



HAL
open science

Biocatalyst and continuous microfluidic reactor for an intensified production of n-butyl levulinate: kinetic model assessment

Alexandre Cordier, Marcel Klinksiek, Christoph Held, Julien Legros, Sébastien Leveneur

► To cite this version:

Alexandre Cordier, Marcel Klinksiek, Christoph Held, Julien Legros, Sébastien Leveneur. Biocatalyst and continuous microfluidic reactor for an intensified production of n-butyl levulinate: kinetic model assessment. *Chemical Engineering Journal*, In press, pp.138541. <10.1016/j.cej.2022.138541>. <hal-03748632>

HAL Id: hal-03748632

<https://normandie-univ.hal.science/hal-03748632v1>

Submitted on 9 Aug 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

1 Biocatalyst and continuous microfluidic reactor for an intensified production of *n*-butyl
2 levulinate: kinetic model assessment

3 Alexandre Cordier¹, Marcel Klinksiek³, Christoph Held³, Julien Legros^{1*}, Sébastien
4 Leveneur^{2*}

5 ¹*INSA Rouen, CNRS, Normandie Université, UNIROUEN, COBRA laboratory, F-*
6 *76000 Rouen, France, E-mail: julien.legros@univ-rouen.fr*

7 ²*INSA Rouen, UNIROUEN, Normandie Univ, LSPC, UR4704, 76000 Rouen, France,*
8 *E-mail: sebastien.leveneur@insa-rouen.fr*

9 ³ *Laboratory of Thermodynamics, Department of Biochemical and Chemical*
10 *Engineering, TU Dortmund University, Emil-Figge. Str.70, 44227 Dortmund, Germany*

11

12 **Abstract**

13 The use of enzymes to catalyze chemical reactions has increased these recent years.
14 Several models have been developed to express the kinetics over these biocatalysts.
15 The most well-known of them, Michaelis-Menten, is used when only one substrate
16 adsorbs on the enzyme. In the case of the esterification reaction, i.e., bimolecular
17 system, a more complex kinetic model such as the Ping-Pong Bi-Bi should be applied.
18 The use of such advanced models is essential for reactor scaleup and to optimize
19 production. However, these models usually do not consider the reaction temperature.
20 To fill this gap, a Ping-Pong Bi-Bi model was developed to produce butyl levulinate
21 from the esterification of levulinic acid over an immobilized enzyme, Novozym®435.
22 Microfluidic technology was used to ensure ideal mixing conditions. The Ping-Pong
23 model, considering inhibition mechanisms, fits the experimental concentrations. ePC-
24 SAFT equation of state was used to estimate the equilibrium constants.

25 **Keywords**

26 Kinetic modeling, ePC-SAFT, Enzyme, Ping-Pong mechanism

27

28 **1. Introduction**

29 Lignocellulosic biomass (LCB) is seen as the best alternative to fossil raw materials to
30 make the chemical and fuel industries sustainable [1–3]. Compared to first-generation
31 biomass, LCB is not competing with the food sector, avoiding the fuel versus food
32 dilemma. The chemistry of this LCB valorization focuses on the production of platform
33 molecules [4–6] and lignin valorization [4–6].

34 The platform molecule levulinic acid production has gained much interest these last
35 years [7–9]. Levulinic acid or alkyl levulinates are starting materials for producing
36 another platform molecule γ -valerolactone (GVL) [10–17]. Alkyl levulinates can be
37 used directly as blending components for biodiesel or as a fuel oxygenate additives
38 [18,19], and can also find applications as additives, solvents, and intermediates in fine
39 chemistry [9]. In the study of Christensen et al., they showed that butyl levulinate (BL)
40 improved conductivity, cold flow properties, and lubricity of diesel fuel and reduced its
41 vapor pressure [20]. Moreover, it was found that BL remains in solution with diesel
42 down to the fuel cloud point and has more compatibility with elastomers, compared to
43 ethyl levulinate, which tends to separate from diesel at a temperature below 0 °C and
44 results to be more corrosive [20]. Frigo et al., demonstrated that diesel fuel blended
45 with a mixture of dibutyl ether and BL could reduce particulate emissions without
46 changing engine power efficiency or increasing the NO_x emission [21].

47 Alkyl levulinates can be produced via the alcoholysis of sugar monomers or LA
48 esterification [7,8,22]. The latter route can be done via homogenous [23],
49 heterogeneous [24–30] or enzymatic catalysis [31–37]. The use of heterogeneous
50 catalysts such as resins, zeolite or immobilized enzymes should be favored to avoid
51 additional separation stages [23]. From a chemical engineering viewpoint, using a
52 continuous reactor for biomass valorization should be favored for large production [38].

53 Microreactor or microfluidic technology has raised interest in the scientific community
54 because concentration and temperature gradients are reduced.

55 Production of n-butyl levulinate from LA esterification over lipase catalysis has been
56 scarcely studied [32,34]. Yadav and Borkar [34] developed a kinetic model of LA
57 esterification by butanol over Novozym®435, without considering the reversibility of
58 this reaction or the temperature effect. Bhavsar and Yadav [34] showed that a
59 continuous packed bed reactor with immobilized Novozym can be used to produce BL.

60 There is a need to intensify this reaction from an industrial [39] and fundamental
61 standpoint. The use of a microreactor enables to operate in the absence of gradients
62 allowing to use plug-flow model and thus simplifying the kinetic modeling stage [40].

63 The developed models proposed in the literature for the esterification of carboxylic acid
64 over enzyme do not consider the temperature effect, and the knowledge of this effect
65 on kinetic constants is mandatory for an industrial scaleup. In this manuscript, using
66 microfluidic technology, kinetic models for the synthesis of BL over commercial
67 Novozym®435 were developed and assessed at different temperatures.

68

69

70

71

72

73

74 **2. Experimental section**

75

76 2.1 Chemicals

77 All the chemicals were used as provided, without further purification. Butan-1-ol (wt%
78 $\geq 99.9\%$), levulinic acid (wt% $\geq 99\%$), (trimethylsilyl)diazomethane (2.0 M in hexane)
79 and the commercial supported *Candida Antarctica* lipase B (CAL-B), Novozym 435
80 (5000 U.mg⁻¹), were purchased from Sigma-Aldrich. Dichloromethane stabilized by
81 ethanol was purchased from CARLO ERBA Reagents. Deionized water from the
82 Aquadem™ system (Veolia) was used.

83 2.2 Analytical method

84 The aliquots taken at each residence time (t^R) for the analysis of butyl levulinate
85 concentration were analyzed on a Thermo Scientific™ TRACE™ 1310 GC-FID
86 equipped with an apolar column (DB-5MS, 30 m \times 0.250 mm ID \times 0.250 μ m film
87 thickness). The initial temperature of the analysis method was set at 50 °C for 2 min to
88 reach 250 °C with a temperature rate of 25 °C/min. The aliquots were diluted in
89 dichloromethane, and an excess of trimethylsilyl diazomethane as carboxylic acid
90 scavenger was added (to protect the GC column from corrosion); 1 μ L of the resulting
91 solution was injected into the GC.

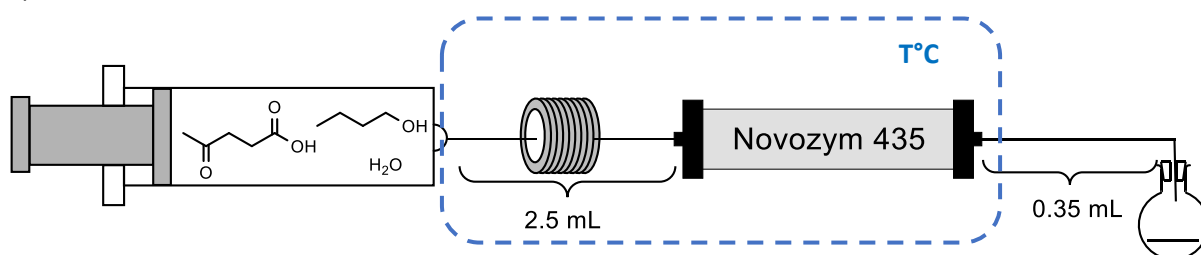
92 High field ¹H NMR analyses were performed on a 300 MHz Bruker Spectrospin
93 spectrometer. Chemical shifts (δ) are given with regard to TMS using residual CHCl₃
94 solvent as an internal reference.

95

96

97 2.3 Procedure for the biocatalyzed esterification of levulinic acid with butanol in a
98 flow reactor

99 The Luer-lock syringe, filled with levulinic acid, butanol and water, was connected to a
100 preheating loop (PTFE tubing, ID = 1.59 mm, L = 126 cm) before the packed-bed
101 reactor with an internal diameter of 6.6 mm. The packed-bed reactor was composed
102 of an Omnifit™ column filled with Novozym®435 immersed in a thermostated bath (Fig.
103 1).



104

105 Fig.1. Process for the butyl levulinate synthesis with Omnifit cartridge.

106

107 The kinetic measurements were performed. First, the flow system was filled with the
108 solution at a flow rate of 5 mL/min. Then the flow-rate was increased step by step to
109 reach the desired t^R . The collection of aliquots began after running the system for 2 mL
110 of reaction product collected, in order to reach the steady-state. The volume of the
111 microreactor is related to the amount of the Novozym®435: 50 mg, 100 mg and 150
112 mg of supported CAL-B were packed 195 μ L, 390 μ L and 520 μ L, respectively. The
113 kinetic monitoring was performed as described in Table 1.

114

115

Table 1. Different flow-rates used for the kinetic monitoring.

Mass of Novozym®435 (mg) Residence Time (min)	50 mg	100 mg	150 mg
30	6.5 $\mu\text{L}/\text{min}$	13 $\mu\text{L}/\text{min}$	17.33 $\mu\text{L}/\text{min}$
20	9.75 $\mu\text{L}/\text{min}$	19.5 $\mu\text{L}/\text{min}$	26 $\mu\text{L}/\text{min}$
15	13 $\mu\text{L}/\text{min}$	26 $\mu\text{L}/\text{min}$	34.66 $\mu\text{L}/\text{min}$
10	19.5 $\mu\text{L}/\text{min}$	39 $\mu\text{L}/\text{min}$	52 $\mu\text{L}/\text{min}$
7	27.85 $\mu\text{L}/\text{min}$	55.71 $\mu\text{L}/\text{min}$	74.28 $\mu\text{L}/\text{min}$
5	39 $\mu\text{L}/\text{min}$	78 $\mu\text{L}/\text{min}$	104 $\mu\text{L}/\text{min}$
3	65 $\mu\text{L}/\text{min}$	130 $\mu\text{L}/\text{min}$	173.33 $\mu\text{L}/\text{min}$
2	97.5 $\mu\text{L}/\text{min}$	195 $\mu\text{L}/\text{min}$	260 $\mu\text{L}/\text{min}$
1	195 $\mu\text{L}/\text{min}$	390 $\mu\text{L}/\text{min}$	520 $\mu\text{L}/\text{min}$
0	0 $\mu\text{L}/\text{min}$	0 $\mu\text{L}/\text{min}$	0 $\mu\text{L}/\text{min}$

116

117 Repeatability was assessed by collecting three samples after the steady-state, and it
 118 was found that the standard deviation was lower than $0.05 \text{ mol}\cdot\text{L}^{-1}$. Furthermore, one
 119 run was repeated two times (Run 4) on different days to verify the repeatability of the
 120 whole system (Fig. S1.1). Fig. S1.1 shows that the repeatability is good.

121 Table 2 shows the experimental matrix used in this study.

122

Table 2. Experimental matrix for esterification.

Run	Temperature (°C)	Mass of catalyst (mg)	Void volume (μL)	Inlet (mol/L)				Ratio [BuOH] _{in} /[LA] _{in}
				BL	LA	BuOH	W	
1	20	100	390	0.00	2.61	7.81	0.34	3.00
2	35	100	390	0.00	2.61	7.81	0.34	3.00
3	50	100	390	0.00	2.61	7.81	0.34	3.00
4	65	100	390	0.00	2.61	7.82	0.34	3.00
5	80	100	390	0.00	2.61	7.81	0.34	3.00
6	65	150	520	0.00	2.61	7.81	0.34	2.99
7	65	50	195	0.00	2.61	7.81	0.34	2.99
8	65	100	390	0.00	5.09	5.09	0.31	1.00
9	50	100	390	0.00	5.16	5.16	0.32	1.00
10	35	100	390	0.00	5.09	5.09	0.31	1.00
11	20	100	390	0.00	5.09	5.09	0.31	1.00
12	65	150	520	0.00	5.09	5.09	0.31	1.00
13	65	50	195	0.00	5.09	5.09	0.31	1.00
14	65	100	390	0.00	1.74	8.71	0.32	4.99
15	50	100	390	0.00	1.77	8.83	0.33	5.00
16	35	100	390	0.00	1.79	8.95	0.33	4.99
17	20	100	390	0.00	1.82	9.07	0.34	4.99
18	65	50	195	0.00	1.74	8.71	0.32	4.99
19	65	150	520	0.00	1.74	8.71	0.32	4.99
20	65	100	390	0.00	2.59	7.76	0.70	3.00
21	50	100	390	0.00	2.59	7.76	0.70	3.00
22	35	100	390	0.00	2.59	7.76	0.70	3.00
23	20	100	390	0.00	2.59	7.76	0.70	3.00
24	65	100	390	0.00	2.46	7.78	1.20	3.17
25	50	100	390	0.00	2.57	7.69	1.20	3.00
26	35	100	390	0.00	2.46	7.78	1.20	3.17
27	20	100	390	0.00	2.46	7.78	1.20	3.17

126 2.4 Stability of Novozym 435

127 It is fundamental to evaluate the Novozym 435 stability to know if there are enzymes
128 or another product leaching from support denaturation. Two experiments were
129 performed: a degradability test for Novozym 435 in butanol solvent in a batch reactor
130 and deactivation in the microfluidic system.

131 The degradability test was performed as follows: 100 mg of Novozym 435 were placed
132 into 390 μL of a solution containing a $[\text{BuOH}]_{\text{inlet}}/[\text{LA}]_{\text{inlet}}$ ratio = 5. The mixture was
133 heated at 65°C. After 1 h, the sample was filtered through a 30 μm PTFE frit of the
134 Omnifit column (the exact same one that we used for the kinetic study), then CDCl_3
135 was added, and a ^1H NMR analysis was performed. The obtained spectrum was
136 compared to those of authentic samples of butyl levulinate, n-butanol and levulinic
137 acid, as well as those from PMMA and methyl methacrylate from the literature [41].

138 The deactivation test was carried out at 65 °C with 100 mg of catalyst and inlet levulinic
139 acid concentration of 2.61 mol.L⁻¹. The outlet concentration of BL was followed with
140 time-on-stream at 65°C.

141 **3. Results**

142 *3.1 External and internal mass transfer evaluation*

143 Besides the effect of mass transfer resistance on the kinetics, the presence or absence
144 of flow maldistribution should be determined [42–44]. According to Doraiswamy and
145 Tajbl [42], if the ratio reactor diameter on particle diameter is higher than 4, then they
146 conclude that there is a proper liquid distribution with no channeling. In this system,
147 this ratio is higher than 10. Thus, we concluded the absence of flow maldistribution.

148 To evaluate the influence of both effect, the same methodology presented by Leveneur
149 et al. [45] was applied.

150 External mass transfer for each experiment was evaluated throughout the coefficient
151 f_e (Equation (1)) defined by Villiermaux [46]. If f_e is lower than 5%, then the external
152 mass transfer is negligible.

153
$$f_e = \frac{\overline{r_{Obs}} \cdot L}{k_D \cdot C_b} \quad (1)$$

154 where, L is the ratio particle volume (V_P) on the external particle surface (A_P), $\overline{r_{Obs}}$ is
155 the initial observed rate of esterification, k_D is the mass transfer coefficient and C_b the
156 concentration in the bulk phase. In Equation (1), the concentration of LA in the bulk
157 phase was used because butanol is in excess. The mean particle size of
158 Novozym®435 is equal to 0.65 mm, and thus L is equal to $1.08 \cdot 10^{-4}$ m [47].

159 The mass transfer coefficient k_D can be estimated via the Sherwood number (Sh)
160 expressed as

161
$$Sh = \frac{k_D \cdot \overline{d_P}}{D} = 2 + 1.8 \cdot Re_P^{\frac{1}{2}} \cdot Sc^{\frac{1}{3}} \quad (2)$$

162 where, d_p is the mean diameter of the catalyst particle, and Sc stands for the
163 Schmidt number expressed as

$$164 \quad Sc = \frac{\mu_f}{\rho_f \cdot D} \quad (3)$$

165 D is the molecular diffusion coefficient of LA in butanol calculated by the Wilke-Chang
166 equation [48]. For instance, the molecular diffusion of LA in butanol at 50°C was found
167 to be $9.02 \cdot 10^{-10} \text{ m}^2 \cdot \text{s}^{-1}$. The terms μ_f and ρ_f represent the viscosity and density of the
168 fluid, i.e., butanol. These physicochemical properties were calculated from Ariba et al.
169 work [49]. The f_e values were found to be lower than 5% for each experiment showing
170 the absence of external mass transfer.

171 The internal mass transfer effect was evaluated via the Thiele modulus number ϕ_S
172 defined by Equation (4) [46].

$$173 \quad \phi_S^2 = \frac{\overline{r_{Obs}} \cdot L^2}{D_e \cdot C_S} \quad (4)$$

174 If ϕ'_S is lower than 0.1, hence internal mass transfer can be assumed to be negligible.
175 C_S is the LA concentration at the particle surface and in this study $C_S=C_B$ because
176 there is no external mass transfer. The term D_e represents the effective diffusion
177 coefficient defined as $D_e = \frac{\varepsilon_P \sigma}{\tau} \cdot D$, where ε_P , σ and τ represent the porosity,
178 constriction factor and tortuosity of the particle, respectively. From Ravelo et al. [47],
179 Novozym[®]435 porosity is equal to 0.5. The tortuosity and constriction factor values
180 were fixed to 6 and 1 [50]. Based on the ϕ'_S values of each experiment, the internal
181 mass transfer can be assumed to be negligible.

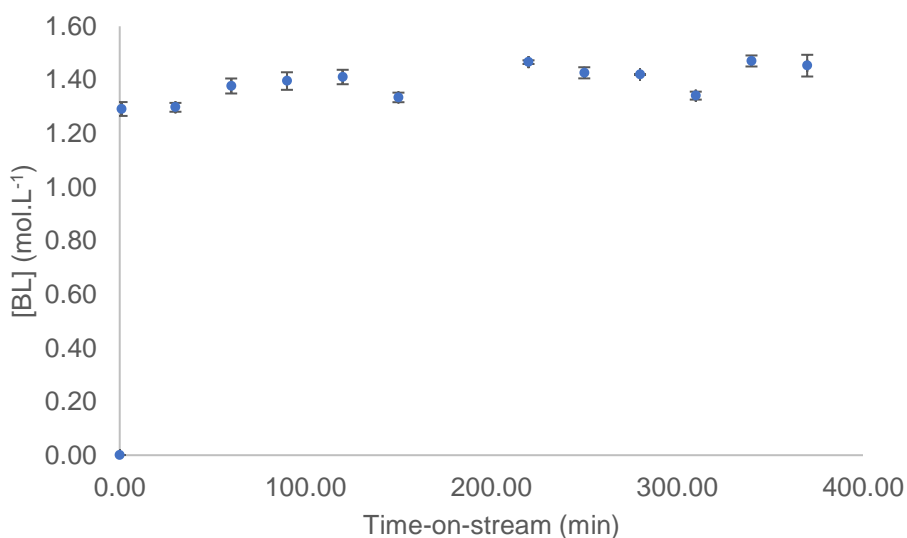
182

183

184 3.2 *Novozym 435 stability study*

185 Novozym 435 degradability test (S2) is an essential study because its acrylic
186 support/matrix tends to dissolve in many organic solvents [51–53]. By spectrum
187 comparison of the mixture with a ¹H NMR analysis of different isolated products (butyl
188 levulinate, n-butanol and levulinic acid), the mixture just contains n-butanol, levulinic
189 acid and butyl levulinate and absolutely no traces of PMMA (or associate compound)
190 were detected. Thus, our measurements are perfectly reliable with no interference from
191 external chemicals.

192 Fig. 2 shows the BL concentration at the outlet versus time-on-stream. From Fig. 2,
193 one can notice that enzyme deactivation can be neglected during the experiment.



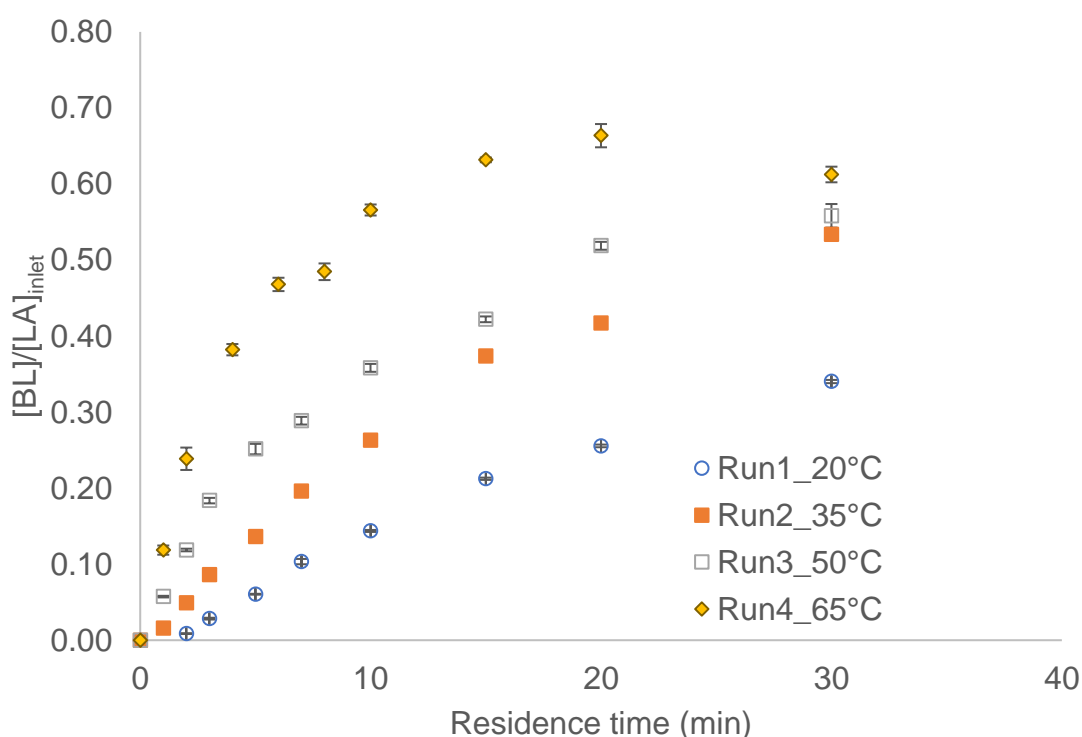
194
195 Fig. 2. Evolution of experimental BL concentration (blue circle) with error bars (black)
196 versus time-on-stream at 65°C, [LA]_{inlet}=2.61 mol.L⁻¹ and 100 mg of catalyst.

197 3.3 *Temperature effect*

198 Fig. 3 shows the effect of temperature on the ratio $\frac{[BL]}{[LA]_{inlet}}$, where [BL] is the
199 experimental outlet concentration of BL. To evaluate this effect, experimental data

200 obtained from Runs 1-4 were compared, because there were carried out in the same
 201 operating conditions, except for the reaction temperature (Table 2). As expected, the
 202 kinetics of esterification increases with temperature.

203 These data can also be used to evaluate the effect of mass transfer. The natural
 204 logarithm of the initial rate constants versus $1/T$ was plotted (Fig. S3.1). From Fig. S3.1,
 205 the linearity between the natural logarithm of the initial rate constants and $1/T$ confirms
 206 the absence of mass transfer resistance.



207
 208 Fig.3. Effect of temperature on the experimental concentration ratio $[BL]/[LA]_{inlet}$
 209 (Table 2): Run 1 at 20 °C (light blue circle), Run 2 at 35 °C (orange square), Run 3 at
 210 50 °C (grey square), Run 4 at 65 °C (yellow diamond) and error bars (black).

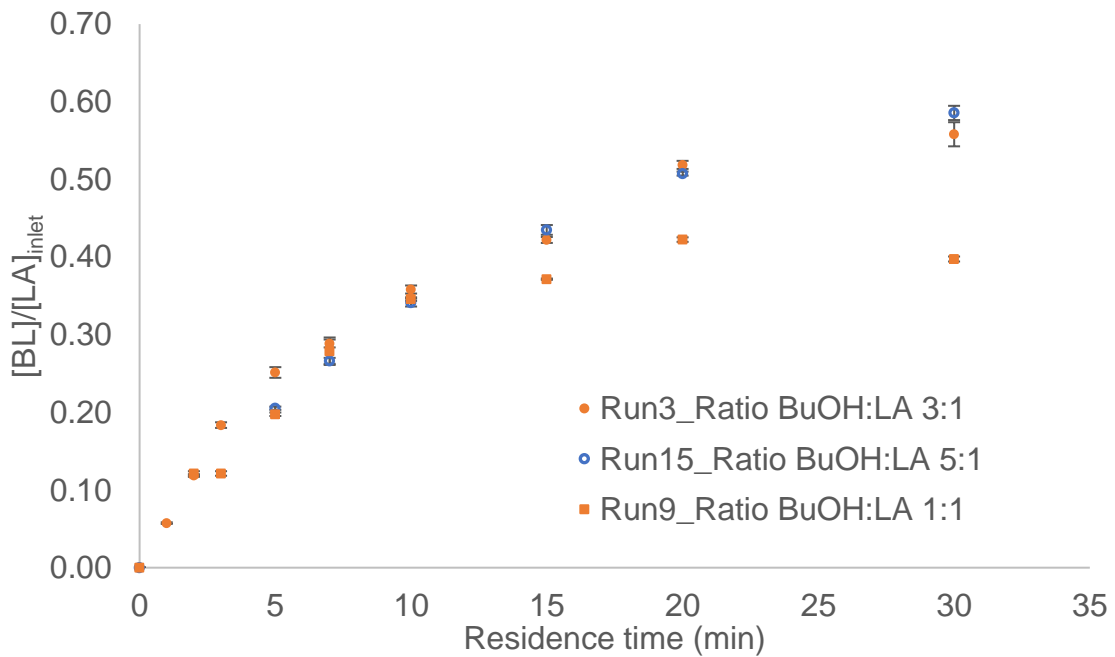
211 3.4 Molar ratio effect

212 The effect of $\frac{[BuOH]_{inlet}}{[LA]_{inlet}}$ can affect the thermodynamics and kinetics of esterification [31].

213 Figs 4 and 5 show the effect of this ratio on the kinetics, via the normalized ratio $\frac{[BL]}{[LA]_{inlet}}$.

214 Different experiments carried out in similar operating conditions, except the ratio
 215 $\frac{[BuOH]_{inlet}}{[LA]_{inlet}}$, were compared (Table 2). One can notice that when $\frac{[BuOH]_{inlet}}{[LA]_{inlet}}$ is equal to
 216 3:1 or 5:1, then the reaction rates and equilibrium values are similar. For a $\frac{[BuOH]_{inlet}}{[LA]_{inlet}}$
 217 equal to 1:1, there is a deviation when the reaction reaches the equilibrium.

218



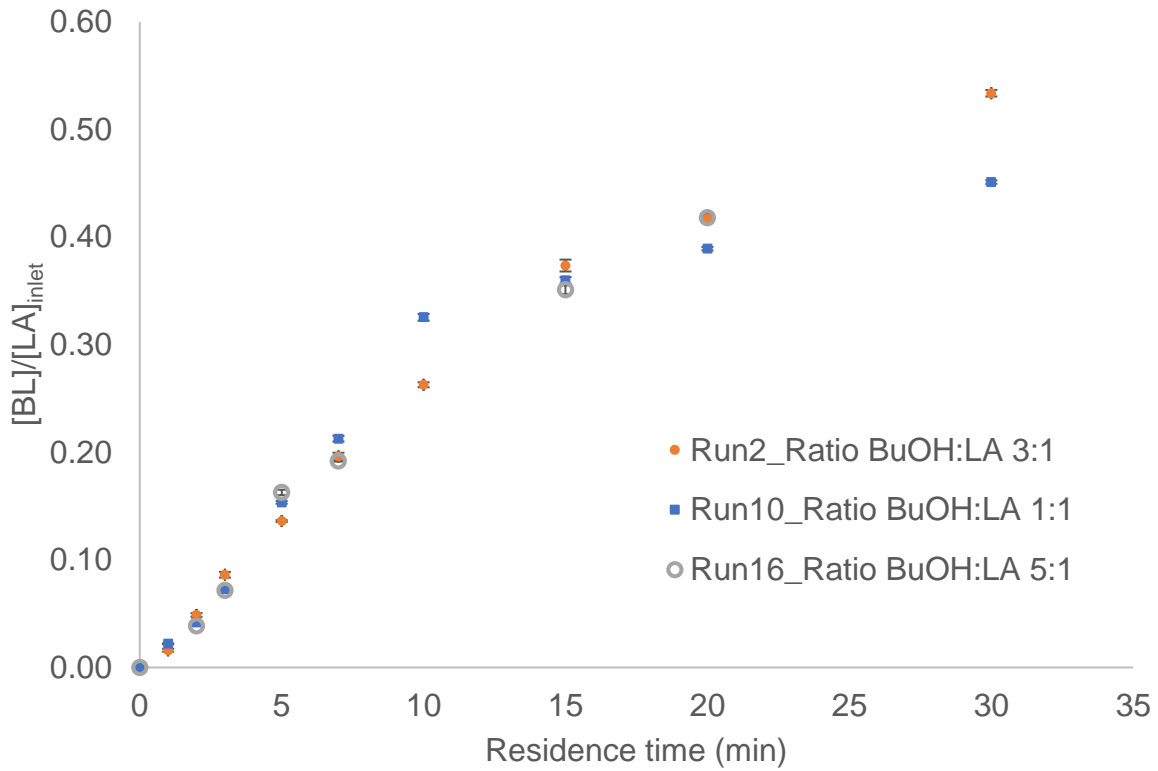
219

220 Fig.4. Effect of $\frac{[BuOH]_{inlet}}{[LA]_{inlet}}$ ratio on experimental concentration ratio $[BL]/[LA]_{inlet}$ at 50°C

221 (Table 2): Run 3 at $\frac{[BuOH]_{inlet}}{[LA]_{inlet}} = 3:1$ (orange circle), Run 15 at $\frac{[BuOH]_{inlet}}{[LA]_{inlet}} = 5:1$ (blue

222 circle), Run 9 at $\frac{[BuOH]_{inlet}}{[LA]_{inlet}} = 1:1$ (orange square) and error bars (black).

223



224

225 Fig.5. Effect of $\frac{[BuOH]_{inlet}}{[LA]_{inlet}}$ ratio on experimental concentration ratio $[BL]/[LA]_{inlet}$ at 35

226 °C (Table 2): Run 2 at $\frac{[BuOH]_{inlet}}{[LA]_{inlet}} = 3:1$ (orange circle), Run 10 at $\frac{[BuOH]_{inlet}}{[LA]_{inlet}} = 1:1$

227 (blue square), Run 16 at $\frac{[BuOH]_{inlet}}{[LA]_{inlet}} = 1:1$ (grey circle) and error bars (black).

228

229 3.5 Evaluation of equilibrium constant

230 The PC-SAFT equation of state first published by Gross and Sadowski [54]
231 expresses the residual Helmholtz energy a^{res} as shown in Equation 5.

$$232 \quad a^{res} = a^{hc} + a^{disp} + a^{assoc} \quad (5)$$

233 Thereby the hard-chain (a^{hc}) reference system represents the repulsive interactions
234 between the molecules. The attractive interactions, such as the dispersion (a^{disp}),
235 association (a^{assoc}), are described as perturbations of the reference system. More
236 details on the modeling procedure and parameters used can be found in
237 Supplementary Information (S4). Thermodynamic modeling of the reaction equilibrium
238 is based on the temperature and pressure-dependent equilibrium constant K_{th} . It is
239 calculated by the reacting agent concentrations in the equilibrium and activity
240 coefficients according to Equation 6.

241 Based on Equation 5, we can calculate the temperature and pressure-dependent
242 equilibrium constant K_{th} .

$$243 \quad K_{th}(T, p) = K_{eq}(T, p, x) \cdot K_{\gamma}(T, p, x) = \prod_i (x_i \cdot \gamma_i)^{\nu_i} \quad (6)$$

244 where, K_{eq} is determined from the experimental equilibrium concentrations and the
245 activity coefficients are obtained by PC-SAFT [54–59]. The activity coefficient of each
246 reactant i in the mixture is calculated from the ratio of the fugacity coefficients in the
247 mixture and of the fugacity coefficient of the pure component.

$$248 \quad \gamma_i = \frac{\varphi_i(T, p, x)}{\varphi_{oi}(T, p, x_i=1)} \quad (7)$$

249 The equilibrium constant K_{th} for each temperature enables the calculation of
250 equilibrium concentrations at different conditions, i.e., molar ratios. The equilibrium

251 constant K_{th} was calculated based on the experiments with a molar ratio of BuOH:LA
252 3:1 (Table 3).

253 Table 3. Calculated equilibrium constant K_{th} based on the equilibrium concentrations
254 of Runs 1-5 (Table 2).

T / °C	K_{eq}	K_Y	K_{th}
20	0.157	3.578	0.56
35	0.309	2.793	0.67*
50	0.356	2.308	0.82
65	0.491	1.969	0.97
80	0.695	1.732	1.20

255 *interpolated value

256

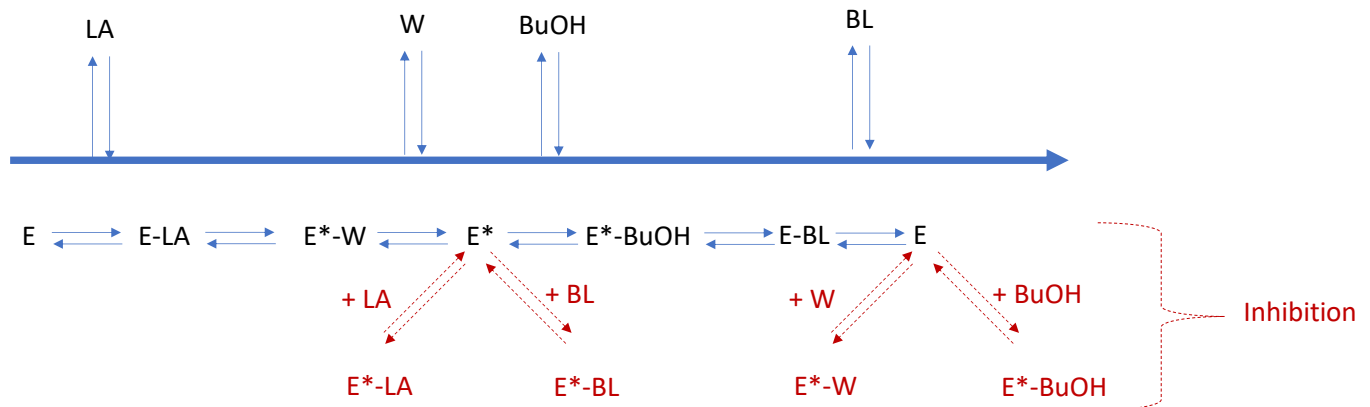
257

258 **4. Discussion**

259 **4.1 Ping-Pong Models**

260 Several authors showed that the Ping-Pong Bi-Bi mechanism can be used for the
 261 esterification reactions [32,60–66]. Several of them showed that alcohol and carboxylic
 262 acid can inhibit the enzyme, but none developed a kinetic model considering the
 263 reversibility of this reaction and the temperature effect on the kinetic constants.

264 Fig. 6 shows the Ping-Pong Bi-Bi mechanism for the esterification of LA by butanol.



265

266 Fig. 6. Ping-Pong Bi-Bi and inhibition mechanism for the esterification of LA.

267 From Varma and Madras study [65], the rate equation can be derived as

268

$$r_{Esterification} = \frac{k_f \cdot k_b \cdot [E]_0^2 \cdot \left([LA] \cdot [BuOH] - \frac{[BL] \cdot [W]}{K_{eq}} \right)}{D} \quad (8)$$

269 where, $[LA]$, $[BuOH]$, $[BL]$ and $[W]$ are the outlet concentrations of levulinic acid,
 270 butanol, butyl levulinate and water, respectively. The term $[E]_0$ stands for the initial
 271 concentration of enzyme. The denominator D is expressed as

$$\begin{aligned}
D = & k_b \cdot [E]_0 \cdot [LA] \cdot [BuOH] + k_b \cdot [E]_0 \cdot K_{BuOH} \cdot [LA] \cdot \left(1 + \frac{[LA]}{K_{ILA}}\right) \\
& + k_b \cdot [E]_0 \cdot K_{LA} \cdot [BuOH] \cdot \left(1 + \frac{[BuOH]}{K_{IBuOH}}\right) + \frac{k_f \cdot [E]_0 \cdot K_W}{K_{eq}} \cdot [BL] \cdot \left(1 + \frac{[BL]}{K_{IBL}}\right) \\
& + \frac{k_f \cdot [E]_0 \cdot K_{BL}}{K_{eq}} \cdot [W] \cdot \left(1 + \frac{[W]}{K_{IW}}\right) \\
& + \frac{k_f \cdot [E]_0}{K_{eq}} \cdot [W] \cdot [BL] + \frac{k_f \cdot K_{BL} \cdot [E]_0}{K_{IIA} \cdot K_{eq}} \cdot [W] \cdot [LA] + \frac{k_b \cdot K_{LA} \cdot [E]_0}{K_{IIBL}} \cdot [LA] \cdot [BL] \\
& + \frac{k_b \cdot K_{LA} \cdot [E]_0}{K_{BuOH-W}} \cdot [W] \cdot [BuOH] + \frac{k_b \cdot K_{BuOH} \cdot [E]_0}{K_{LA-BL}} \cdot [LA] \cdot [BL] \quad (9)
\end{aligned}$$

273 The terms $k_f \cdot [E]_0$ and $k_b \cdot [E]_0$ represent kinetic rate constants. The terms K_{LA} , K_{BL} ,
274 K_{BuOH} and K_W are the Michaelis constants for LA, BL, BuOH, and W, respectively. The
275 inhibition constants by levulinic acid and BL are defined by K_{IIA} and K_{IIBL} . The
276 dissociation constants, representing the dissociation of the inhibitor from the
277 corresponding enzyme-inhibitor, are K_{IIA} , K_{IBL} , K_{IBuOH} and K_{IW} . The adsorption
278 constants K_{BuOH-W} and K_{LA-BL} are lumped constants.

279 Mitchell and Krieger proposed a new rate expression of this Ping-Pong Bi-Bi
280 mechanism [63],

$$r_{Esterification} = \frac{(k_{LA} \cdot [LA] \cdot k_{BuOH} \cdot [BuOH] - k_{BL} \cdot [BL] \cdot k_W \cdot [W]) \cdot [E]_0}{D'} \quad (10)$$

282 where, the denominator D' is

$$\begin{aligned}
D' = & k_{LA} \cdot [LA] \cdot \left(1 + \frac{[W]}{K_{IW}} + \frac{[BuOH]}{K_{BuOH}}\right) + k_{BuOH} \cdot [BuOH] + k_W \cdot [W] \\
& + k_{BL} \cdot [BL] \cdot \left(1 + \frac{[W]}{K_W} + \frac{[BuOH]}{K_{IBuOH}}\right) \quad (11)
\end{aligned}$$

284

285

286 The terms k_{LA} , k_{BuOH} , k_{BL} and k_W are the specific constants of the enzyme for levulinic
 287 acid, butanol, butyl levulinate and water, respectively. K_{BuOH} and K_W are Michaelis-
 288 type constants for butanol and water, respectively. K_{IBuOH} and K_{IW} are the inhibition
 289 constants for butanol and water, respectively.

290 By considering the binding of BuOH or water with the free enzyme, D' becomes D''

$$291 \quad D'' = k_{LA} \cdot [LA] \cdot \left(1 + \frac{[W]}{K_{IW}} + \frac{[BuOH]}{K_{BuOH}}\right) + (k_{BuOH} \cdot [BuOH] + k_W \cdot [W]) \cdot \left(1 + \frac{[BuOH]}{K_{siBuOH}}\right) \cdot \left(1 + \frac{[W]}{K_{siW}}\right) \\ + k_{BL} \cdot [BL] \cdot \left(1 + \frac{[W]}{K_W} + \frac{[BuOH]}{K_{IBuOH}}\right) \quad (12)$$

292 where, K_{siBuOH} and K_{siW} stand for the constants between BuOH and water with the
 293 free enzyme.

294 According to Mitchell and Krieger [63], these equations are mathematically symmetric
 295 and less-lumped parameters. Different models were evaluated based on reaction rates
 296 developed by Mitchell and Krieger [63] and Varma and Madras [65].

297 4.2 Kinetic modeling

298 Experiments were performed in isothermal conditions, and internal and external mass
 299 transfers were found to be negligible. Plug-flow model was used; thus, material
 300 balances for each species can be written as

$$301 \quad \frac{d[BL]}{d\tau} = r_{Esterification} \quad (13)$$

$$302 \quad \frac{d[BuOH]}{d\tau} = -r_{Esterification} \quad (14)$$

$$303 \quad \frac{d[LA]}{d\tau} = -r_{Esterification} \quad (15)$$

$$304 \quad \frac{d[W]}{d\tau} = r_{Esterification} \quad (16)$$

305 where, τ is the space time defined as $\frac{V_L}{Q}$, V_L and Q are the volume of the liquid in the
306 reactor and volumetric flow-rate, respectively.

307 Ordinary differential equations ODEs (13)-(16) were solved by the solver DDALPUS
308 algorithm, via a damped Newton method [67].

309 For the non-linear regression, the concentration of BL was used as an observable. The
310 estimation of the different kinetic constants (Equations (8)-(12)) was done via the
311 minimization of the objective function $S(\theta)$ expressed as

$$312 \quad S(\theta) = \sum_{u=1}^n w_u \cdot ([BL]_{exp,u} - [BL]_{sim,u})^2 = SSR \quad (17)$$

313 where, w_u is the weigh factor for the experimental value u .

314 The objective function is expanded as a quadratic function of the parameters around
315 the initial parameter values of the current iteration. The resulting quadratic minimization
316 problem is solved with a modified Gauss-Jordan algorithm within a user-defined
317 feasible region; then, a weak line search is conducted to establish an improved
318 objective value and initial parameter vector for the next iteration. Interval estimates for
319 the individual estimated parameters are then calculated from the final quadratic
320 expansion of the objective function. This minimization is done by the package
321 GREGPLUS to provide optimal parameter estimates with the 95% confidence
322 intervals, expressed by the highest probability density (HPD). GREGPLUS provides
323 the normalized parameter covariance matrix.

324 The GREGPLUS package and DDAPLUS solver are implemented in the Athena Visual
325 Studio® 14.2 [68] used in this study.

326 Different models were evaluated based on the Ping-Pong Bi-Bi mechanism developed
 327 by the Varma and Madras study [65] and the Mitchell and Krieger study [63]. The term
 328 $[E]_0$ was expressed by the catalyst loading ρ_{Enzyme} , i.e., the mass of catalyst divided by
 329 the volume of liquid in the reactor.

330 The general equation for esterification can be derived as

$$331 \quad r_{Esterification} = k_{Esterification} \cdot \rho_{Enzyme} \cdot \frac{1}{D} \cdot \left([LA] \cdot [BuOH] - \frac{[BL] \cdot [W]}{K_{eq}} \right) \quad (18)$$

332 Different models were assessed, as summarized in Table 4.

333 Model 1 is the simplest one, by letting the denominator D equal to 1.

334 Models 2-4 are derived from Varma and Madras. Equation (8) was divided by k_b . To
 335 ease the parameter estimation and avoid division by very low number (close to zero)
 336 or high number, the following modification were included

$$337 \quad K''_{ILA} = \frac{K_{BuOH}}{K_{ILA}}, K''_{IBuOH} = \frac{K_{LA}}{K_{IBuOH}}, K''_W = \frac{K_W}{K_{eq}}, K''_{BL} = \frac{K_{BL}}{K_{eq}}, K''_{IBL} = \frac{K''_W}{K_{IBL}}, K'_W = k_f \cdot K_W \cdot \frac{1}{k_b} \cdot \frac{1}{K_{eq}},$$

$$K''_{IW} = \frac{K''_{BL}}{K_{IW}},$$

$$K'_{BL} = k_f \cdot K_{BL} \cdot \frac{1}{k_b} \cdot \frac{1}{K_{eq}}$$

$$K_{Lump} = \frac{k_f}{k_b \cdot K_{eq}}, K''_{IIBL} = \frac{K_{LA}}{K_{IIBL}}, K''_{BuOH-W} = \frac{K_{LA}}{K_{BuOH-W}} \text{ and } K''_{LA-BL} = \frac{K_{BuOH}}{K_{LA-BL}}$$

338 Model 2 ignores the inhibition mechanism, hence K''_{ILA} , K''_{IBL} , K''_{IW} , K''_{IBuOH} , K''_{IILA} , K''_{IIBL} ,
 339 K''_{LA-BL} , and K''_{BuOH-W} were fixed to zero.

340 Model 3 considers the inhibition by butanol and ignores the other inhibition mechanism,
 341 hence K''_{ILA} , K''_{IBL} , K''_{IW} , K''_{IILA} , K''_{IIBL} , K''_{LA-BL} , and K''_{BuOH-W} were fixed to zero.

342 Model 4 considers all inhibition mechanisms.

343 Models 5-6 are derived from Mitchell and Krieger.

344 Model 5 is based on Equation (10) and Equation (11) divided by k_{BuOH} . The following
 345 notations are used

$$346 \quad K_{eq} = \frac{k_{LA} \cdot k_{BuOH}}{k_{BL} \cdot k_W}, K_1 = \frac{k_{LA}}{k_{BuOH}}, K_2 = \frac{k_W}{k_{BuOH}}, K_3 = \frac{k_{BL}}{k_{BuOH}}, K''_{IBuOH} = \frac{1}{K_{IBuOH}}, K''_W = \frac{1}{K_W},$$

$$K''_{BuOH} = \frac{1}{K_{BuOH}}, K''_{IW} = \frac{1}{K_{IW}}, K''_{BuOH-W} = \frac{1}{K_{BuOH-W}} \text{ and } K''_{LA-BL} = \frac{1}{K_{LA-BL}}$$

347 Model 6 is based on Equation (10) and divided Equation (12) by k_{BuOH} . The following
 348 notations are included

$$349 \quad K''_{siBuOH} = \frac{1}{K_{siBuOH}} \text{ and } K''_{siW} = \frac{1}{K_{siW}} \quad (19)$$

350 Table 4. Kinetic models tested in this study.

Model	Kinetic term	Denominator
Model 1	$k_{Esterification} \cdot \rho_{Enzyme}$	1
Model 2	$k_f \cdot \rho_{Enzyme}$	$[LA] \cdot [BuOH] + K_{BuOH} \cdot [LA]$ $+ K_{LA} \cdot [BuOH]$ $+ K''_W \cdot [BL]$ $+ K''_{BL} \cdot [W]$ $+ K_{Lump} \cdot [W] \cdot [BL]$
Model 3	$k_f \cdot \rho_{Enzyme}$	$[LA] \cdot [BuOH] + K_{BuOH} \cdot [LA]$ $+ K_{LA} \cdot [BuOH] + K''_{IBuOH} \cdot [BuOH]^2$ $+ K''_W \cdot [BL]$ $+ K''_{BL} \cdot [W]$ $+ K_{Lump} \cdot [W] \cdot [BL]$

Model 4	$k_f \cdot \rho_{\text{Enzyme}}$	$ \begin{aligned} & [LA] \cdot [BuOH] + K_{BuOH} \cdot [LA] + K''_{ILA} \cdot [LA]^2 \\ & + K_{LA} \cdot [BuOH] + K''_{IBuOH} \cdot [BuOH]^2 \\ & + K''_W \cdot [BL] + K'_{IBL} \cdot [BL]^2 \\ & + K''_{BL} \cdot [W] + K''_{IW} \cdot [W]^2 \\ & + K_{Lump} \cdot [W] \cdot [BL] + K'''_{ILLA} \cdot [W] \cdot [LA] \\ & + K'''_{IIBL} \cdot [LA] \cdot [BL] \\ & + K''_{BuOH-W} \cdot [W] \cdot [BuOH] \\ & + K''_{LA-BL} \cdot [LA] \cdot [BL] \end{aligned} $
Model 5	$k_{LA} \cdot \rho_{\text{Enzyme}}$	$ \begin{aligned} & K_1 \cdot [LA] + K''_{IW} \cdot [W] \cdot [LA] + K''_{BuOH} \cdot [BuOH] \cdot [LA] \\ & + [BuOH] + K_2 \cdot [W] \\ & + K_3 \cdot [BL] + K'_W \cdot [W] \cdot [BL] + K'_{IBuOH} \cdot [BuOH] \cdot [BL] \end{aligned} $
Model 6	$k_{LA} \cdot \rho_{\text{Enzyme}}$	$ \begin{aligned} & K_1 \cdot [LA] + K''_{IW} \cdot [W] \cdot [LA] + K''_{BuOH} \cdot [BuOH] \cdot [LA] \\ & + ([BuOH] + K_2 \cdot [W]) \cdot (1 + K''_{siBuOH} \cdot [BuOH]) \\ & \cdot (1 + K''_{siW} \cdot [W]) \\ & + K_3 \cdot [BL] + K'_W \cdot [W] \cdot [BL] + K'_{IBuOH} \cdot [BuOH] \cdot [BL] \end{aligned} $

351

352 To decrease the correlation between the pre-exponential factor and activation energy
353 and ease the parameter estimation stage, the following modified Arrhenius equation
354 was used [69].

$$355 \quad k_c(T) = \exp \left[\ln(k_c(T_{ref})) + \frac{E_a}{R \cdot T_{ref}} \cdot \left(1 - \frac{T_{ref}}{T} \right) \right] \quad (20)$$

356 where, T_{ref} is a reference temperature which is the average temperature of the
357 experimental matrix (Table 1).

358 The following constants were estimated: $\ln(k_c(T_{ref}))$, $\frac{E_a}{R \cdot T_{ref}}$, Michaelis-Menten and
359 inhibition constants. Michaelis-Menten and inhibition constants were assumed to be
360 temperature independent.

361 The effect of the number of estimated parameters on the models was evaluated via
362 the AIC number standing for Akaike Information Criterion [16,17,70].

$$363 \quad AIC = \text{Number of independant event} \cdot \ln\left(\frac{SSR}{\text{number of independant event}}\right) \\ + 2 \cdot \text{Number of estimated parameters} \quad (21)$$

364

365 *4.3 Modeling results*

366 In the first step, preliminary modeling results showed that some parameters tend to
367 zero. Thus, these parameters were discarded:

368 -For Model 2, K''_W , K_{Lump} and K''_{BL} were discarded in the modeling.

369 -For Model 3, K_{LA} , K''_W and K''_{BL} were not considered.

370 -For Model 4, K_{BuOH} , K_{LA} , K''_W , K''_{BL} , K_{Lump} , K'''_{IIA} , K'_{IBL} , K''_{IW} and K'''_{IBL} and K''_{LA-BL} were
371 neglected.

372 -For Model 5, K''_W , K''_{IW} , K_1 , K_2 , K'_{IBuOH} and K_3 were neglected.

373 -For Model 6, K'_{IBuOH} , K''_{IW} , K''_{BuOH} , K_2 , K'_W and K_3 were neglected.

374 By discarding these parameters, the reduced models are displayed in Table 5. Table
375 6 is a summary of the modeling output for the different models. SSR is the sum of
376 squared residuals, the difference between the experimental and simulated
377 concentrations. AIC values showed that Model 4 is the most probable one (Table 4).
378 Due to space limitation of the journal, the modeling results of the other models are
379 displayed in Supporting Information (S5).

380

381

Table 5. Reduced kinetic models for the esterification of levulinic acid over

382

immobilized enzyme.

Model	Kinetic term	Denominator
Model 1	$k_{\text{Esterification}} \cdot \rho_{\text{Enzyme}}$	1
Model 2	$k_f \cdot \rho_{\text{Enzyme}}$	$[LA] \cdot [BuOH] + K_{BuOH} \cdot [LA]$ $+ K_{LA} \cdot [BuOH]$
Model 3	$k_f \cdot \rho_{\text{Enzyme}}$	$[LA] \cdot [BuOH] + K_{BuOH} \cdot [LA]$ $+ K''_{IBuOH} \cdot [BuOH]^2 + K_{Lump} \cdot [W] \cdot [BL]$
Model 4	$k_f \cdot \rho_{\text{Enzyme}}$	$[LA] \cdot [BuOH] + K''_{LA} \cdot [LA]^2$ $+ K''_{IBuOH} \cdot [BuOH]^2$ $+ K''_{BuOH-W} \cdot [W] \cdot [BuOH]$
Model 5	$k_{LA} \cdot \rho_{\text{Enzyme}}$	$K''_{BuOH} \cdot [BuOH] \cdot [LA]$ $+ [BuOH]$
Model 6	$k_{LA} \cdot \rho_{\text{Enzyme}}$	$K_1 \cdot [LA] + ([BuOH]) \cdot (1 + K''_{siBuOH} \cdot [BuOH])$ $\cdot (1 + K''_{siW} \cdot [W])$

383

384

Table 6. Modeling results for each Model.

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
SSR	15.85	15.16	14.83	13.66	15.48	14.03
Number of estimated parameters	2	4	5	5	3	5
AIC	-2311.47	-2335.50	-2347.36	-2398.81	-2324.11	-2381.86

385

386

387

388 Table 7 shows the estimated values with their confidence intervals. One can notice
389 that the confidence intervals for $\ln(k_c(T_{ref}))$, $\frac{E_a}{R \cdot T_{ref}}$ and
390 K''_{ILA} are small, meaning that the initial operating condition variation was well designed
391 to estimate these parameters. Based on our experimental data, it was not possible to
392 calculate the credible interval for K''_{IBuOH} , the optimum value was 51.52 mol.L⁻¹.

393 Table 7 presents the Normalized parameter covariance matrix for Model 4. According
394 to Toch et al. [71], two parameters are correlated if their binary correlation coefficient
395 is higher than 0.95. From Table 8, one can notice that the estimated parameters are
396 not correlated.

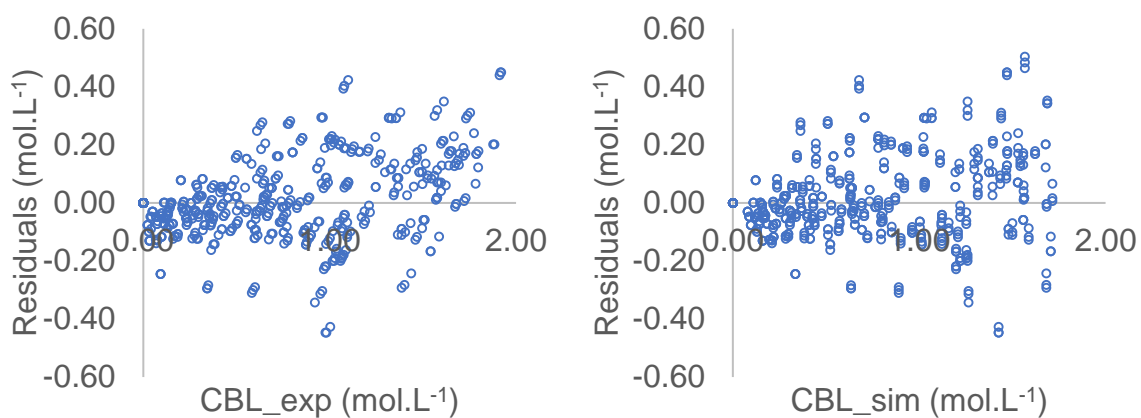
397 Fig. 7 shows that the residuals, $[BL]_{exp,u} - [BL]_{sim,u}$, are randomly distributed versus
398 the experimental concentration of BL ($[BL]_{exp,u}$) and the simulated by Model 4
399 ($[BL]_{sim,u}$). This means that there are no trends pertaining to the errors.

400 Fig. 8 shows the parity plot, and one can notice that Model 4 can predict the
401 experimental data correctly.

402 Figs 9 show the fit of model 4 to some experimental concentrations of BL with the 95%
403 prediction intervals and the mean estimated values. From Figs 9, one can notice that
404 Model 4 fits well the experimental concentrations, and most of the experimental
405 concentrations lie between the intervals. The fact that some experimental
406 concentration points, in the majority at the beginning, are outside the prediction
407 intervals can be because the LA dissociation is not considered in the modeling or the
408 adsorption and inhibition terms were not correctly defined.

409 However, the fit of Model 4 to experimental concentration for experiments carried out
410 with a molar ratio LA/BuOH: 1:1 is lower near to the equilibrium than for the other ratio.

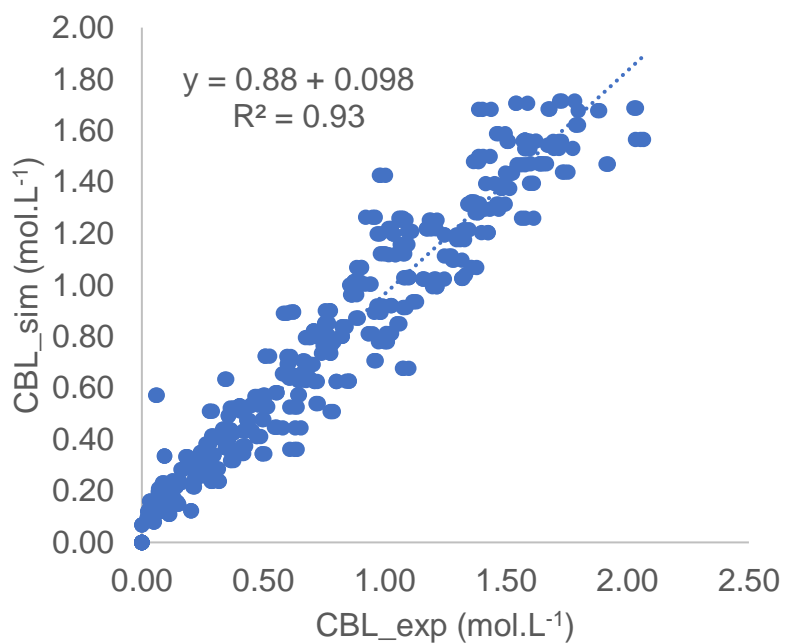
411 This observation is because the equilibrium constant predicted by ePC-SAFT is less
412 reliable for this ratio.



413

414

Fig. 7. Residual plots for Model 4.



415

416

Fig. 8. Parity plot for Model 4.

417

418

419

420

421 Table 7. Estimated values at $T_{ref} = 51^\circ\text{C}$ and statistical data for Model 4.

	Units	Estimates	HPD%
$\ln(k_f(T_{ref}))$	$\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	-2.02	6.25
$\frac{Ea_f}{R \cdot T_{ref}}$	-	11.01	5.56
K''_{ILA}	$\text{mol}\cdot\text{L}^{-1}$	109.80	19.60
K''_{BuOH}	$\text{mol}\cdot\text{L}^{-1}$	51.52	
K''_{BuOH-W}	$\text{L}\cdot\text{mol}^{-1}$	148.65	45.13

422

423 Table 8. Normalized parameter covariance matrix for Model 4.

	$\ln(k_f(T_{ref}))$	$\frac{Ea_f}{R \cdot T_{ref}}$	K''_{ILA}	K''_{BuOH-W}
$\ln(k_f(T_{ref}))$	1			
$\frac{Ea_f}{R \cdot T_{ref}}$	0.1	1		
K''_{ILA}	0.69	0.09	1	
K''_{BuOH-W}	0.93	0.12	0.53	1

424

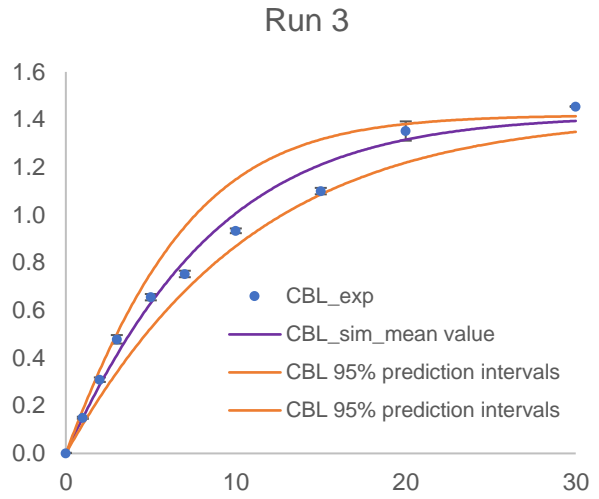
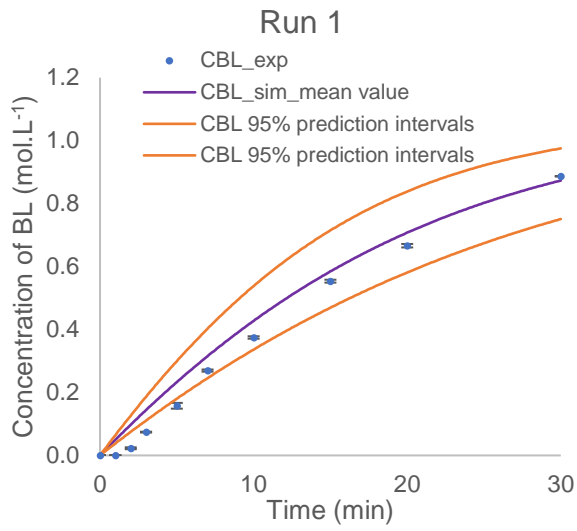
425

426

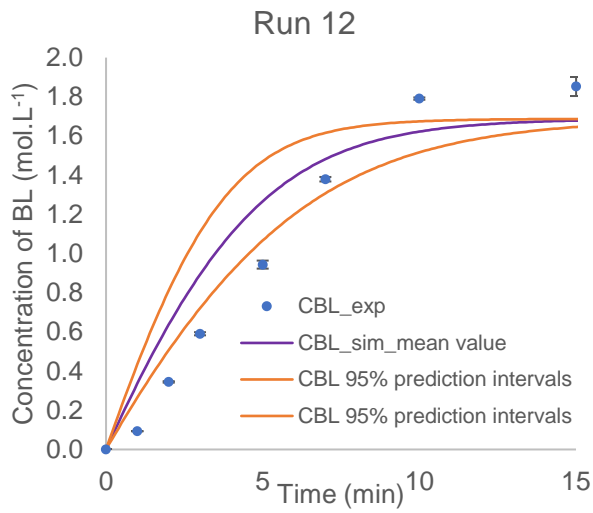
427

428

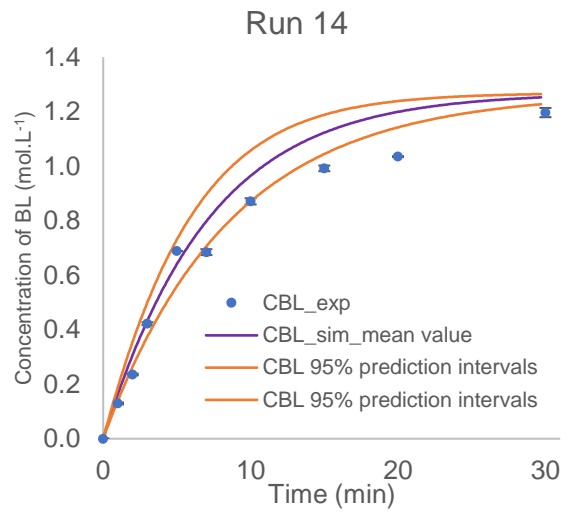
429



430

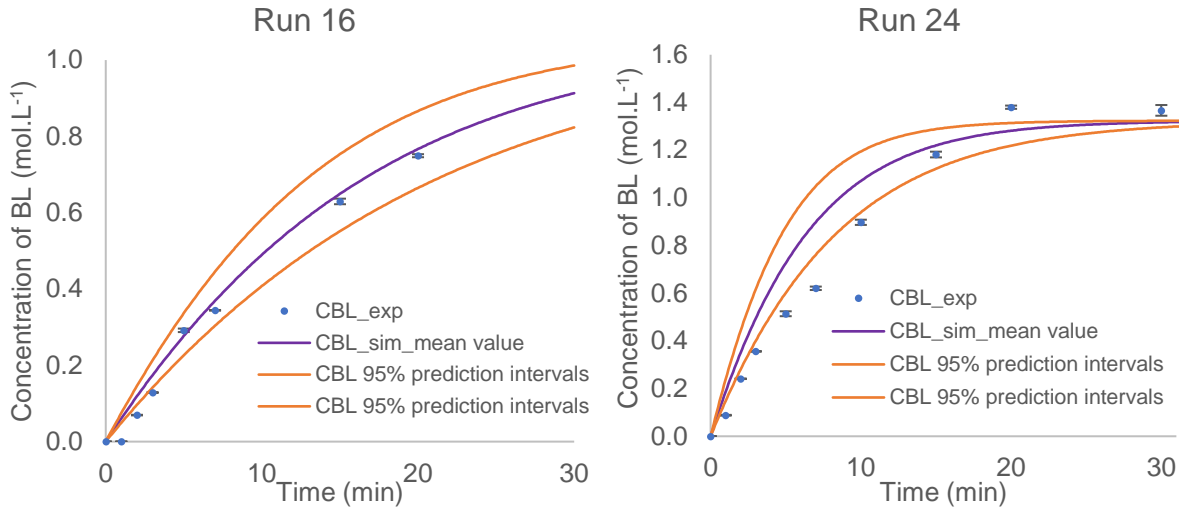


431



432

433



434

435

436

437

438

439

440

Fig. 9. Fit of Model 4 to the experimental concentrations with 95% prediction intervals: experimental concentration of BL (blue circle), error bars (black), simulated concentration of BL using the mean estimated value from Table 7 (purple line), simulated concentration of BL using the estimated values at the extreme of the confidence intervals from Table 7 (orange lines).

441 **5. Conclusions**

442 The synthesis of butyl levulinate from the esterification of levulinic acid over
443 Novozym®435, an immobilized enzyme, was investigated in microfluidic technology in
444 isothermal conditions. The enzyme's catalytic activity was found to be stable for 400
445 minutes. The internal and external mass transfer resistance was found to be negligible.
446 Thus, a plug-flow model was used to estimate the kinetic constants.

447 Several kinetic experiments were performed by varying the reaction temperature from
448 20 to 80 °C, inlet LA concentration from 1.74 to 5.09 mol.L⁻¹, inlet butanol concentration
449 from 5.09 to 9.07 mol.L⁻¹, mass of dried enzyme from 50 to 150 mg and residence time
450 from 1 to 30 minutes.

451 The equilibrium constants were evaluated via the ePC-SAFT equation of state.

452 We evaluated 6 kinetic models based on power law, the classical Ping-Pong Bi-Bi
453 mechanism and the modified one developed by Mitchell and Krieger. Based on the
454 Akaike information criterion, we found that the classical Ping-Pong model, including
455 the inhibition mechanism by butanol and levulinic acid, can fit the experimental
456 concentrations properly. This model can reasonably predict the experimental
457 concentration by considering the temperature effect on the rate constant.

458

459 **Declaration of Competing Interest**

460 The authors declare that they have no known competing financial interests or personal
461 relationships that could have appeared to influence the work reported in this paper.

462 **Acknowledgments**

463 The authors thank INSA Rouen Normandy, University of Rouen Normandy, the Centre
464 National de la Recherche Scientifique (CNRS), European Regional Development Fund
465 (ERDF) N° HN0001343, Labex SynOrg (ANR-11-LABX-0029), Carnot Institute I2C, the
466 graduate school for reasearch XL-Chem (ANR-18-EURE-0020 XL CHEM) and Region
467 Normandie for their support. This research was funded, in whole or in part, by the ANR
468 (French National Research Agency) and the DFG (German Research Foundation)
469 through the project MUST (MicroflUidics for Structure-reactivity relationships aided by
470 Thermodynamics & kinetics) [ANR-20-CE92-0002 & Project number 446436621].

471

472 **References**

- 473 [1] M.S. Singhvi, D. V. Gokhale, Lignocellulosic biomass: Hurdles and challenges
474 in its valorization, *Appl. Microbiol. Biotechnol.* 103 (2019) 9305–9320.
475 <https://doi.org/10.1007/s00253-019-10212-7>.
- 476 [2] Y.N. Guragain, P. V. Vadlani, Renewable Biomass Utilization: A Way Forward
477 to Establish Sustainable Chemical and Processing Industries, *Clean Technol.* 3
478 (2021) 243–259. <https://doi.org/10.3390/cleantechnol3010014>.
- 479 [3] P. Zhu, O.Y. Abdelaziz, C.P. Hulteberg, A. Riisager, New synthetic approaches
480 to biofuels from lignocellulosic biomass, *Curr. Opin. Green Sustain. Chem.* 21
481 (2020) 16–21. <https://doi.org/10.1016/j.cogsc.2019.08.005>.
- 482 [4] X. Lu, L. Lagerquist, K. Eränen, J. Hemming, P. Eklund, L. Estel, S. Leveneur,
483 H. Grénman, Reductive Catalytic Depolymerization of Semi-industrial Wood-
484 Based Lignin, *Ind. Eng. Chem. Res.* (2021) [acs.iecr.1c03154](https://doi.org/10.1021/acs.iecr.1c03154).
485 <https://doi.org/10.1021/acs.iecr.1c03154>.
- 486 [5] W. Schutyser, T. Renders, S. Van Den Bosch, S.F. Koelewijn, G.T. Beckham,
487 B.F. Sels, Chemicals from lignin: an interplay of lignocellulose fractionation,
488 depolymerisation, and upgrading, *Chem. Soc. Rev.* 47 (2018) 852–908.
489 <https://doi.org/10.1039/C7CS00566K>.
- 490 [6] R. Rinaldi, R. Jastrzebski, M.T. Clough, J. Ralph, M. Kennema, P.C.A.
491 Bruijninx, B.M. Weckhuysen, Paving the Way for Lignin Valorisation: Recent
492 Advances in Bioengineering, Biorefining and Catalysis, *Angew. Chemie Int. Ed.*
493 55 (2016) 8164–8215. <https://doi.org/10.1002/ANIE.201510351>.
- 494 [7] K.C. Badgujar, V.C. Badgujar, B.M. Bhanage, A review on catalytic synthesis of

- 495 energy rich fuel additive levulinate compounds from biomass derived levulinic
496 acid, *Fuel Process. Technol.* 197 (2020) 106213.
497 <https://doi.org/10.1016/j.fuproc.2019.106213>.
- 498 [8] D. Di Menno Di Bucchianico, Y. Wang, J.-C. Buvat, Y. Pan, V. Casson Moreno,
499 S. Leveneur, Production of levulinic acid and alkyl levulinates: a process
500 insight, *Green Chem.* 24 (2022) 614–646. <https://doi.org/10.1039/d1gc02457d>.
- 501 [9] A. Démolis, N. Essayem, F. Rataboul, Synthesis and applications of alkyl
502 levulinates, *ACS Sustain. Chem. Eng.* 2 (2014) 1338–1352.
503 <https://doi.org/10.1021/sc500082n>.
- 504 [10] L. Yan, Q. Yao, Y. Fu, Conversion of levulinic acid and alkyl levulinates into
505 biofuels and high-value chemicals, *Green Chem.* 19 (2017) 5527–5547.
506 <https://doi.org/10.1039/c7gc02503c>.
- 507 [11] Y. Wang, M. Cipolletta, L. Vernières-Hassimi, V. Casson-Moreno, S. Leveneur,
508 Application of the concept of Linear Free Energy Relationships to the
509 hydrogenation of levulinic acid and its corresponding esters, *Chem. Eng. J.* 374
510 (2019) 822–831. <https://doi.org/10.1016/j.cej.2019.05.218>.
- 511 [12] S. Capecchi, Y. Wang, V. Casson Moreno, C. Held, S. Leveneur, Solvent effect
512 on the kinetics of the hydrogenation of n-butyl levulinate to γ -valerolactone,
513 *Chem. Eng. Sci.* 231 (2021) 116315.
514 <https://doi.org/10.1016/j.ces.2020.116315>.
- 515 [13] D.M. Alonso, S.G. Wettstein, J.A. Dumesic, Gamma-valerolactone, a
516 sustainable platform molecule derived from lignocellulosic biomass, *Green*
517 *Chem.* 15 (2013) 584–595. <https://doi.org/10.1039/C3GC37065H>.

- 518 [14] L. Negahdar, M.G. Al-Shaal, F.J. Holzhäuser, R. Palkovits, Kinetic analysis of
519 the catalytic hydrogenation of alkyl levulinates to γ -valerolactone, *Chem. Eng.*
520 *Sci.* (2017). <https://doi.org/10.1016/j.ces.2016.11.007>.
- 521 [15] W.R.H. Wright, R. Palkovits, Development of heterogeneous catalysts for the
522 conversion of levulinic acid to γ -valerolactone, *ChemSusChem*. 5 (2012) 1657–
523 1667. <https://doi.org/10.1002/cssc.201200111>.
- 524 [16] S. Capecchi, Y. Wang, J. Delgado, V. Casson Moreno, M. Mignot, H. Grénman,
525 D.Y. Murzin, S. Leveneur, Bayesian Statistics to Elucidate the Kinetics of γ -
526 Valerolactone from n-Butyl Levulinate Hydrogenation over Ru/C, *Ind. Eng.*
527 *Chem. Res.* 60 (2021) 11725–11736. <https://doi.org/10.1021/acs.iecr.1c02107>.
- 528 [17] J. Delgado, W.N. Vasquez Salcedo, G. Bronzetti, V. Casson Moreno, M.
529 Mignot, J. Legros, C. Held, H. Grénman, S. Leveneur, Kinetic model
530 assessment for the synthesis of γ -valerolactone from n-butyl levulinate and
531 levulinic acid hydrogenation over the synergy effect of dual catalysts Ru/C and
532 Amberlite IR-120, *Chem. Eng. J.* 430 (2022) 133053.
533 <https://doi.org/10.1016/j.ces.2021.133053>.
- 534 [18] L. Peng, X. Gao, K. Chen, Catalytic upgrading of renewable furfuryl alcohol to
535 alkyl levulinates using $AlCl_3$ as a facile, efficient, and reusable catalyst, *Fuel*.
536 160 (2015) 123–131. <https://doi.org/10.1016/J.FUEL.2015.07.086>.
- 537 [19] A.F. Peixoto, R. Ramos, M.M. Moreira, O.S.G.P. Soares, L.S. Ribeiro, M.F.R.
538 Pereira, C. Delerue-Matos, C. Freire, Production of ethyl levulinate fuel
539 bioadditive from 5-hydroxymethylfurfural over sulfonic acid functionalized
540 biochar catalysts, *Fuel*. 303 (2021) 121227.
541 <https://doi.org/10.1016/J.FUEL.2021.121227>.

- 542 [20] E. Christensen, A. Williams, S. Paul, S. Burton, R.L. McCormick, Properties
543 and Performance of Levulinate Esters as Diesel Blend Components, *Energy*
544 and *Fuels*. 25 (2011) 5422–5428. <https://doi.org/10.1021/EF201229J>.
- 545 [21] S. Frigo, G. Pasini, G. Caposciutti, M. Antonelli, A.M.R. Galletti, S. Gori, R.
546 Costi, L. Arnone, Utilisation of advanced biofuel in CI internal combustion
547 engine, *Fuel*. 297 (2021) 120742. <https://doi.org/10.1016/J.FUEL.2021.120742>.
- 548 [22] D. Di Menno Di Bucchianico, J.C. Buvat, M. Mignot, V. Casson Moreno, S.
549 Leveneur, Role of solvent the production of butyl levulinate from fructose, *Fuel*.
550 318 (2022) 123703. <https://doi.org/10.1016/j.fuel.2022.123703>.
- 551 [23] A. Harwardt, K. Kraemer, B. Rüngeler, W. Marquardt, Conceptual Design of a
552 Butyl-levulinate Reactive Distillation Process by Incremental Refinement,
553 *Chinese J. Chem. Eng.* 19 (2011) 371–379. [https://doi.org/10.1016/S1004-](https://doi.org/10.1016/S1004-9541(09)60223-8)
554 [9541\(09\)60223-8](https://doi.org/10.1016/S1004-9541(09)60223-8).
- 555 [24] V. Russo, R. Tesser, C. Rossano, T. Cogliano, R. Vitiello, S. Leveneur, M. Di
556 Serio, Kinetic study of Amberlite IR120 catalyzed acid esterification of levulinic
557 acid with ethanol: From batch to continuous operation, *Chem. Eng. J.* 401
558 (2020) 126126. <https://doi.org/10.1016/J.CEJ.2020.126126>.
- 559 [25] M.A. Tejero, E. Ramírez, C. Fité, J. Tejero, F. Cunill, Esterification of levulinic
560 acid with butanol over ion exchange resins, *Appl. Catal. A Gen.* 517 (2016) 56–
561 66. <https://doi.org/10.1016/J.APCATA.2016.02.032>.
- 562 [26] A.F. Peixoto, S.M. Silva, P. Costa, A.C. Santos, B. Valentim, J.M. Lázaro-
563 Martínez, C. Freire, Acid functionalized coal fly ashes: New solid catalysts for
564 levulinic acid esterification, *Catal. Today*. 357 (2020) 74–83.
565 <https://doi.org/10.1016/j.cattod.2019.07.038>.

- 566 [27] S. Dharne, V. V. Bokade, Esterification of levulinic acid to n-butyl levulinate
567 over heteropolyacid supported on acid-treated clay, *J. Nat. Gas Chem.* 20
568 (2011) 18–24. [https://doi.org/10.1016/S1003-9953\(10\)60147-8](https://doi.org/10.1016/S1003-9953(10)60147-8).
- 569 [28] K.Y. Nandiwale, V. V. Bokade, Esterification of Renewable Levulinic Acid to n-
570 Butyl Levulinate over Modified H-ZSM-5, *Chem. Eng. Technol.* 38 (2015) 246–
571 252. <https://doi.org/10.1002/ceat.201400326>.
- 572 [29] K.Y. Nandiwale, V. V. Bokade, Environmentally benign catalytic process for
573 esterification of renewable levulinic acid to various alkyl levulinates biodiesel,
574 *Environ. Prog. Sustain. Energy.* 34 (2015) 795–801.
575 <https://doi.org/10.1002/ep.12042>.
- 576 [30] K.C. Maheria, J. Kozinski, A. Dalai, Esterification of Levulinic Acid to n-Butyl
577 Levulinate Over Various Acidic Zeolites, *Catal. Lett.* 2013 14311. 143 (2013)
578 1220–1225. <https://doi.org/10.1007/S10562-013-1041-3>.
- 579 [31] M. Song, X. Di, Y. Zhang, Y. Sun, Z. Wang, Z. Yuan, Y. Guo, The effect of
580 enzyme loading, alcohol/acid ratio and temperature on the enzymatic
581 esterification of levulinic acid with methanol for methyl levulinate production: A
582 kinetic study, *RSC Adv.* 11 (2021) 15054–15059.
583 <https://doi.org/10.1039/d1ra01780b>.
- 584 [32] G.D. Yadav, I. V. Borkar, Kinetic Modeling of Immobilized Lipase Catalysis in
585 Synthesis of n-Butyl Levulinate†, *Ind. Eng. Chem. Res.* 47 (2008) 3358–3363.
586 <https://doi.org/10.1021/IE800193F>.
- 587 [33] V.N. Emel'yanenko, E. Altuntepe, C. Held, A.A. Pimerzin, S.P. Verevkin,
588 Renewable platform chemicals: Thermochemical study of levulinic acid esters,
589 *Thermochim. Acta.* 659 (2018) 213–221.

- 590 <https://doi.org/10.1016/j.tca.2017.12.006>.
- 591 [34] K. V. Bhavsar, G.D. Yadav, n-Butyl levulinate synthesis using lipase catalysis:
592 comparison of batch reactor versus continuous flow packed bed tubular
593 microreactor, *J. Flow Chem.* 8 (2018) 97–105. [https://doi.org/10.1007/s41981-](https://doi.org/10.1007/s41981-018-0014-5)
594 [018-0014-5](https://doi.org/10.1007/s41981-018-0014-5).
- 595 [35] L. Zhou, Y. He, L. Ma, Y. Jiang, Z. Huang, L. Yin, J. Gao, Conversion of
596 levulinic acid into alkyl levulinates: Using lipase immobilized on meso-molding
597 three-dimensional macroporous organosilica as catalyst, *Bioresour. Technol.*
598 247 (2018) 568–575. <https://doi.org/10.1016/J.BIORTECH.2017.08.134>.
- 599 [36] S. Zhai, L. Zhang, X. Zhao, Q. Wang, Y. Yan, C. Li, X. Zhang, Enzymatic
600 synthesis of a novel solid–liquid phase change energy storage material based
601 on levulinic acid and 1,4-butanediol, *Bioresour. Bioprocess.* 9 (2022) 1–10.
602 <https://doi.org/10.1186/s40643-022-00502-w>.
- 603 [37] K.C. Badgujar, V.C. Badgujar, B.M. Bhanage, Lipase as a green and
604 sustainable material for production of levulinate compounds: State of the art,
605 *Mater. Sci. Energy Technol.* (2022). <https://doi.org/10.1016/j.mset.2022.02.005>.
- 606 [38] R. Gérardy, D.P. Debecker, J. Estager, P. Luis, J.C.M. Monbaliu, Continuous
607 Flow Upgrading of Selected C2–C6 Platform Chemicals Derived from Biomass,
608 *Chem. Rev.* 120 (2020) 7219–7347.
609 <https://doi.org/10.1021/ACS.CHEMREV.9B00846>.
- 610 [39] H. Zhang, Y. Bai, N. Zhu, J. Xu, Microfluidic reactor with immobilized enzyme-
611 from construction to applications: A review, *Chinese J. Chem. Eng.* 30 (2021)
612 136–145. <https://doi.org/10.1016/J.CJCHE.2020.12.011>.

- 613 [40] C.J. Taylor, J.A. Manson, G. Clemens, B.A. Taylor, T.W. Chamberlain, R.A.
614 Bourne, Modern advancements in continuous-flow aided kinetic analysis,
615 *React. Chem. Eng.* (2022). <https://doi.org/10.1039/d1re00467k>.
- 616 [41] H. Hajifatheali, E. Ahmadi, A. Wojtczak, Z. Jaglicic, The synthesis of N-
617 methylbis[2-(dodecylthio)ethyl]amine (SNS) and investigation of its efficiency
618 as new mononuclear catalyst complex in copper-based ATRP, *Macromol. Res.*
619 2015 2311. 23 (2015) 977–985. <https://doi.org/10.1007/S13233-015-3132-Z>.
- 620 [42] L.K. Doraiswamy, D.G. Tajbl, *Laboratory Catalytic Reactors*, *Catal. Rev.* 10
621 (1974) 177–219. <https://doi.org/10.1080/01614947408079629>.
- 622 [43] J.-M. Commenge, M. Saber, L. Falk, Methodology for multi-scale design of
623 isothermal laminar flow networks, *Chem. Eng. J.* 173 (2011) 541–551.
624 <https://doi.org/10.1016/J.CEJ.2011.07.060>.
- 625 [44] A.A. Hassankiadeh, *Dynamic Assessment and Optimization of Catalytic*
626 *Hydroprocessing Process: Sensitivity Analysis and Practical Tips*, University of
627 Newfoundland, 2021.
- 628 [45] S. Leveneur, J. Wärnå, K. Eränen, T. Salmi, Green process technology for
629 peroxy-carboxylic acids: Estimation of kinetic and dispersion parameters aided
630 by RTD measurements: Green synthesis of peroxy-carboxylic acids, *Chem.*
631 *Eng. Sci.* 66 (2011) 1038–1050. <https://doi.org/10.1016/j.ces.2010.12.005>.
- 632 [46] J. Villiermaux, *Génie de la réaction chimique*, Technip, Tec & Doc Lavoisier,
633 Paris, 1993.
634 <http://www.lavoisier.fr/livre/notice.asp?depuis=e.lavoisier.fr&id=978285206759>
635 2.

- 636 [47] M. Ravelo, M. Wojtusik, M. Ladero, F. García-Ochoa, Synthesis of ibuprofen
637 monoglyceride in solventless medium with novozym®435: Kinetic analysis,
638 Catalysts. 10 (2020) 76. <https://doi.org/10.3390/catal10010076>.
- 639 [48] C.R. Wilke, P. Chang, Correlation of diffusion coefficients in dilute solutions,
640 AIChE J. 1 (1955) 264–270. <https://doi.org/10.1002/aic.690010222>.
- 641 [49] H. Ariba, Y. Wang, C. Devouge-Boyer, R.P. Stateva, S. Leveneur,
642 Physicochemical Properties for the Reaction Systems: Levulinic Acid, Its
643 Esters, and γ -Valerolactone, J. Chem. Eng. Data. 65 (2020) 3008–3020.
644 <https://doi.org/10.1021/acs.jced.9b00965>.
- 645 [50] D.M. Chesterfield, P.L. Rogers, E.O. Al-Zaini, A.A. Adesina, Production of
646 biodiesel via ethanolysis of waste cooking oil using immobilised lipase, Chem.
647 Eng. J. 207–208 (2012) 701–710. <https://doi.org/10.1016/J.CEJ.2012.07.039>.
- 648 [51] C. Ortiz, M.L. Ferreira, O. Barbosa, J.C.S. Dos Santos, R.C. Rodrigues, Á.
649 Berenguer-Murcia, L.E. Briand, R. Fernandez-Lafuente, Novozym 435: The
650 “perfect” lipase immobilized biocatalyst?, Catal. Sci. Technol. 9 (2019) 2380–
651 2420. <https://doi.org/10.1039/c9cy00415g>.
- 652 [52] D. Kowalczykiewicz, K. Szymańska, D. Gillner, A.B. Jarzębski, Rotating bed
653 reactor packed with heterofunctional structured silica-supported lipase.
654 Developing an effective system for the organic solvent and aqueous phase
655 reactions, Microporous Mesoporous Mater. 312 (2021) 110789.
656 <https://doi.org/10.1016/j.micromeso.2020.110789>.
- 657 [53] H. Zhao, Z. Song, Migration of reactive trace compounds from Novozym® 435
658 into organic solvents and ionic liquids, Biochem. Eng. J. 49 (2010) 113–118.
659 <https://doi.org/10.1016/j.bej.2009.12.004>.

- 660 [54] J. Gross, G. Sadowski, Perturbed-Chain SAFT: An Equation of State Based on
661 a Perturbation Theory for Chain Molecules, *Ind. Eng. Chem. Res.* 40 (2001)
662 1244–1260. <https://doi.org/10.1021/IE0003887>.
- 663 [55] M. Lemberg, G. Sadowski, Predicting the Solvent Effect on Esterification
664 Kinetics, *ChemPhysChem.* 18 (2017) 1977–1980.
665 <https://doi.org/10.1002/cphc.201700507>.
- 666 [56] E. Altuntepe, V.N. Emel'yanenko, M. Forster-Rotgers, G. Sadowski, S.P.
667 Verevkin, C. Held, Thermodynamics of enzyme-catalyzed esterifications: II.
668 Levulinic acid esterification with short-chain alcohols, *Appl. Microbiol.*
669 *Biotechnol.* 101 (2017) 7509–7521. [https://doi.org/10.1007/s00253-017-8481-](https://doi.org/10.1007/s00253-017-8481-4)
670 4.
- 671 [57] C. Held, G. Sadowski, Thermodynamics of Bioreactions, *Annu. Rev. Chem.*
672 *Biomol. Eng.* 7 (2016) 395–414. [https://doi.org/10.1146/annurev-chembioeng-](https://doi.org/10.1146/annurev-chembioeng-080615-034704)
673 080615-034704.
- 674 [58] F. Tumakaka, G. Sadowski, Application of the Perturbed-Chain SAFT equation
675 of state to polar systems, in: *Fluid Phase Equilib.*, American Chemical Society,
676 2004: pp. 233–239. <https://doi.org/10.1016/j.fluid.2002.12.002>.
- 677 [59] D. Fuchs, J. Fischer, F. Tumakaka, G. Sadowski, Solubility of Amino Acids:
678 Influence of the pH value and the Addition of Alcoholic Cosolvents on Aqueous
679 Solubility, *Ind. Eng. Chem. Res.* 45 (2006) 6578–6584.
680 <https://doi.org/10.1021/IE0602097>.
- 681 [60] G.D. Yadav, S. V. Pawar, Synergism between microwave irradiation and
682 enzyme catalysis in transesterification of ethyl-3-phenylpropanoate with n-
683 butanol, *Bioresour. Technol.* 109 (2012) 1–6.

- 684 <https://doi.org/10.1016/j.biortech.2012.01.030>.
- 685 [61] S.R. Bansode, M.A. Hardikar, V.K. Rathod, Evaluation of reaction parameters
686 and kinetic modelling for Novozym 435 catalysed synthesis of isoamyl butyrate,
687 J. Chem. Technol. Biotechnol. 92 (2017) 1306–1314.
688 <https://doi.org/10.1002/jctb.5125>.
- 689 [62] S.A. Zulkeflee, S.A. Sata, F.S. Rohman, N. Aziz, Modelling of immobilized
690 *Candida rugosa* lipase catalysed esterification process in batch reactor
691 equipped with temperature and water activity control system, Biochem. Eng. J.
692 161 (2020) 107669. <https://doi.org/10.1016/j.bej.2020.107669>.
- 693 [63] D.A. Mitchell, N. Krieger, Looking through a new lens: Expressing the Ping
694 Pong bi bi equation in terms of specificity constants, Biochem. Eng. J. 178
695 (2022) 108276. <https://doi.org/10.1016/j.bej.2021.108276>.
- 696 [64] C.G. Lopresto, V. Calabrò, J.M. Woodley, P. Tufvesson, Kinetic study on the
697 enzymatic esterification of octanoic acid and hexanol by immobilized *Candida*
698 *antarctica* lipase B, J. Mol. Catal. B Enzym. 110 (2014) 64–71.
699 <https://doi.org/10.1016/j.molcatb.2014.09.011>.
- 700 [65] M.N. Varma, G. Madras, Kinetics of synthesis of butyl butyrate by esterification
701 and transesterification in supercritical carbon dioxide, J. Chem. Technol.
702 Biotechnol. 83 (2008) 1135–1144. <https://doi.org/10.1002/jctb.1897>.
- 703 [66] M. Rizzi, P. Stylos, A. Riek, M. Reuss, A kinetic study of immobilized lipase
704 catalysing the synthesis of isoamyl acetate by transesterification in n-hexane,
705 Enzyme Microb. Technol. 14 (1992) 709–714. [https://doi.org/10.1016/0141-](https://doi.org/10.1016/0141-0229(92)90110-A)
706 [0229\(92\)90110-A](https://doi.org/10.1016/0141-0229(92)90110-A).

- 707 [67] M. Caracotsios, W.E. Stewart, Sensitivity analysis of initial value problems with
708 mixed odes and algebraic equations, *Comput. Chem. Eng.* 9 (1985) 359–365.
709 [https://doi.org/10.1016/0098-1354\(85\)85014-6](https://doi.org/10.1016/0098-1354(85)85014-6).
- 710 [68] W.E. Stewart, M. Caracotsios, *Computer-Aided Modeling of Reactive Systems*,
711 First, New Jersey, 2008. <https://doi.org/10.1002/9780470282038>.
- 712 [69] G. Buzzi-Ferraris, Planning of experiments and kinetic analysis, *Catal. Today*.
713 52 (1999) 125–132. [https://doi.org/10.1016/S0920-5861\(99\)00070-X](https://doi.org/10.1016/S0920-5861(99)00070-X).
- 714 [70] M.A. McDonald, L. Bromig, M.A. Grover, R.W. Rousseau, A.S. Bommarius,
715 Kinetic model discrimination of penicillin G acylase thermal deactivation by
716 non-isothermal continuous activity assay, *Chem. Eng. Sci.* 187 (2018) 79–86.
717 <https://doi.org/10.1016/j.ces.2018.04.046>.
- 718 [71] K. Toch, J.W. Thybaut, G.B. Marin, A systematic methodology for kinetic
719 modeling of chemical reactions applied to n-hexane hydroisomerization, *AIChE*
720 *J.* 61 (2015) 880–892. <https://doi.org/10.1002/aic.14680>.
- 721
- 722