From a Sequential Chemo-Enzymatic Approach to a Continuous Process for HMF Production from Glucose

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Article

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Abstract: Notably available from the cellulose contained in lignocellulosic biomass, glucose is a highly attractive substrate for eco-efficient processes towards high-value chemicals. A recent strategy for biomass valorization consists on combining biocatalysis and chemocatalysis to realise the so-called chemo-enzymatic or hybrid catalysis. Optimisation of the glucose conversion to 5-hydroxymethylfurfural (HMF) is the object of many research efforts. HMF can be produced by chemo-catalyzed fructose dehydration, while fructose can be selectively obtained from enzymatic glucose isomerization. Despite recent advances in HMF production, a fully integrated efficient process remains to be demonstrated. Our innovative approach consists on a continuous process involving enzymatic glucose isomerization, selective arylboronic-acid mediated fructose complexation/transportation and chemical fructose dehydration to HMF. We designed a novel reactor based on two aqueous phases dynamically connected via an organic liquid membrane, which enabled substantial enhancement of glucose conversion (70%) while avoiding intermediate separation steps. Further, in the as-combined steps, the use of an immobilized glucose isomerase and an acidic resin facilitates catalyst recycling.

1. Introduction

In a context of fast depletion of fossil resources, lignocellulosic biomass possesses a high potential as a sustainable raw material for fuels [1] and fine chemicals [2, 3] production in industrially significant volumes. Upon specific physical, chemical and/or biological treatments of lignocellulose, access can be given to monomeric sugars [4], like glucose as its main saccharidic component. Glucose is, as a result, currently considered as one of the most promising starting materials for the conversion of biomass into drop-in or new high-value platform chemicals [5].

Efficient and eco-responsible valorization routes for this new renewable carbon source are highly desirable, and every field of catalysis should be mobilized as the key to success for a selective valorization of such a polyfunctional substrate. Biocatalytic processes are already or about to be industrialized [6] and heterogeneous or homogeneous catalysis, alone or in combination, have shown high efficiency for biomass valorization, but lack of selectivity still causes problems in the products separation steps [7]. In the very last years, a new paradigm of catalysis has emerged to

44 answer the challenges of selectivity and productivity by combining biocatalysis and chemocatalysis
45 [8-10]. This combination is known as chemo-enzymatic catalysis or hybrid catalysis [11, 12].

46 In the field of sustainable and biosourced chemistry, glucose conversion to 5-hydroxymethylfurfural
47 (HMF) drives a specific attention as a platform molecule [13]. Its hydrogenation, for instance, can
48 lead to 2,5-dimethylfuran (2,5-DMF), a new fuel additive [14] and a precursor of terephthalic acid
49 after dehydrative Diels-Alder reaction with ethylene to p-xylene and subsequent oxidation [15, 16].
50 HMF oxidation is also envisioned to obtain 2,5-furandicarboxylic acid (FDCA), a biosourced
51 alternative to terephthalic acid in the polyesters plastic production with a high market potential [17,
52 18]. Unfortunately, abundant glucose is not the substrate of choice for efficient dehydration to HMF
53 compared to its more expensive fructose isomer. The latter presents a fructofuranose mutamer more
54 prone to dehydration to a furanic ring with lower energetic barriers [19]. Isomerization of glucose to
55 fructose thus appears as a key step that can hardly be circumvented. A well-known transformation
56 of glucose to fructose is the enzymatic glucose isomerization, employed in the industrial production
57 of high-fructose corn syrup "HFCS" [20]. Despite the need for expensive high purity glucose, for
58 buffers and multiple ion-exchange resins to get rid of all the metallic residues in alimentary HFCS,
59 enzymatic isomerization remains the preferred process compared to chemical isomerization, even if
60 the latter receives a recent renewed interest [21-25]. The aforementioned enzymatic reaction reaches
61 a thermodynamic equilibrium ($K_{eq} \sim 1$), which limits glucose conversion [26-28]. Whereas this
62 equilibrium is particularly well-adapted for HFCS production involving glucose/fructose mixtures,
63 HMF synthesis requires a pure fructose feed for the subsequent dehydration step, where
64 compatibility issues between catalysts arise: Huang & al indeed shown incompatibility between a
65 thermophilic glucose isomerase immobilized on aminopropyl-functionalized mesoporous silica
66 (FMS) and a heterogeneous Brønsted acid propylsulfonic-FMS-SO$_3$H in a THF:H$_2$O (4:1 v/v) mixture
67 [29]. Isomerization is carried out at 363 K to reach 61% fructose yield, and then the temperature has
68 to be increased to 403 K to reach a 30% HMF yield. In the meantime, glucose isomerase is fully
69 denatured.

70 Strategies have thus been set up to combine enzymatic isomerization of glucose to fructose and
71 chemical by separating bio- and chemo-catalysis involving fructose transportation between the
72 isomerization aqueous phase and an organic phase for subsequent reactivity. Huang *et al* [30]
73 describe this concept for the first time by adding sodium tetraborate in the aqueous isomerization
74 medium to form a fructoborate compound by complexation with fructose. Transportation of this
75 complex to a separated organic phase is assisted by a cationic quaternary ammonium and enables
76 enhanced glucose conversion by shifting the isomerization equilibrium towards fructose. The
77 separate dehydration of fructose in the organic phase gives an increase in HMF yield up to 63%
78 compared to a yield of 28% HMF without borate addition, associated to a glucose conversion of 88%
79 instead of 53%. However, in this work, the complexation selectivity between glucose and fructose
80 was not satisfactory and was thus further improved by Palkovits & al, who optimized the chemical
81 nature of the complexing boronate species [31]. A global process exploiting this concept for glucose
82 to HMF production has concomitantly been proposed by Alipour *et al* [32] Therein, fructose is
83 complexed with phenylboronic acid and transferred to an organic phase, which is separated and
84 contacted with an acidic ionic liquid phase to promote the release of free fructose. This fructose-rich
85 ionic liquid is further used for dehydration to HMF in a biphasic medium. The produced HMF is
86 then back-extracted to a last low-boiling point organic phase. This method involves the use of four
87 different media with intermediate phase separations. Inspired by these sequential processes, we
88 propose here to circumvent this drawback by setting up an innovative integrated implementation
89 minimizing the number of steps and preventing among others the use of costly and hard to recover
90 ionic liquids.

91 Herein, we highlight an efficient and fully-integrated cascade reactions process to convert glucose to
92 HMF using an organic liquid membrane separating two aqueous phases for fructose transportation.
93 This approach that we first proposed as a concept exemplifying the potential of hybrid catalysis [33]

94   is based on continuous aqueous glucose isomerization, fructose complexation, extraction and
95   transportation towards an intermediate organic phase. Fructose is then released at the interface with
96   the second aqueous phase (aqueous receiving phase), where it is subsequently dehydrated to HMF.
97   This integrated process without intermediate phase recovery, separation and recycling is presented
98   in Figure 1. Before fully designing the entire process, where all the reactions are carried out
99   simultaneously, we separately investigated each reaction to notably determine their respective
100 optimal ranges of conditions before combination: (i) Glucose isomerization to fructose occurs in a
101 primary aqueous feed phase; (2) The as-formed fructose transportation is then made possible
102 through selective complexation with phenylboronic acid as a carrier associated to a quaternary
103 ammonium into an organic solvent (4-methyl-2-pentanone "MIBK"); (3) Eventually, the
104 fructoboronate complex is hydrolyzed by acidic conditions in a receiving aqueous phase containing
105 an acidic resin, which also promotes dehydration of fructose to HMF. Our approach is not only
106 focused on the compatibility issues of bio- and chemocatalytic reactions with the aim of overcoming
107 the isomerization equilibrium limitation, but also pays a particular attention in integrating all the
108 steps to minimize separation and recycling burdens, which can be detrimental for the overall
109 economics and efficiency of the process. The methodology envisioned to move from a sequential
110 approach towards an integrated continuous process is schematized in Figure 2.
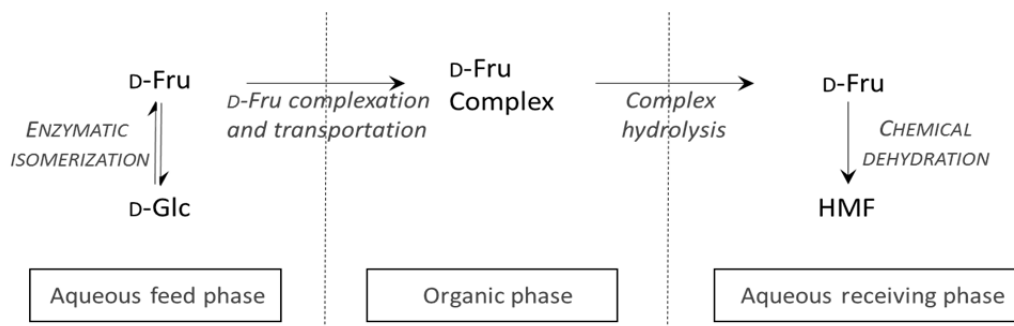
111

112 **Figure 1.** Hybrid catalysis simultaneous process applied to the transformation of glucose to HMF
113 (D-Glc = D-glucose, D-Fru = D-Fructose, HMF = 5-hydroxymethylfurfural)

114 **Figure 2.** Methodology from a sequential approach towards an integrated continuous process.

115
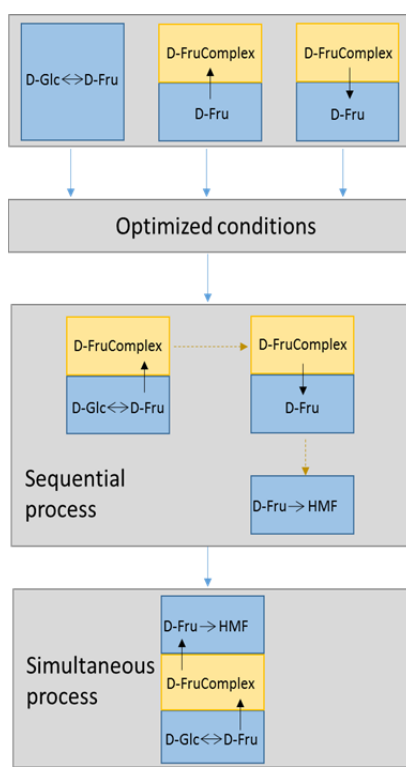
2. Results and Discussion

2.1. Study of D-glucose enzymatic isomerization in the aqueous feed phase

The optimal temperature of use ranges from 333 to 353 K and the optimum pH ranges from pH 7.0 to 9.0 [34]. We first checked the influence of pH and temperature using the lot of IGI received from our supplier. A glucose solution at pH 7.5 (Tris-HCl buffer, 100 mM) was prepared and isomerized from 323 K to 363 K. The relative enzymatic activity is presented in Figure 3, which highlights an optimal enzymatic activity at 343 K. A glucose solution of a pH from 4.5 to 9 (Tris-HCl buffer, 100 mM from pH 7 to 9; sodium phosphate buffer, 100 mM pH 6.5; sodium citrate buffer, 100 mM pH 4.5) was thus prepared and isomerized at 343 K. The relative enzymatic activity presented in Figure 4 shows an optimal IGI activity at a pH around 7.5. It is noteworthy that the IGI maintained more than 80% of its optimal activity between pH 7 and 9. Below a pH of 6.5, the IGI lost more than 50% of its activity. The selected pH in the aqueous feed phase for the enzymatic isomerization reaction was 8.5. A D-glucose enzymatic isomerization was then performed in the selected conditions (Tris-HCl 100 mM, pH 8.5, 343 K). The results are presented in Figure 5 as a function of time. A blank reaction (grey dots) was ran in the absence of IGI and showed the absence of D-glucose isomerization or degradation in the chosen experimental conditions. When IGI was present in the system, the D-fructose amount increased from 0% to 42% during the first 30 min. From 30 to 60 min, the D-glucose conversion was slower, while the D-Fructose amount increased from 42% to 55%. A maximum D-glucose conversion of 55% was finally reached in agreement with the glucose-fructose thermodynamic equilibrium expected at this temperature (Keq = 1,23), as already observed by
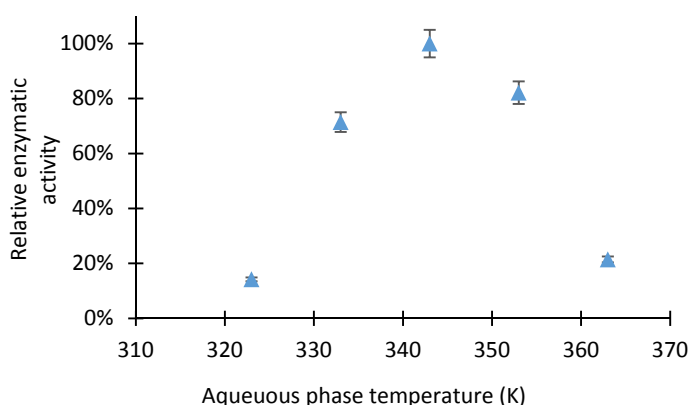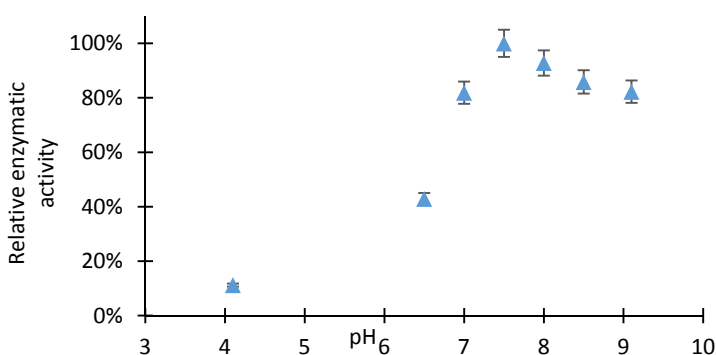

McKay *et al* [35].

**Figure 3.** Relative enzymatic activity of IGI in D-glucose isomerization at pH 7.5 as a function of temperature.

Figure 4. Relative enzymatic activity of IGI in *D*-glucose isomerization at 343 K as a function of pH

Figure 5. D-glucose consumption during the isomerization step as a function of time; (Δ), D-fructose

142 formation (□), D-glucose amount in the blank experiment at pH 8.5 (○). Initial conditions: D-Glucose
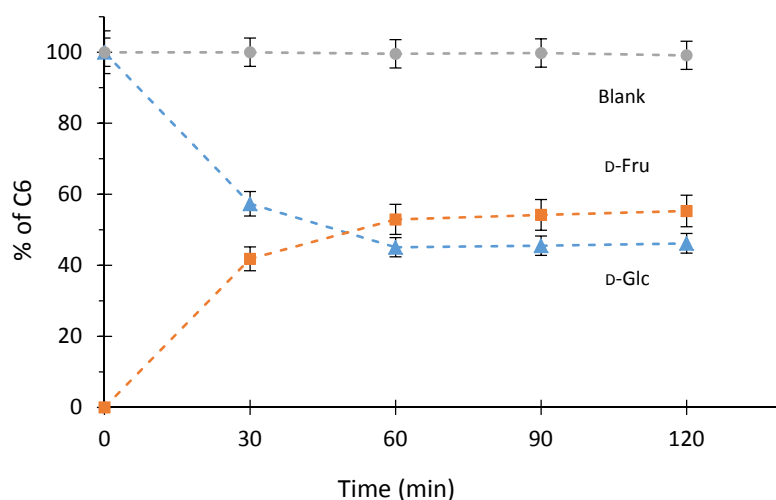
143 (100 mM), IGI (0,5 g), Tris-HCl 100 mM, pH 8.5, V = 100 mL, 343 K, 750 rpm.

144 *2.2. Organic liquid membrane for D-fructose complexation/transportation*

145 In order to enhance the isomerization yield, simultaneous isomerization and
146 complexation/transportation of D-fructose in a liquid/liquid aqueous/organic biphasic system was
147 further studied. The temperature was set at 343 K and the pH was chosen as a trade-off to
148 simultaneously maximize the enzymatic activity (7 < pH < 9), to avoid monosaccharide degradation,
149 and to enhance the extraction process as reported previously [36]: the pH was thus kept at a value of
150 8.5 by using a Tris-HCl 100 mM buffer. The extraction was conducted in an organic solvent with low
151 water miscibility (MIBK) containing a lipophilic arylboronic acid (carrier) and a quaternary
152 ammonium salt (Aliquat336®) as a phase transfer agent [37]. At pH 8.5, aryl boronic acid $ArB(OH)_2$
153 was actually present under its hydroxylated anionic form, as a tetrahedral aryltrihydroxyborate
154 $ArB(OH)_3^-$ [38]. At the interface between the aqueous and organic phases, D-fructose further reacts
155 with the arylborate to form a tetrahedral fructoboronate ester (Figure 6) [38]. The fructoboronate
156 complex then forms an intimate ion pair with Aliquat336®, which enables its transportation to the
157 organic phase [39].
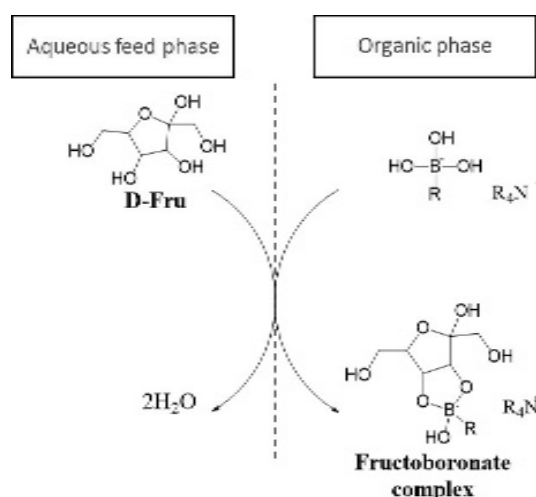
158 **Figure 6.** Fructoboronate complex formation at the interphase between the first aqueous phase and
159 the organic liquid membrane.

160

161 2.2.1. Influence of the boronic acid structure

162 The influence of the boronic acid structure was investigated in order to optimize kinetics and
163 maximize selectivity of D-fructose complexation/transportation. Seven arylboronic acids differing
164 by the electronic properties of their substituents and thus by their pKa were screened in the
165 complexation reaction with D-fructose: 2,3-DCPBA, 2,4-DCPBA, 3,4-DCPBA, 3,5-DCPBA, PBA,
166 4-TBPBA, 4-TFMPBA. The complexation/transportation, which will be referred to as "extraction" in
167 the following sections, was carried out in a biphasic system, as described before. The pKa of the
168 different boronic acids and the relative extraction yields are summarized in Table 1 and reported in
169 Figure 7.

170

171 **Table 1**. Influence of the boronic acid structure on the extraction yield and rate.

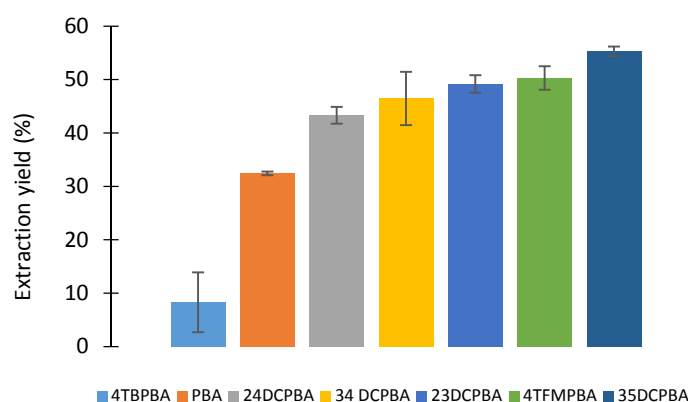| Boronic acid	pK_a	Extraction yield %		Extraction rate µmol/min	
4-TBPBA	9.3	8.3	± 5.6	0.52	± 0.08
PBA	9.1	32.4	± 0.3	1.27	± 0.39
2,4-DCPBA	8.9	43.3	± 1.6	1.56	± 0.34
3,4-DCPBA	7.4	46.5	± 4.9	1.48	± 0.23
2,3-DCPBA	7.4	49,2	± 1.6	1.99	± 0.17
4-TFMPBA	9.1	50.3	± 2.2	1.26	± 0.12
3,5-DCPBA	7.4	55.3	± 0.9	1.94	± 0.28

Figure 7. Influence of the arylboronic acid structure on the complexation/transportation process
after 45 min. [D-Fructose]I = 100 mM, [Boronic acid] = 100 mM, [Aliquat336®] = 200 mM, Tris HCl 100
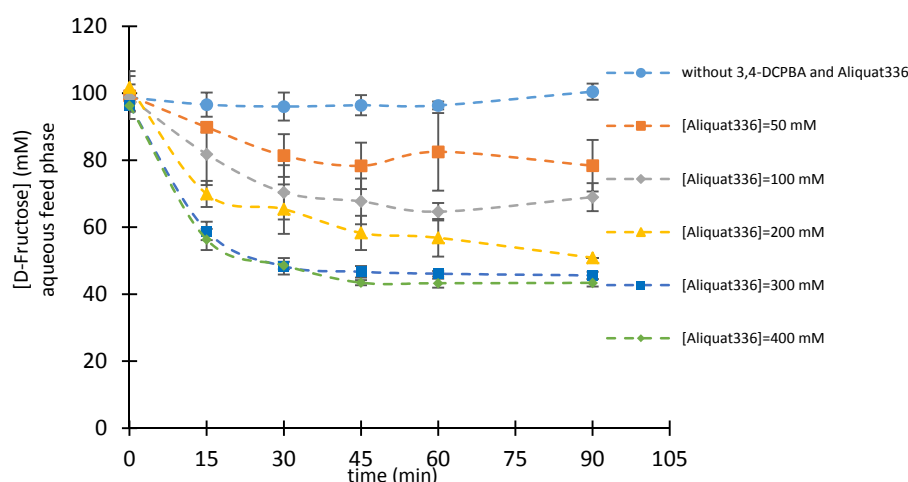mM pH 8.5, MIBK, 343 K, 750 rpm.

192 A group composed by 2,4-DCPBA, 3,4-DCPBA, 2,3-DCPBA, 4TFMPBA and 3,5-DCPBA showed the
193 best results with extraction yields between 43% and 55%, while with 4-TBPBA and PBA only 8% and
194 32% were achieved, respectively. These results also show that the D-fructose extraction yield
195 increases when the pKa of the boronic acid used decreases. Better extraction yields were observed
196 when the pKa of the boronic acid was lower than (or close to) the pH of the aqueous feed phase (8.5).
197 This analysis supports the hypothesis from Morin et al. [36] and Karpa *et al* [37] indicating that
198 higher extraction yields are obtained when pH ≥ pKa, favoring a predominant tetrahedral anionic
199 borate form of the complex, which enables its transportation by association with the ammonium
200 carrier. An exception was however observed with 4-TFMPBA with a pKa of 9.1. This might be due to
201 the fact that 4-TFMPBA has a higher dipolar moment (2.90 D, against 0.43 D to 1.86 D for the other
202 used boronic acids) that could increase the reactivity. The extraction rates and the pKas (Table 1)
203 seem to be correlated. A higher pKa of the boronic acid was linked with a lower initial extraction
204 rate. Among the most effective boronic acids, 3,4-DCPBA was selected for further studies.

205

206 2.2.2. Influence of the boronic acid:Aliquat336® molar ratio

207 With 3,4-DCPBA as the selected boronic acid, the influence of the molar ratio between the boronic
208 acid and the ammonium salt Aliquat336® in the organic solvent was investigated. Figure 8 shows the
209 evolution of D-Fructose concentration in the aqueous phase as a function of time for different
210 Aliquat336® concentrations (from 0 to 400 mM). D-Fructose and 3,4-DCPBA were initially
211 introduced at concentrations of 100 mM. In the first 15 minutes of reaction, the concentration of
212 D-Fructose decreased irrespective of the Aliquat336® concentration with various initial rates. An
213 equilibrium seemed to be progressively reached between 15 and 45 minutes, as no more evolution of
214 the D-Fructose concentration was then observed. A blank reaction (blue dots) without Aliquat336®
215 nor 3,4-DCPBA was also tested, which showed no evolution. From these observations, it can be
216 considered that the molar ratio 3,4-DCPBA:Aliquat336® is a relevant parameter to control the initial
217 D-fructose extraction rate. Figure 9 shows the evolution of the initial extraction rate and of the
218 D-fructose extraction yield for different 3,4-DCPBA:Aliquat336® molar ratios (from 1:0.5 to 1:4).The
219 initial extraction rate continuously increased when the 3,4-DCPBA:Aliquat336® molar ratio
220 increased from 1:0.5 to 1:2 with respectively 0.37 µmol/min and 1.50 µmol/min. Above a 1:2 molar
221 ratio, no further kinetic benefits could be clearly observed from such an excess of Aliquat336® in the
222 reaction medium. This result is consistent with an ion-pairing phenomenon between the
223 fructoboronate complex and the Aliquat336® occurring at the interphase enabling D-fructose
224 transportation. The D-fructose extraction yield increased when the 3,4-DCPBA:Aliquat336® molar
225 ratio increased from 1:0.5 to 1:2 with respectively 21.5% and 50.0%. Like in the case of the initial
226 extraction rate, above a 1:2 molar ratio no further benefits on the extraction yield were displayed by
227 using an excess of Aliquat336®. Therefore, in order to maximize the initial extraction yield and the
228 initial rate while optimizing the quantity of ammonium salt added, an arylboronic acid:Aliquat336®
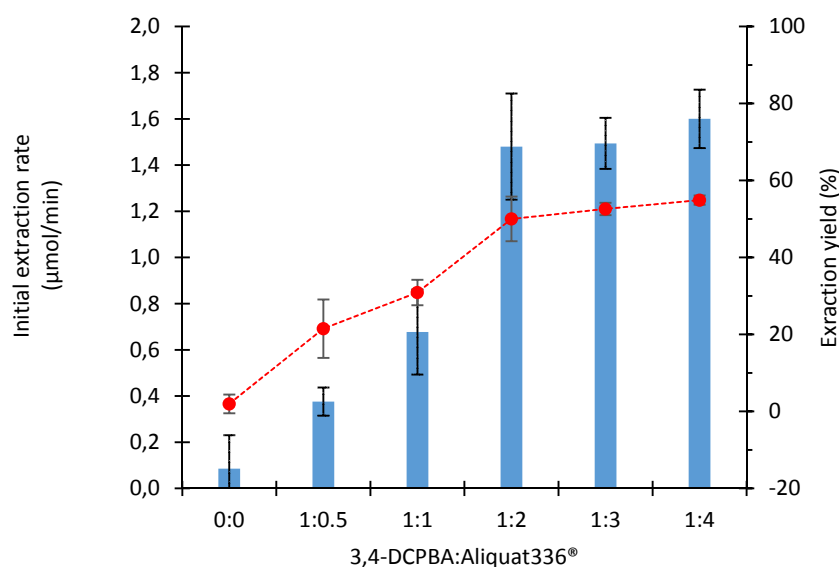229 ratio of 1:2 was selected for the following studies.

230 **Figure 8.** Evolution of [D-fructose] in the aqueous phase as a function of time for different
231 3,4-DCPBA/Aliquat336® ratios. [D-fructose]i = 100 mM, [3,4-DCPBA] = 100 mM, [Aliquat336®] = X
232 mM (X varies from 0 to 400 mM), Tris-HCl 100 mM pH 8.5, MIBK, 343 K, 750 rpm.

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236 **Figure 9.** Initial extraction rate in aqueous phase (blue rods) and extraction yield (red dots) in
237 function of 3,4-DCPBA:Aliquat336® molar ratio. [D-Fructose]I = 100 mM, [3,4-DCPBA] = 100 mM,
238 [Aliquat336®] = X mM (X varies from 0 to 400 mM), Tris-HCl

239 2.2.3. Influence of boronic acid and Aliquat336® concentrations

240 Keeping a molar ratio of arylboronic acid:Aliquat336® equal to 1:2 and at fixed initial D-Fructose
241 amount, both 3,4-DCPBA and Aliquat336® concentrations were varied, considering the best
242 compromise between component concentrations and costs (Figure 10). From 0 to 15 min, the
243 D-Fructose extraction yield increases rapidly and then stabilizes for lowest 3,4-DCPBA
244 concentrations less than 100 mM. The best extraction yields are thus obtained for higher 3,4-DCPBA
245 concentrations, the final yield being the highest at 300 mM. The slight difference in extraction yields
246 between 100 mM and 300 mM 3,4-DCPBA concentration does not justify the use of such a
247 concentrated solution. From Figure 11, the initial extraction rate values for different
248 3,4-DCPBA:Aliquat336® concentrations have been calculated (Table 2). Two behaviors can be
249 observed, the first one for the 25:50 ratio and the second one for all the other ratios, namely from
250 50:100 to 300:600. We observed that the larger the 3,4-DCPBA amount, the higher the initial
251 extraction rate. However, after a certain amount of 3,4-DCPBA, the interface seems to be saturated
252 by 3,4-DCPBA:Aliquat336® complexes and then the initial rate does not increase anymore.
253 Therefore, further optimization steps considered a concentration of 100 mM while keeping a molar
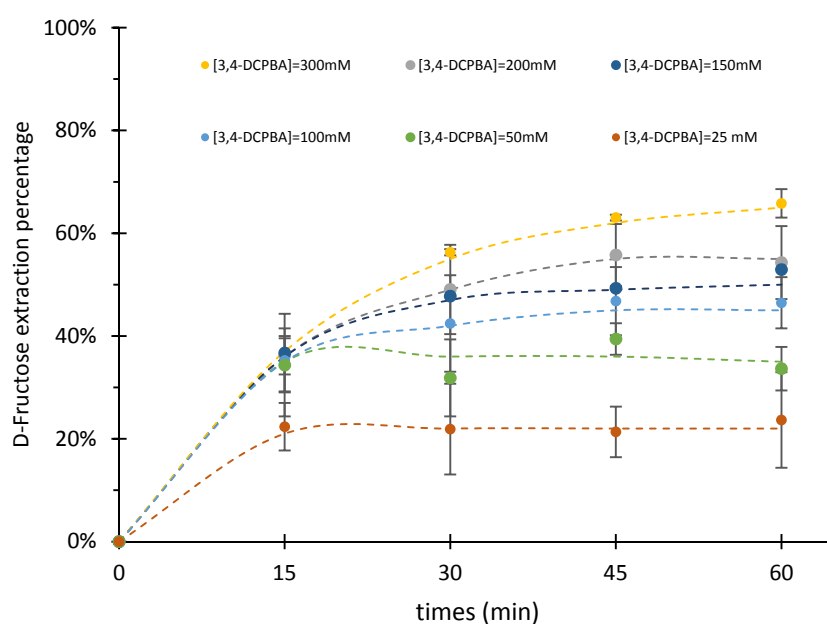254 ratio of 1:2 with Aliquat 336®.

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260 **Table 2**. Initial extraction rates for various concentrations in the system. [D-Fructose]I = 100 mM,
261 [3,4-DCPBA] = Y mM (Y varies from 25 to 300 mM), [Aliquat336®] = Yx2 mM, Tris-HCl 100 mM, pH
262 8.5, MIBK, 343 K, 750 rpm.

[3,4-DCPBA] (mM)	Extraction rate (μmol/min)	
25	0.89	±0.15
50	1.37	±0.45
150	1.45	±0.05
100	1.22	±0.15
200	1.40	±0.21
300	1.33	±0.14

268

269 **Figure 10.** Evolution of the D-Fructose extraction yield for different 3,4-DCPBA and Aliquat336®
270 concentrations for a 1:2 molar ratio. [D-Fructose]I = 100 mM, [3,4-DCPBA] = Y mM (Y varies from 25
271 to 300 mM), [Aliquat336®] = Y x 2 mM, Tris-HCl 100 mM, pH 8.5, MIBK, 343 K, 750 rpm.

272 2.2.4. Influence of the D-fructose:boronic acid molar ratio

273 The influence of the D-Fructose:3,4-DCPBA molar ratio was further investigated. Figure 11 shows
274 the D-fructose extraction yield as a function of this molar ratio. The yield ranged between 40% and
275 60%, for ratios from 0.25:1 to 1:1 and between 18% and 28% for ratios from 2:1 to 10:1. When the
276 3,4-DCPBA was introduced in excess or stoechiometrically compared to D-Fructose (red rods), the
277 extraction yield was larger than 40%, reaching a maximum of 60% for a two-fold excess of boronic

278   acid. However, when it was introduced in default (blue rods), the extraction yield did not exceed
279   35% without any significant further evolution between a molar ratio of 2:1 to 10:1.
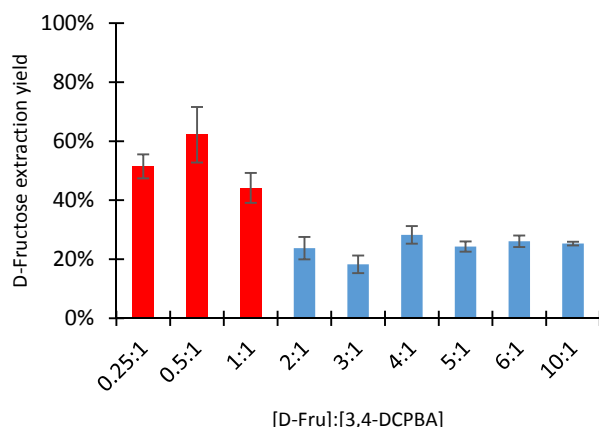
280

281   **Figure 11.** Influence of the initial D-Fructose:3,4-DCPBA molar ratio on the the extraction yield.
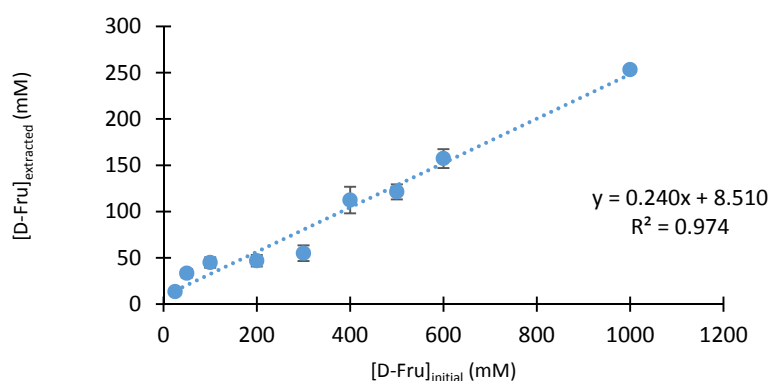282   [D-Fructose]I = X mM, [3,4-DCPBA] = 100 mM, [Aliquat336®] = 200 mM, Tris-HCl 100 mM, pH 8.5,
283   MIBK, 343 K, 750 rpm.

284   2.2.5. Influence of D-fructose and boronic acid concentrations

285   The evolution of the extracted D-fructose concentration as a function of the initial D-fructose
286   concentration, for the same 3,4-DCPBA concentration, is shown in Figure 12. Logically, when the
287   initial concentration of D-fructose increased from 25 mM to 1000 mM, the extracted concentration
288   linearly increased, according to a straight correlation between the initial amount of D-fructose and
289   the amount of extracted D-fructose. Keeping a molar D-fructose:boronic acid ratio equal to 1:1 and a
290   molar ratio of arylboronic acid:Aliquat336® equal to 1:2, a benchmark experiment was set with the
291   following parameters: T = 34 K, [D-Fructose]I = 100 mM, [3,4-DCPBA] = 100 mM, [Aliquat 336®] = 200
292   mM. The other experiments have been set up by doubling all concentrations. Table 3 presents the
293   extraction yields and initial extraction rates depending on the variation of the initial D-fructose
294   concentration while keeping all the molar ratios relative to 3,4-DCPBA and Aliquat336® as constant.
295   An increase in the concentrations (D-Fructose, 3,4-DCPBA and Aliquat336®) led to a decrease in the
296   D-Fructose extraction yield. Indeed, at 100 mM of D-Fructose, 43.3% of the fructoboronate complex
297   were extracted whereas only 32.7% and 27.3% were respectively extracted at 200 mM and 300 mM of
298   D-fructose. Therefore, the optimal conditions ([D-Fru]/[3,4-DCPBA]/[Aliquat336] ratio of
299   100/100/200 mM) were kept for the following studies.

300

The chart shows a scatter plot with y = 0.240x + 8.510 and R² = 0.974, with [D-Fru]extracted (mM) on the y-axis and [D-Fru]initial (mM) on the x-axis.

301 **Figure 12.** Influence of the initial D-Fructose concentration on the amount of D-fructose extracted.
302 [D-Fructose]I = X mM (X varies from 25 to 1000), [3,4-DCPBA] = 100 mM, [Aliquat336®] = 200 mM,
303 Tris-HCl 100 mM, pH 8.5, MIBK, 343 K, 750 rpm.

304 **Table 3.** D-fructose extraction yield and initial extraction rate for different concentrations.

[D-Fru]	[3,4-DCPBA]	[Aliquat336®]	Extraction yield	
mM	mM	mM	%	
100	100	200	43.34	± 4.22
200	200	400	32.61	± 1.22
300	300	600	27.31	± 4.6

305

306 2.2.6. Hydrolysis of the fructoboronate complex for D-fructose release in the second aqueous phase

307 The further stage of the D-fructose transportation is the fructoboronate complex hydrolysis for
308 D-fructose release in the second aqueous phase (the aqueous receiving phase). Therefore, the
309 influence of the pH of the second aqueous phase on the release yield was investigated. pHs of 1, 3, 5
310 and 8 were tested by using with H_2SO_4 for pH 1, citrate buffer for pH 3 and 5 and Tris HCl buffer for
311 pH 8. Prior to these experiments, a D-fructose extraction experiment was performed
312 complexation/transportation) in the optimized conditions (100 mM D-fructose and a
313 D-fructose:3,4-DCPBA:Aliquat336® molar ratio of 1:1:2). The organic phase was then recovered and
314 contacting with the second aqueous phase for the release step. As observed by Paugam *et al* [40] and
315 keeping in mind the final objective of this work that is the obtaining of a simultaneous process, the
316 saccharide flow from the basic aqueous donor phase to the acidic receiving phase through an
317 organic phase is favored when a pH gradient is applied between the two phases. Figure 13 shows
318 the evolution of the D-fructose release yield as a function of time for different pH of the aqueous
319 receiving phase. In the cases of pH 8 and pH 1, an increase was observed until 30 minutes (with an
320 initial rate of about 0.6 and 1.8 µmol/min, respectively, Table 4) and a steady-state was reached for a
321 yield of 22.7% and 54.5% for pH 8 and pH 1, respectively. Concerning pH 3 and pH 5, an increase
322 was observed up to 60 minutes (with an initial rate of 1.5 µmol/min, Figure 13) to obtain respectively
323 91% and 100% of release. Considering that the pKa of 3,4-DCPBA is around 7.4, when the pH of the
324 receiving aqueous phase is 8, the pH is then superior to pKa. Then, the release mechanism is
325 unfavorable [41]. With pH 3 and 5, more than 90% of the extracted D-Fructose were released.
326 Moreover, the experiment with H_2SO_4 at pH 1 shows full hydrolysis of the fructoboronate complex
327 followed by conversion of D-Fructose to HMF, levulinic and formic acid, which explains the high
328 release rate and the decrease in the actual D-fructose concentration after 15 minutes.

329
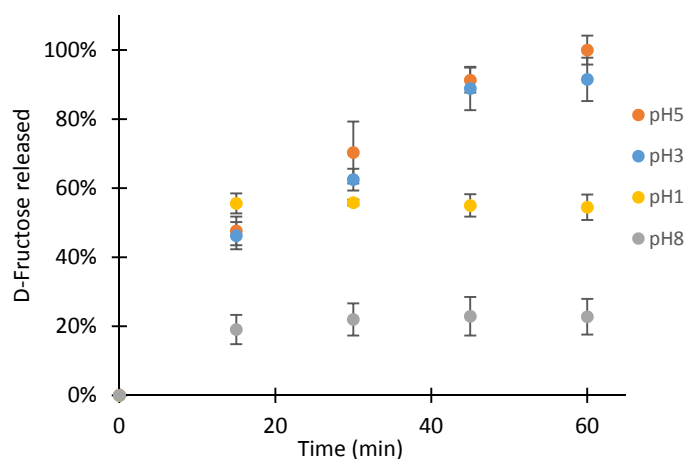
330 **Figure 13.** Influence of the pH of the aqueous receiving phase on the D-fructose complex
331 transportation. Extraction process: Tris-HCl 100 mM pH 8.5, [D-Fru]I = 100 mM, [3,4-DCPBA] = 100
332 mM, [Aliquat336®] = 200 mM, MIBK, 343 K. Receiving phase: Citrate buffer

333 **Table 4.** Transport yield and extraction rate of D-fructoboronate hydrolysis to D-fructose and
334 D-fructose release in the aqueous phase as a function of its pH.

pH	Transport Yield (%)		Extraction rate (µmol/min)	
5	100	± 4.2	1.58	± 0.14
3	91.5	± 6.3	1.53	± 0.13
1	54.5	± 3.7	1.84	± 0.09
8	22.7	± 5.2	0.63	± 0.14

335

336

337 *2.3. Study of D-Fructose dehydration in the receiving aqueous phase*

338 D-Fructose dehydration to HMF was carried out in the receiving aqueous phase. We previously
339 observed when studying fructoboronate complex transportation and hydrolysis in a highly acidic
340 aqueous phase the formation of formic and levulinic acids. This confirmed that solutions to
341 minimize HMF degradation by rehydration in the aqueous phase are requested. An alternative to
342 strong homogeneous acid has been to use an acidic resin containing strong sulfonic groups, its
343 supported character facilitating a potential regenerability and recyclability of the catalyst.

344

345 *2.4. Study of sequential process*

346 In the formerly optimized conditions, a sequential process was tested. The first step consisted on a
347 simultaneous D-glucose isomerization and D-fructose transportation through a fructoboronate
348 complex, followed by the second step characterized by the hydrolysis of the fructoboronate complex
349 to D-fructose and release of D-fructose in the aqueous receiving phase, and finally by the
350 dehydration of fructose to HMF by heterogeneous catalyst in the third step (Figure 14).

351 2.4.1. D-glucose isomerization and D-fructose complexation/transportation

352   The aqueous feed phase contained Tris-HCl buffer 100 mM at pH 8.5, D-Glucose and immobilized
353   glucose isomerase at the required amount. The organic layer contained the system determined by
354   the previous study: [3,4-DCPBA] = 100 mM, [Aliquat336®] = 200 mM. The initial aqueous D-glucose
355   concentration was 100 mM. The system was mixed in a reactor where the enzyme was placed in a
356   basket to avoid any contact with the organic layer. Moreover, the use of MIBK, with a low solubility
357   in water, as an organic solvent contributed to bring the contact between MIBK and enzyme
358   negligible. The system was heated at 343 K for 3 hours. The amount of D-glucose and D-fructose
359   were chromatographically determined in the aqueous phase during the process. Table 5 shows the
360   performances of each step. An interesting isomerization yield of 74.5% was obtained showing the
361   actual shifting of the equilibrium of the enzymatic catalysis (55%) by fructose elimination from the
362   media through complexation with the boronic acid. Moreover, according to the extraction yield of
363   D-fructose and D-glucose, 56.5% and 1%, respectively, a very high selectivity of the complexation
364   reaction with the chosen boronic acid towards the D-fructose was actually demonstrated. The partial
365   D-fructose extraction yield of 56.5% showed that the reaction conditions could be still improved.
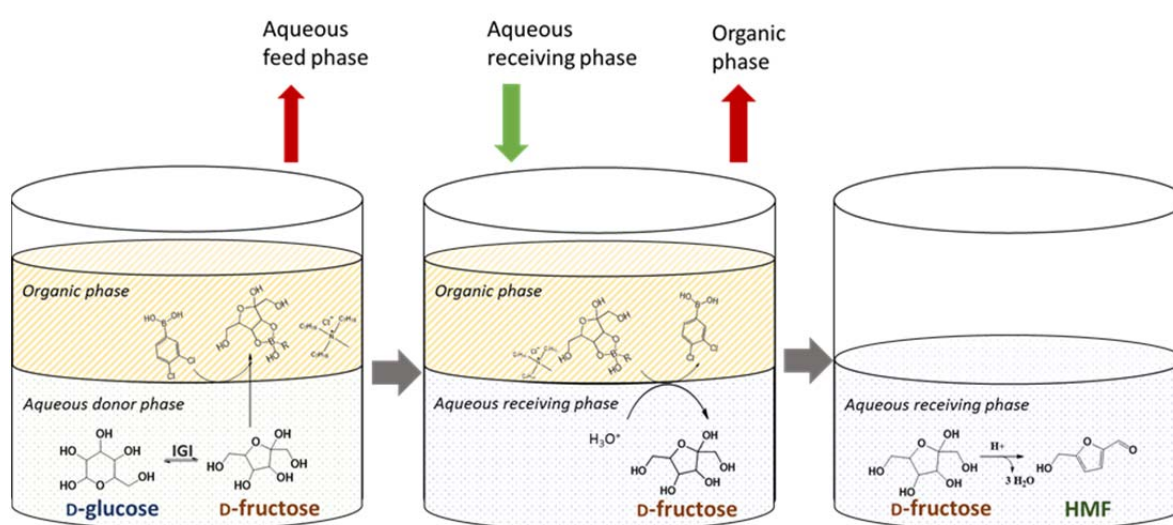
366

367   **Figure 14.** Illustration of the sequential process with isomerization and complexation/transportation
368   step, hydrolysis/release step and dehydration step.

369

370   **Table 5.** Results obtained from sequential process. The D-Fructose released yield was calculated
371   using the amount of D-Fructose in the organic phase at the end of the 1st transport. The fructose
372   conversion yield was calculated using the amount of fructose in the aqueous receiving phase at the
373   end of the 2nd transport. The HMF total amount was calculated using the initial amount of glucose
374   in the aqueous donor phase.

		1st step			2nd step	3rd step		
		isomerization	D-Fructose extracted	D-Glucose extracted	D-Fructose released	Fructose conversion	HMF yield	HMF total
%		74.5	56.5	1.56	57.4	52	21.9	5.3
time			3 h		3 h	35 h		41 h

375

376

377   2.4.2. Fructoboronate complex hydrolysis, release of D-fructose and dehydration to HMF

378   The basket of IGI was removed and the aqueous feed phase was discarded and replaced by the
379   receiving aqueous phase with a citrate buffer at 100 mM keeping a pH 3. The system was heated at

343 K for 3 hours. In these conditions, the hydrolysis step led to 63% of fructose release in the aqueous phase. Then, after 3 hours at 343 K, the organic layer was discarded and the acidic resin was introduced. The system was heated at 363 K. The dehydration step led to a HMF yield of 20% after 10 h. The total HMF yield was 5%.

2.5. Study of continuous process

In the optimal conditions previously determined, a continuous integrated process was tested [33]. The reactor, phases and matter flows are presented in Figure 15. The "coaxial" reactor is composed of two cylinders coaxially placed, a cover enabling introduction of the stirring system and a refrigerant for temperature control. The inner cylinder contains the donor aqueous phase and has a height less than that of the outer cylinder which contains the receiving aqueous phase, which allows the organic phase to be in contact with both aqueous phases. The experiment was conducted with a donor aqueous phase formed by 50 mL of 100 mM Tris-HCl pH 8.5 containing 100 mM of D-glucose, 200 mg IGI, 20 mM $MgSO_4$, 8 mM Na_2SO_3. The receiving aqueous phase was formed by 55 mL of 100 mM sodium citrate buffer, pH 3. The organic phase consisted of 100 mL of MIBK containing 100 mM of 3,4-DCPBA and 200 mM of Aliquat336®. All the phases were maintained at 343 K. The stirring was carried out at 180 rpm, which is the maximum rate to avoid the mixing of the phases, thanks to a stirring blade immersed in the organic phase. This "coaxial" reactor was used to simultaneously perform the isomerization, extraction, hydrolysis and dehydration steps. The monitoring of the D-fructose concentration in the aqueous phases was carried out, making it possible to calculate the efficiency of the continuous process for the simultaneous isomerization, fructose extraction and dehydration. The results are shown in Table 6.
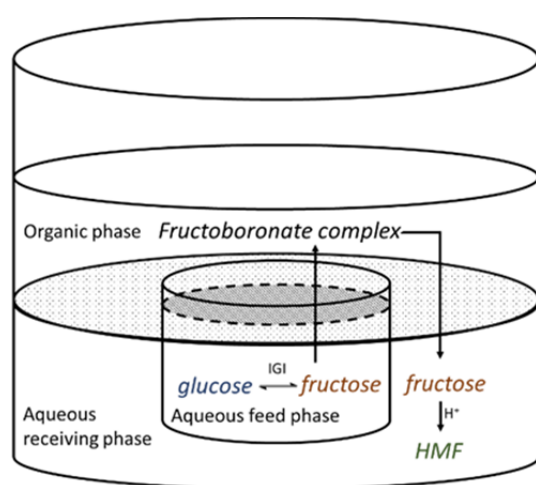
Figure 15. Illustration of the continuous process.

Table 6. Results obtained by continuous process.

	Isomerization	Fructose extraction	HMF	Selectivity
Yield (%)	70.1	50.2	4.1	70.4

408 The D-glucose isomerization yield was 70%, with 50% of the formed D-fructose transported and a
409 global HMF yield of 4% (table 8). Those results are comparable to those obtained by the sequential
410 process. Therefore, this process demonstrates the efficiency of using a cascade chemo-enzymatic
411 catalysis to continuously obtain HMF from D-glucose. However, insufficient agitation of each phase
412 seems to be the main limitation of this reactor configuration. Further improvements of this proof of
413 concept towards enhanced HMF yields and global performances will be reported in due course.

414 **3. Materials and Methods**

415 *3.1. Chemicals and reagents*

416 D-Glucose (≥99.5%), D-Fructose (≥99%), N-methyl-N,N,N-trioctylammonium chloride
417 (Aliquat336®), 3,4-dichlorophenylboronic acid (3,4-DCPBA), 3,5-dichlorophenylboronic acid
418 (3,5-DCPBA) (98%), 2,4-dichlorophenylboronic acid (2,4-DCPBA), 2,3-dichlorophenylboronic acid
419 (2,3-DCPBA), 4-tert-butylphenylboronic acid (4-TBPBA) (≥ 95%), 4-(trifluoromethyl)phenylboronic
420 acid (4-TFMPBA), Trizma base (≥ 99.9%), hydrochloric acid (36.8-38%), sulfuric acid (95-97%),
421 Sweetzyme IT®Extra (IGI) (≥ 350 U/g), Dowex monosphere 650C, levulinic acid (LA) (98%), formic
422 acid (FA) (95%), trisodium citrate (≥ 99%), 5-hydroxymethylfurfural (HMF) (99%) were purchased
423 from Sigma Aldrich Co. (St. Louis, MO). Phenylboronic acid (PBA) (95%) and 4-methyl-2-pentanone
424 (MIBK) (≥ 99%) were purchased from Sigma-Fluka. Citric acid (≥ 99%) was purchased from Merck.
425 Distilled-deionized water (Mili-Q) grad was used whenever necessary, obtained from a MilliQ water
426 purification system (Millipore, Academic, SA, France).

427

428 *3.2. Characterization*

429 In the conversion/complexation experiments, the determination of reactants, intermediates and
430 products quantities (Table 7) was carried out using an HPLC Shimadzu equipped with a
431 LC-20ADXR pump, a DGU-20A5R degasser, a SIL-20ACXR autosampler, a SPD-MD20A diode array
432 detector, a CTO-20AC oven and a CBM-20A communicator module piloted by a LabSolution
433 software The column was an Aminex HPX-87H (300 mm x 7.8 mm, 9 μm; Bio-Rad). The mobile
434 phase was 5 mM H2SO4, using isocratic mode with 0.4 mL.min-1. The chosen wavelength for HMF
435 and levulinic acid detection were 284 nm and 266 nm, respectively. The chosen wavelength for
436 detection of formic acid, D-Glucose and D-Fructose was 195 nm. In the case of monosaccharides, a
437 Refractive Index Detector was also used (analytical standards are listed in Table 7).

438

439 **Table 7.** Concentration range of products used for analytical standards.

Analytical method	RID				UV 284 nm	UV 195 nm		
Product	HMF	D-Glc	D-Fru	LA	HMF	D-Glc	D-Fru	FA
Concentration range (mM)	0.14-30	0.5-25	0.5-25	0.5-25	0.007-0.5	0.5-25	0.5-25	0.5-35

440

441

442 *3.3. Aqueous phase D-glucose isomerization (aqueous feed phase) and D-fructose complexation/transportation*

443 3.3.1. D-glucose isomerization in aqueous feed phase

444 We used one of commercial enzymes the most largely used for the conversion of starches to high
445 fructose syrup, namely immobilized glucose isomerase (IGI, Sweetzyme®IT extra).31 1 mL of a
446 D-glucose solution was prepared by mixing D-glucose (100 mM), Na2SO3 (8 mM) and MgSO4 (20
447 mM) in a buffer solution (100 mM) at a selected pH (Tris-HCl buffer for pH 7, 7.5, 8, 8.5, 9, phosphate
448 buffer for pH 6.5, or citrate buffer for pH 4.5) and then introduced in an Eppendorf tube. The
449 solution was incubated at the desired temperature (323 to 363 K) in a Mixing Block (BIOER-102

450 Thermocell) at 900 rpm. After rehydration in water for 12 h, IGI (10 mg) was put in contact with the
451 aforementioned prepared D-glucose solution. The reaction time was set at 90 min.

452 3.3.2. D-fructose transport in the organic phase

453 First, a D-fructose/D-glucose (25 to 600 mM) solution was prepared in Tris-HCl buffer (100 mM). 600
454 µL of this solution were introduced in an Eppendorf tube (solution 1). Aliquat336® (25-300 mM), and
455 arylboronic acid (25-300 mM) were dissolved in MIBK as a solvent to prepare solution 2. 600 µL of
456 solution 2 were then introduced in a second Eppendorf tube. Solutions 1 and 2 were incubated at the
457 desired temperature in a Mixing Block (BIOER-102 Thermocell) at 750 rpm. After 5 min, both
458 solutions were mixed in an Eppendorf tube placed in the Mixing Block in the same conditions. After
459 D-fructose transport, the aqueous and organic phases were separated by pipetting.

460

461

462 3.3.3. Aqueous phase D-fructose complex hydrolysis in receiving aqueous phase

463 600 µL of citrate buffer were introduced in an Eppendorf tube and incubated at the required
464 temperature (343 K) in a Mixing Block at 750 rpm. After 5 min, 600 µL of the organic phase obtained
465 after the extraction procedure and containing the fructoboronate complex were introduced in the
466 tube. After a reaction time of 45 min, the aqueous and organic phases were separated by pipetting.

467 3.3.4. Study of sequential process from glucose isomerization to fructose dehydration

468 A 250 mL vessel (Reactor-ReadyTM Lab Reactor, Radley, Figure 16) equipped with a temperature
469 control system (Ministat, Huber temperature control system, HUBER, UK) was used for these
470 experiments.

471 • Simultaneous D-glucose isomerization by enzymatic catalysis in the aqueous feed phase and
472 D-fructose complexation/transportation in the organic phase

473 IGI (0.5 g) was loaded in a stirring basket introduced in a first reactor (Figure 16 – Left) with 100 mL
474 of a aqueous solution containing D-Glucose (100 mM), Na_2SO_4 (8 mM), $MgCl_2$ (20 mM), in Tris-HCl
475 buffer (100 mM, pH 8.5) at 343 K. Then, 100 mL of MIBK containing 3,4-DCPBA (100 mM) and
476 Aliquat336® (200 mM) were introduced in the reactor. The mixture was stirred via the stirring basket
477 at 200 rpm, with the temperature kept at 343 K for 180 min, before discarding the aqueous feed
478 phase.

479 • D-fructose complex hydrolysis in the aqueous receiving phase

480 100 mL of the organic phase issued from the first reactor and containing the fructoboronate complex
481 were transferred in a second reactor (Figure 16 – Middle) and heated at 343 K. Then, 100 mL of a
482 citrate buffer solution (100 mM, pH 3) were introduced. The mixture was stirred with a turbine
483 stirring shaft at 200 rpm and set up at 343 K for 180 min.

484

485 **4. Conclusion**

486 In this work, we have first provided the conditions of the key parameters (temperature, pH,
487 arylboronic acid structure and concentrations, Aliquat336® concentrations) applied for a sequential
488 process from glucose to HMF through fructose transportation in an organic liquid membrane. The

489 optimized conditions have been then applied to an unprecedented integrated process in a
490 specifically designed reactor. Therein, enzymatic glucose isomerization and fructose dehydration to
491 HMF, involving an intermediate fructose transportation by complexation with an aryboronic
492 acid-Aliquat336® complex, could be carried out simultaneously in independent aqueous phases.
493 These phases were both in contact with a single organic phase enabling the transportation of fructose
494 from the first aqueous phase to the second. In this process, we thus succeeded in simultaneously
495 enzymatically isomerizing glucose to fructose and dehydrating fructose to HMF by minimizing the
496 number of reaction media and separation steps. Indeed, no intermediate product or phase isolation
497 was necessary. The use of numerous organic solvents or ionic liquid, as previously described in the
498 literature, is limited to a unique organic phase used for fructose transportation. Improvement of
499 performances in terms of HMF yields implying better diffusion of species between the different
500 phases by improving the conception of new reactor systems will be reported in due course.

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