaccharides were increasingly employed as functional or therapeutical drugs.
45 Nevertheless, in most cases, the lack of performing and creative industrial oligosaccharides
46 processes is considered as the main scientific bottleneck limiting their exploitation as
47 biological molecules. For this reason, development of oligosaccharides engineering strategies
48 is of primary importance. Natural polysaccharides can be depolymerized by physical (Zou et
49 al., 2012; Wolff, Watson, Sloan & Rist, 1953), chemical (Zief, Brunner & Metzendor, 1956)
50 and enzymatic procedures (Delattre, Michaud, Courtois & Courtois, 2005; Khalikova, Susi &
51 Korpela, 2005). However the physical and chemical strategies are known for their random and
52 hardly controllable cleavage (Delattre, Michaud, Courtois & Courtois, 2005).
53 Therefore, enzymatic cleavage by using glycoside hydrolases (EC 3.2.1.-) and polysaccharide
54 lyases (EC 4.2.2.-), which are easily available at low cost, are one of the best ways to selectively
55 obtain oligosaccharides from natural polysaccharides. Controlling the degree of polymerization
56 (DP) of oligosaccharides is moreover possible if an endolytic mechanism is involved. The
57 production of oligosaccharides from polysaccharides by highly specific enzymes has been
58 abundantly studied during the last decade notably in nutraceutical, agronomy or agro-industry
59 (Akpinar, Erdogan & Bostanci, 2009; Laroche & Michaud, 2007; Pierre et al., 2011; Pierre et al.,
60 2013; Wang, 2009). However, few authors have worked on polysaccharides degradations through
61 the use of immobilized enzymes (Aslan & Tanriseven, 2007; Delattre, Michaud &
62 Vijayalakshmi, 2008; Lali, Manudhane, Motlekar & Karandijar, 2002; Turecek, Pittner &
63 Birkner, 1990). Even, immobilized enzymes generally overpass classical limitations described for
64 free enzymes (Sheldon, 2007).

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65 IMobilized Enzymes Reactors (IMER) possess several advantages such as reusability, high
66 enzyme stability, scale-up reduction cost and the possibility to work in automatic continuous
67 flow system for long periods (Markoglou & Wainer, 2003). They also turn out to be
68 economically beneficial for the production of high value molecules (Fu et al., 2012). During
69 the design of IMERs, one of the most important parameters is the ideal choice of the matrix
70 for the enzymes immobilization. This choice is closely dependant on the surface area, the
71 cost, the thermal and chemical stabilities and the mass transfer characteristics (Bartolini,
72 Cavrini & Andrisano, 2005). In most of cases, the main drawback of porous beads IMERs
73 was the low mass transfer observed by authors (Petro, Svec & Fréchet, 1996). Consequently,
74 recent polymeric macroporous monolithic supports have been developed to improve (*i*) the
75 large surface area, (*ii*) the good mass transfer characteristics and (*iii*) the low back-pressure
76 (Champagne et al., 2007; Josic, Buchacher & Jungbauer, 2001). Monolith matrices are well
77 characterized for their low column back pressure and high efficiency in terms of
78 chromatographic parameters. In fact, the diffusion path is lower due to the high convective
79 effect allowing a very good mass transfer (Champagne et al., 2007; Josic, Buchacher &
80 Jungbauer, 2001). These last years, original monolith matrices have been employed for
81 enzymes immobilization. Convective Interaction Media® (CIM) disks are macroporous
82 poly(glycidyl methacrylate-co-ethylene dimethacrylate) (Figure 1A) and were already
83 suggested for IMERs design (Delattre & Vijayalakshmi, 2009; Josic, Buchacher & Jungbauer,
84 2001; Vodopivec, Podgornik, Berovič & Štrancar, 2003). In this way, the immobilization of a
85 pectin lyase on an epoxy-activated CIM® disk allowed obtaining large amounts of purified
86 oligogalacturonans within a short delay (Delattre, Michaud & Vijayalakshmi, 2008). Tavernier
87 et al. (2008) also showed the possibility to control the degree of polymerization (DP) of
88 oligoglucuronans through the substrate flow rate by using an immobilized glucuronan lyase.

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89 The main goal of this paper is to demonstrate the potential of macroporous epoxy-activated
90 monolithic support for the covalent immobilization of endodextranases to produce
91 isomaltooligosaccharides (IMOS) from dextran. Dextran is an extracellular
92 homopolysaccharide of α-D-glucopyranose (D-Glc*p*) with mainly α-(1,6) linkages and α-(1,2),
93 α-(1,3) and α-(1,4) branching in the backbone chain. It is produced from sucrose by bacteria
94 such as *Leuconostoc*, *Lactobacillus*, *Streptococcus*, which possess glucansucrase activities
95 (E.C. 2.4.1.5) (Badel, Bernardi & Michaud, 2010). Firstly, IMOS were produced by free
96 endodextranase D8144 from *Penicilium sp.* Enzymatic parameters were calculated in
97 conventional conditions and DPs of IMOS were estimated. Secondly, IMOS were then
98 produced after immobilizing the same enzymes on an epoxy-activated CIM® disk in order to
99 show the potential of this new endodextranase based monolithic IMERs for rapid on-line
100 dextran depolymerization.
101

102 **2. Materials and methods**

103 **2.1. Enzymes and Reagents**

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

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114 **2.2. Protein and reducing sugar analyses**

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

121 **2.3. Production of IMOS by free endodextranase D8144**

122 The time-course of reducing sugar concentrations was performed to determine the free
123 endodextranase activity, using a Radley Carousel 12 plus (Radley Discovery Technologies,
124 Shire Hill, UK). Briefly, 1.6 U of endodextranase D8144 were added to 20 mL of 1 to 8 %
125 (w/v) Dextran T40 solution in potassium phosphate buffer (100 mM; pH 6) at 37°C and under
126 stirring (400 rpm). 500 µL were taken at regular time intervals, then boiled for 30 min at
127 100°C in a dry bath to inactivate the enzymes and finally stored at -20°C before reducing
128 sugar analyses. One unit (U) of endodextranase activity was defined as the amount of
129 enzymes necessary to release reducing sugars equivalent to 1 µmol of isomaltose per min
130 from Dextran T40 used as substrate at optimal temperature and pH (37°C; pH 6) (Aslan &
131 Tanriseven, 2007; Rogalski et al., 1998). Initial velocities (Vi), maximal velocity (Vm) and
132 dissociation constant (Km) were estimated for free endodextranase D8144 by using
133 Michaëlis-Menten and Lineweaver-Burk methods (Johnson & Goody, 2011; Lineweaver &
134 Burk, 1934). All the experiments were done at least in triplicate.

135 **2.4. Production of IMOS by the immobilized endodextranase reactor**

136 **2.4.1. The immobilized endodextranase reactor system**

137 The IMER used in this study (Figure 1B) for both immobilization of endodextranase D8144
138 and dextran hydrolysis, consisted in a Gilson Minipuls 3 peristaltic pump (Middleton, WI,

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139 USA) providing flow rates up to 5.5 $mL.min^{-1}$, an epoxy-activated CIM® disk placed on its
140 support and a 10 mL agitated feed tank. The whole system was placed in an oven to control
141 the temperature.

142 **2.4.2. Immobilization procedure of endodextranase D8144**

143 The CIM® disk was previously equilibrated with a potassium phosphate buffer solution (100
144 mM; pH 8) during 30 min at 20°C and under stirring (120 rpm). 444 µg of proteins
145 corresponding to 92 U of endodextranase D8144 were dissolved in 2.8 mL of potassium
146 phosphate buffer solution (100 mM; pH 8) and placed under circulation at 0.3 $mL.min^{-1}$
147 through the epoxy-activated CIM® disk for 20 h at 20°C. This dynamic immobilization step
148 was then followed by a static one in which the disk was plunged in the same endodextranase
149 D8144 solution for 5 h under gentle stirring (120 rpm). In order to inactivate any residual
150 epoxy sites, the CIM® disk was then flushed for 12 h with 30 mM ethanolamine solution (in
151 100 mM potassium phosphate buffer solution, pH 8) at 0.3 $mL.min^{-1}$ and 20°C. The
152 immobilization procedure was ended by washing the CIM® disk for one hour with a
153 potassium phosphate buffer solution (100 mM; pH6) at 0.3 $mL.min^{-1}$ and 20°C. Three
154 different epoxy CIM® disks were used to confirm the robustness of the immobilization
155 procedures.

156 **2.4.3. Immobilized endodextranase activity assays**

157 Before any activity assay, the IMER was placed at 37°C and flushed during 30 min with
158 potassium phosphate buffer (100 mM; pH 6). As for free enzymes, pH 6 was used since
159 previous authors observed no pH shift after the immobilization of another endodextranase onto
160 an epoxy-activated Eupergit C support (Aslan & Tanriseven, 2007). Four mL of Dextran T40
161 solutions (1 to 8 %; w/v; 100 mM potassium phosphate buffer, pH 6) were then placed under
162 circulation through the immobilized endodextranases CIM® disk at a flow rate of 0.3 $mL.min^{-1}$
163 for 11 h corresponding to 51 cycles at 37°C. One cycle was defined as the time necessary to

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164 flush the 4 mL of the Dextran T40 solution through the endodextranases CIM® disk. Twenty
165 µL were taken at regular time intervals, then boiled for 30 min at 100°C in a dry bath to
166 inactivate the enzymes and finally stored at -20°C before reducing sugar analyses (part 2.3.).
167 Finally, the IMER was flushed for 30 min with 100 mM potassium phosphate buffer (pH 6) at
168 0.3 $mL.min^{-1}$ and 37°C, then stored in the same buffer at 4°C before the next utilization.

169 **2.4.4. Operational and storage stability**

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

176 **2.5. Structural analysis of IMOS by analytic chromatography**

177 **2.5.1. HPLC/ESI-MS analyses**

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

188 **2.5.2. HPAEC-PAD analyses**

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189 HPAEC-PAD analyses were carried out on a Dionex ICS3000 ion chromatography system
190 with a CarboPAC™ PA200 (4 mm x 250 mm) analytical column equipped by a CarboPAC™
191 PA200 (4 mm x 50 mm) guard cartridge. All samples analyzed were filtered at 0.2 μm. The
192 sample volume injection was 25 μL and the system was operated at 25°C at a flow rate of 0.5
193 $mL.min^{-1}$ using a gradient of eluent A (NaOH 100 mM) and eluent B (sodium acetate 250
194 mM in NaOH 100mM). The gradient contained four steps (expressed in percent B in A): 0%
195 during 10 min; 0-100% from 10 to 50 min; 100% from 50 to 70 min; 0% from 70 to 80 min.
196 Glucose (DP 1), isomaltose (DP 2), and isomaltotriose (DP 3) were used as standard for
197 quantification. Carbohydrates were detected on a pulsed amperometric ED50 detector
198 (Dionex Corp., Sunnyvale, CA, USA). Data acquisition and processing were carried out using
199 Chromeleon® software 6.8 (Dionex Corporation, Sunnyvale, CA, USA).
200

201 **3. Results and discussion**

202 **3.1. Kinetic parameters of free and immobilized endodextranase D8144**

203 Convective Interaction Media disk (CIM® disk) was used to immobilize endodextranase
204 D8144. Table 1 presents the yields of immobilized proteins and enzymatic activities obtained
205 after the immobilization of endodextranase D8144 on epoxy-activated CIM® disk. The
206 immobilized proteins content was about 70 μg corresponding to an immobilization yield of
207 15.9 % (w/w) while the theoretical activity of immobilized enzymes was equal to 18.3 U (261
208 $U.mg\ enz^{-1}$) corresponding to an activity yield close to 18.6 % (U/U). These yields were in
209 agreement with other works, *e.g.* Nicoli et al. (2008) who obtained an immobilized trypsin
210 (EC 3.4.21.4) yield above 18 % (w/w). It is noteworthy that a wide range of immobilization
211 yields were described in the literature, varying from 15 to 60 % (w/w) (Tavernier et al., 2008;
212 Vodopivec, Podgornik, Berovič & Štrancar, 2003; Berruex & Freitag, 2002). Johansson,
213 Orgen & Olsson (1983) reported that numerous factors could affect the immobilization

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214 efficiency on CIM® disk as the enzyme loading, the molecular weight of the enzyme, the
215 quantity of free amine groups or the hydrodynamic characteristics of monolith supports.
216 Besides, the procedure of immobilization is of crucial importance since long static incubations
217 allow improving the quantity of immobilized proteins by 25 % (Tavernier et al., 2008;
218 Benčina, Podgornik, Štrancar & Benčina, 2004; Vodopivec, Podgornik, Berovič & Štrancar,
219 2003).
220 Time-courses of enzymatic activities measured by reducing sugars assays are described in
221 Figure 2. Accordingly to the literature for free enzymes in conventional media, reducing
222 sugars concentrations raised during the hydrolysis time of increasing substrate concentrations
223 (Figure 2A). The same tends were observed using the IMER (Figure 2B). Note to mention
224 that the enzymatic degradation using immobilized endodextranases D8144 (0.46 U) was five
225 times faster than using free endodextranase D8144 (1.6 U), which was already described by
226 Tavernier et al. (2008). Beside, approximately 1 % and 19 % of high-weight dextran were not
227 fully degraded after 600 min of hydrolysis with the epoxy CIM® Disk, respectively for 2 %
228 and 8 % of dextran T40 solutions. Kinetic parameters for both free and immobilized
229 endodextranases are detailed in Table 2. The Km value for immobilized enzyme was 4.8 ± 0.2
230 $g.L^{-1}$ and was closely the same for free enzyme, highlighting the absence of diffusional
231 limitation (Vodopivec, Podgornik, Berovič & Štrancar, 2003). Nevertheless, the specific
232 activity measured for immobilized enzyme (6.5 $U.mg\ enz^{-1}$) was 40 times lower than the
233 theoretical one (261 $U.mg\ enz^{-1}$). This result was better than previous studies since Tavernier
234 et al. (2008) measured an enzymatic activity of immobilized glucuronan lyase 1300 times
235 lower than the theoretical immobilized activity. Anyway, this drop was already well described
236 in the literature and was explained by conformational and steric effects involved during
237 random immobilization (Nicoli et al., 2008; Vodopivec, Podgornik, Berovič & Štrancar,
238 2003). Considering that mass transfer effects were negligible in CIM® disk (Vodopivec,

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239 Podgornik, Berovič & Štrancar, 2003), chemical and physical stresses applied on proteins also
240 resulted in modifications of macro- and micro-environments, thus reducing enzymatic activity
241 of immobilized enzymes (Wang, Bhattacharyya & Bachas, 2001; Abou-Rebyeh et al., 1991;
242 Johansson, Orgen & Olsson, 1983).

243 **3.2. Characterization of IMOS produced by free and immobilized**
244 **endodextranase D8144**

245 IMOS obtained during the hydrolysis of Dextran T40 2 or 8 % (w/v), using the IMER
246 operating at a flow rate of 0.3 $mL.min^{-1}$, were monitored by HPAEC-PAD analyses. LC-MS
247 was used for the calibration of the HPAEC-PAD method and allowed the characterization of
248 IMOS from DP 2 + K^+ (m/z = 381), DP 3 + K^+ (m/z = 543), DP 4 + K^+ (m/z = 705), DP 5 +
249 K^+ (m/z = 867) to DP 6+ K^+ (m/z = 1029). HPAEC-PAD profiles are represented in Figure 3.
250 Firstly, a population of IMOS of DPs ranging from 2 to 20 was observed after the enzymatic
251 degradation of Dextran T40 2 and 8% (w/v) using the IMER. We observed a change of DPs
252 population during the enzymatic hydrolysis. Indeed, the longer the hydrolysis time was, the
253 higher was the quantity of small DPs. Different families of DPs were also produced during the
254 enzymatic hydrolysis of Dextran T40 2% and 8%. In order to determine the distribution of
255 these DPs families, relative surface areas for each DP were calculated from HPAEC-PAD
256 results for the enzymatic degradation of Dextran T40 2% (w/v) using the IMER at 0.3
257 $mL.min^{-1}$ (Figure 4). DPs 11 to 20 were generated until 60-120 min then degraded into
258 smaller DPs which probably fed DPs between 6 to 10. DPs 8 to 10 seemed more saved during
259 the hydrolysis time than DPs 6 or 7 which decreased. In the same way, DPs 4 and 5 were
260 probably used by enzymes for generating the lowest DPs, *i.e.* DP 2 and DP 3 whose
261 distribution increased all over the degradation. The same calculation was performed for the
262 enzymatic degradation of Dextran T40 8% using the IMER. Different results were observed
263 concerning DPs 8 to 10, which increased until 120 min then largely decreased, as we already

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264 observed it in Figure 4C. This difference of distribution for DPs 8 to 10 between the
265 enzymatic degradation of Dextran T40 2 and 8% was of primary importance. Indeed, Low
266 Molecular Weight (LMW) IMOS possess interesting and simple structure for chemical
267 modifications (sulfation) and antitumor (Xiao et al., 2011) or nutracreutical activities
268 (Grimoud et al., 2010; Neeser & German, 2004). Thus, these first results showed the
269 possibility to produce high distribution of LMW IMOS (DPs 8 to 10) all over the enzymatic
270 hydrolysis depending on the concentration of substrate. These observations were already
271 suggested by Tavernier et al. (2008) but not experimentally assessed. Therefore, the present
272 study highlighted the potential of this new IMER for the toll-manufacturing production of
273 LMW IMOS.

274 **3.4. Storage and Operating stability**

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

284

285 **4. Conclusion**

286 A new endodextranase based monolithic IMER was developed and allowed the rapid on-line
287 Dextran T40 depolymerization. Although the immobilization efficiency of endodextranase
288 D8144 was rather low, *i.e.* about 15.9 % (w/w), the real specific activity of immobilized

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289 enzymes was better than other works since we obtained an activity close to 6.5 U.mg enz^{-1}.
290 The difference between the real enzymatic activity and the theoretical one (261 U.mg enz^{-1}) of
291 immobilized enzymes was probably due to conformational and steric effects during the
292 immobilization. The Km values (4.8 ± 0.2 g.L^{-1}) for free and immobilized enzymes were
293 closely the same due to the absence of diffusional limitation. DPs of IMOS were investigated
294 and HPAEC-PAD analyses clearly showed its potential as a rapid routine chromatographic
295 method for the detection and quantification of IMOS. Moreover, specific patterns of DPs
296 distributions were observed during the enzymatic hydrolysis. DPs 11 to 20 were generated
297 during the first 120 min then degraded into smaller DPs. Ratio of DPs 8 to 10 increased all
298 over the hydrolysis showing the possibility to produce interesting and sought-after sizes of
299 IMOS in these conditions. Further experiments could be performed to highlight the effect of
300 parameters as the quantity of immobilized enzymes, substrate concentration and flow rate on
301 DPs distribution of IMOS. Besides, the IMER kept more than 77% of its residual activity for
302 78 days (including storage) and 90 hours of hydrolysis, indicating a high stability of the
303 immobilized enzymes. Finally, the easy implementation of the immobilization procedure and
304 the facility to associate the CIM® disk housing with automatic equipment are two major
305 elements making our immobilized endodextranase on epoxy-activated CIM® disk a suitable
306 and attractive way for production of isomaltooligosaccharides from Dextran by IMER.
307

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428 **Table 1:** Protein contents, enzymatic activities and immobilization yields of endodextranase

429 D8144 on epoxy-activated CIM® disk.

430

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 %

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431 **Table 2:** Kinetic parameters for free and immobilized endodextranase D8144.

Endodextranase D8144	Free	Immobilized[1]
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

432 [1] Experiments were performed with a Dextran T40 flow rate of 0.3 $mL.min^{-1}$

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433 **Figure 1:** (A) Schematic conception of convective interaction media (CIM) monolith column
434 described by Champagne et al. (2007) and (B) design of the IMmobilized Endodextranase
435 Reactor (IMER) system: (1) Dextran T40 in phosphate buffer solution (100 mM; pH6), (2)
436 control of pH, temperature, flow rate and concentration, (3) peristaltic pump, (4) immobilized
437 endodextranases on epoxy-activated CIM® disk.

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

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

446 **Figure 4:** Changes in relative surface area (%) of the different DPs of IMOS produced during
447 the enzymatic degradation of Dextran T40 2% (w/v) using the IMER at a flow rate of 0.3
448 $mL.min^{-1}$.

449 **Figure 5:** Operational (A) and storage (B) stability assessments of endodextranase D8144
450 immobilized on an epoxy-activated CIM® disk.

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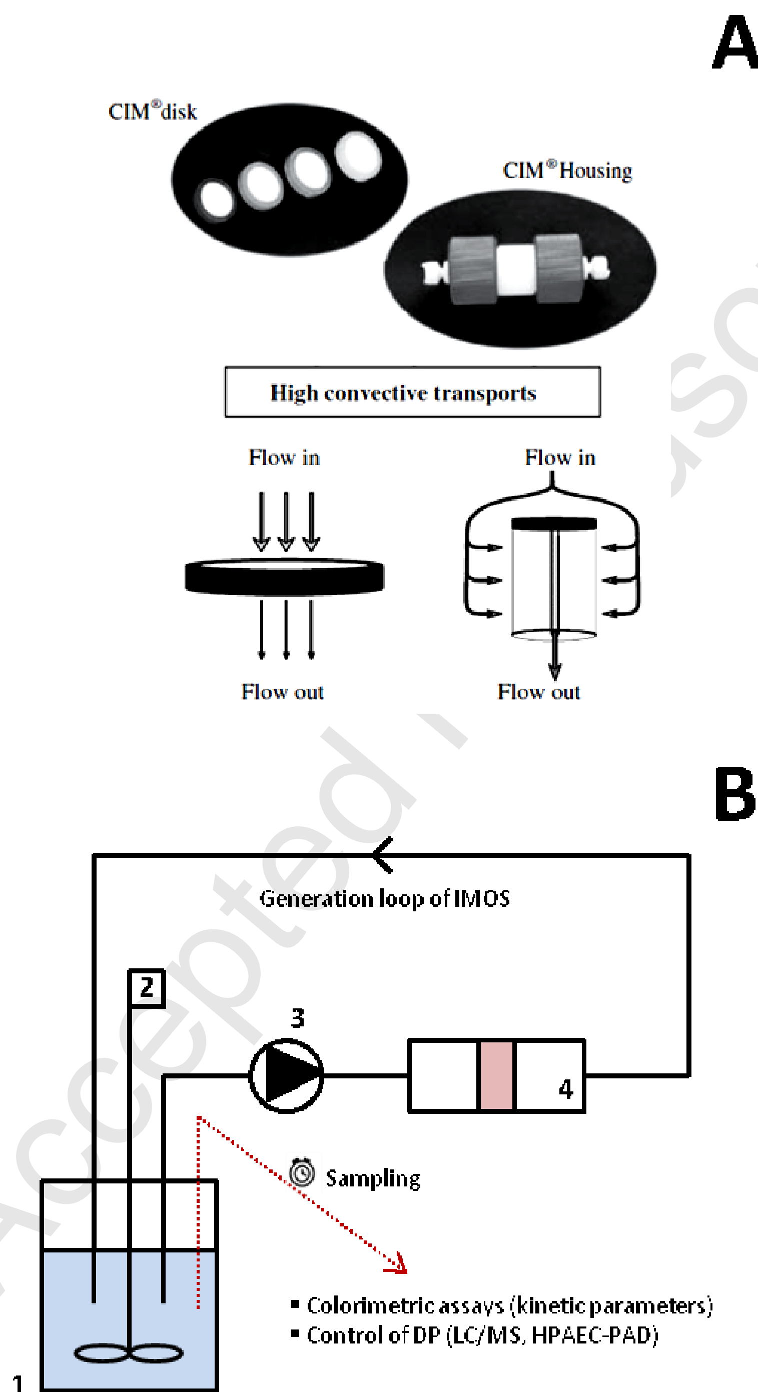

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454 **Figure 1**

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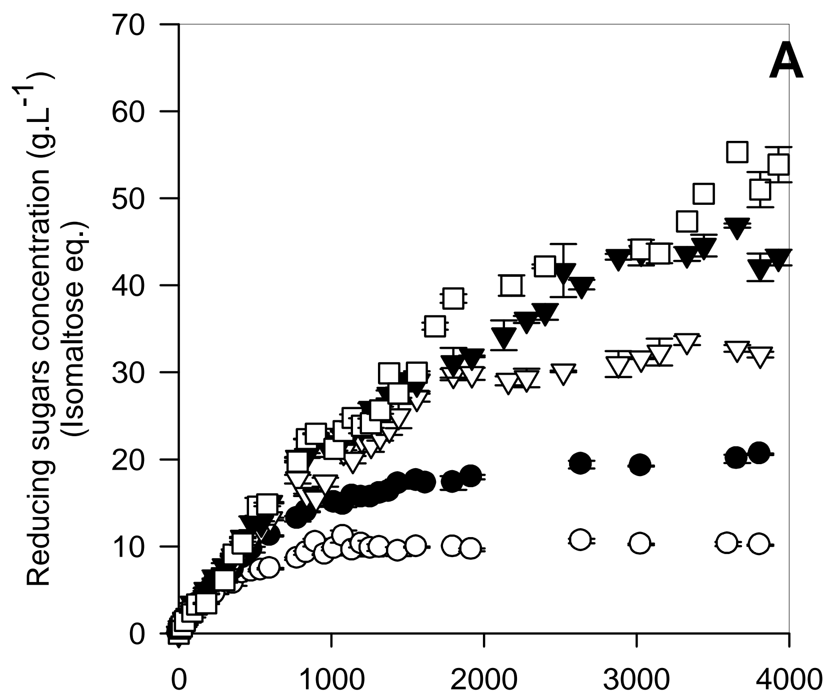

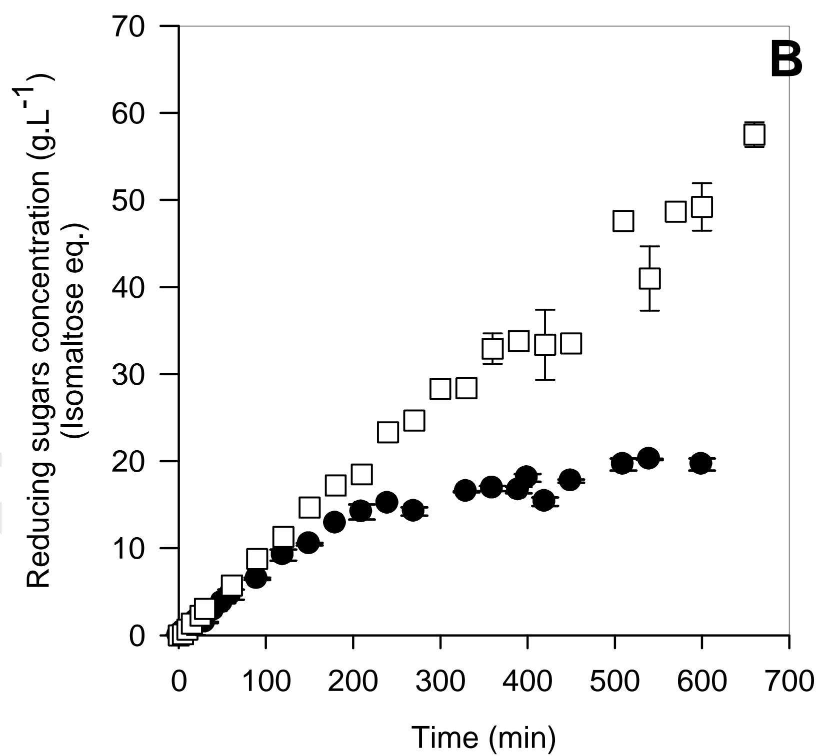

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Figure 2

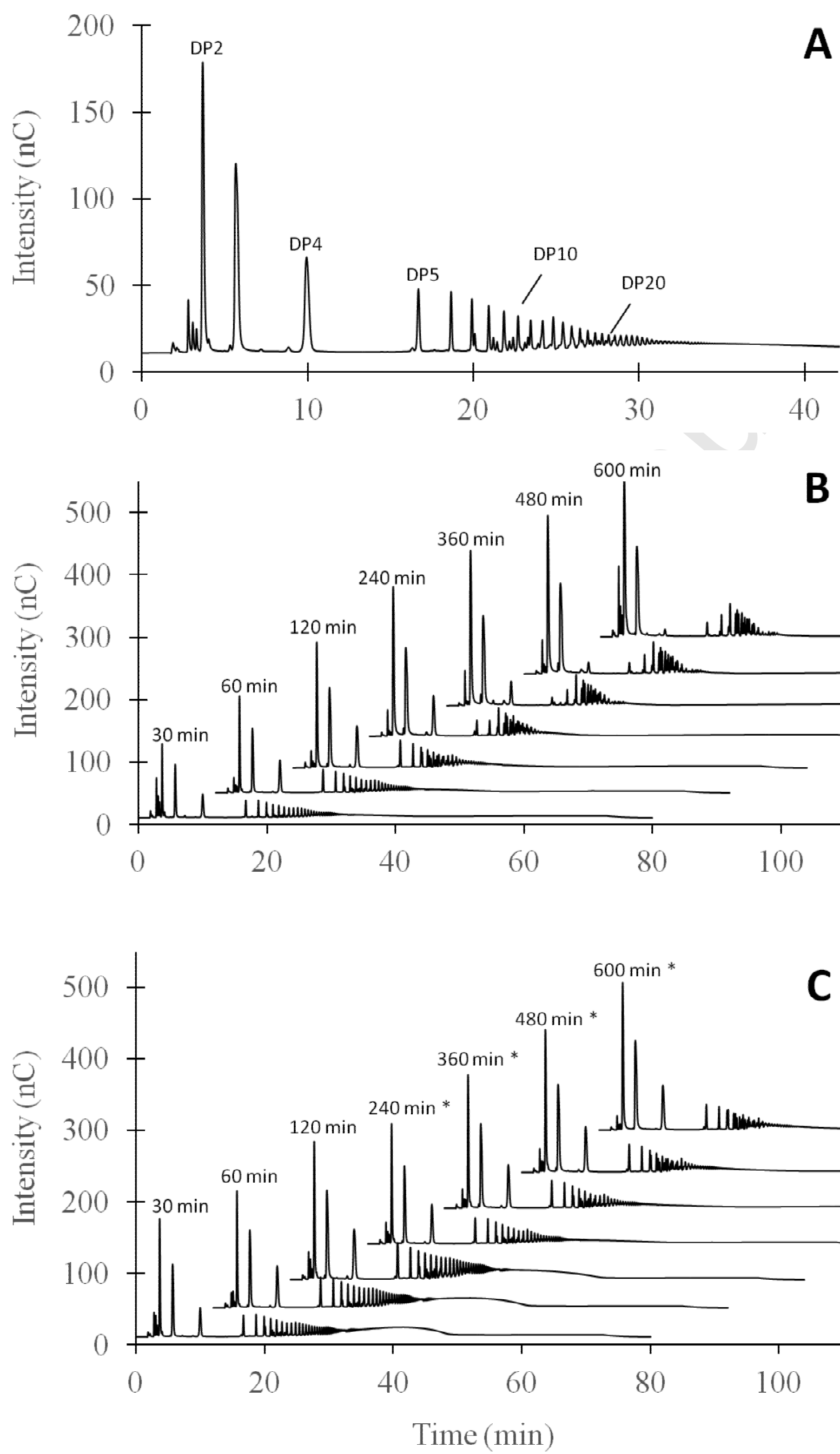

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Figure 3

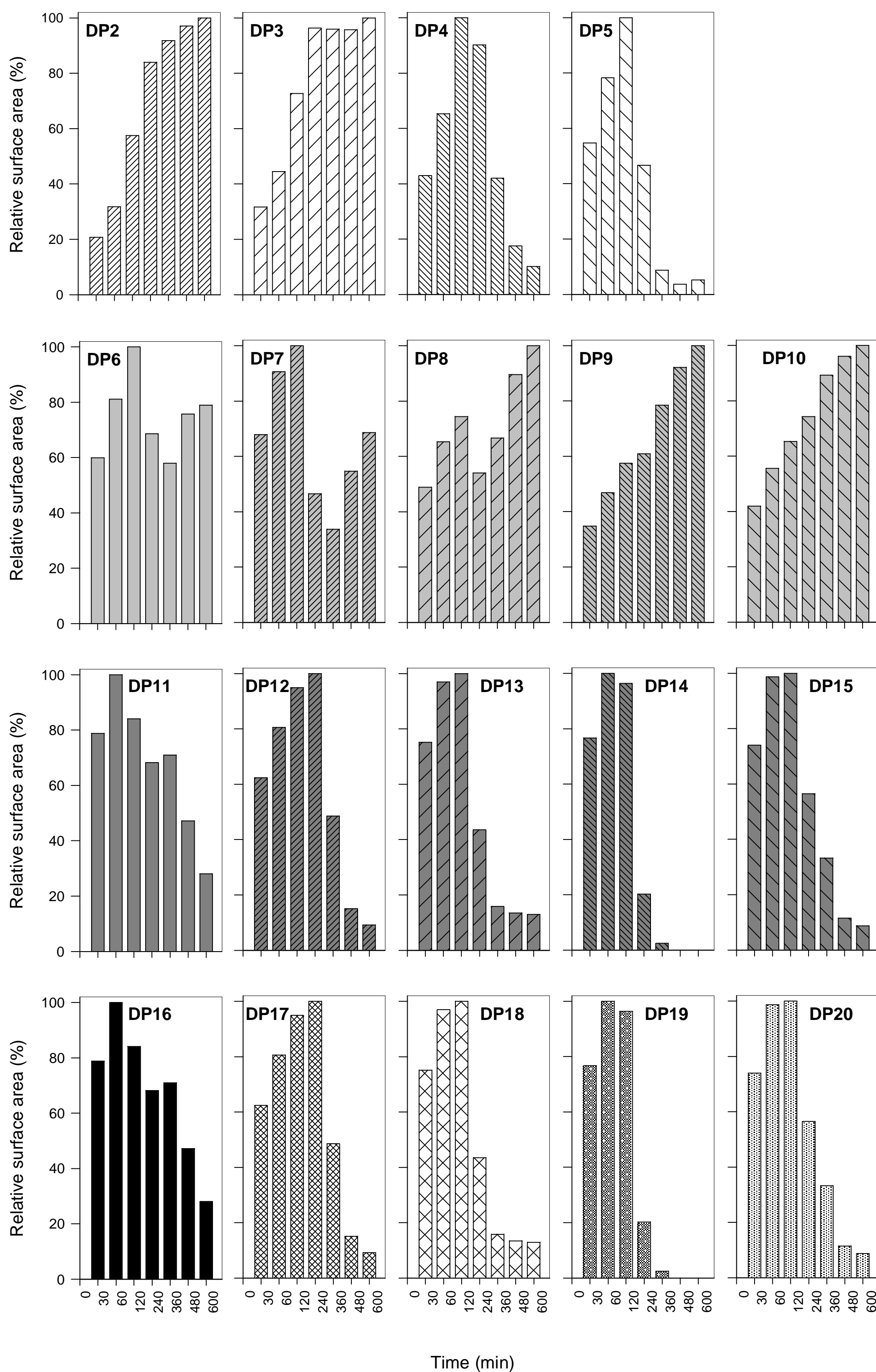

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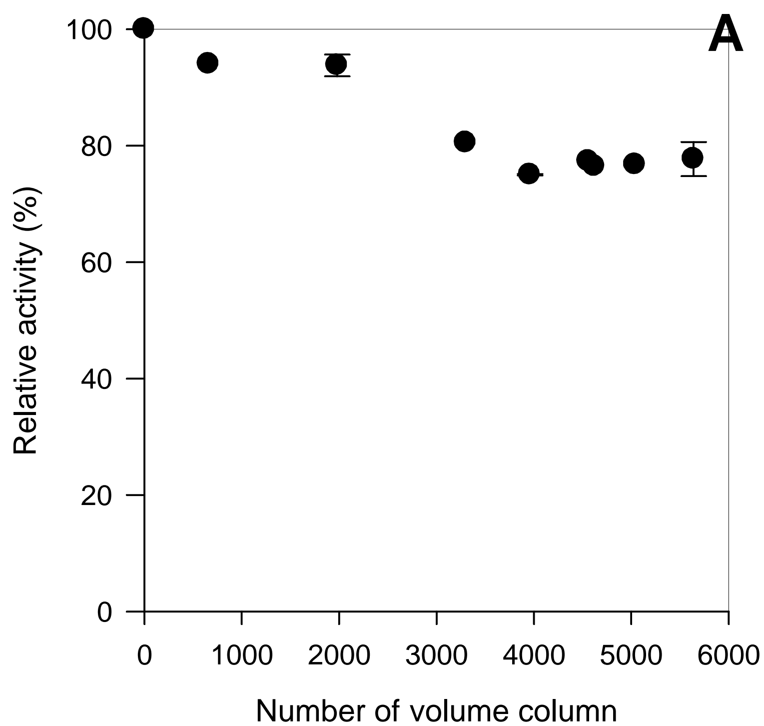
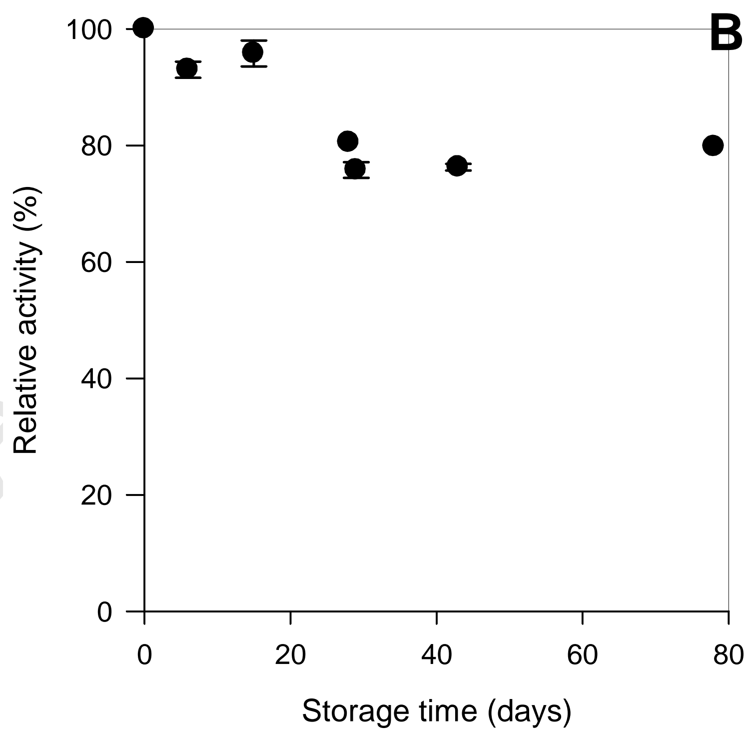
Figure 4





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