Graphic abstract

**Improved production of propionic acid driven by hydrolyzed liquid
containing high concentration of L-lactic acid from co-fermentation of
food waste and sludge**

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18 **Abstract**

19 This study investigated the feasibility of improved production propionic
20 acid-enriched volatile fatty acid (VFA) from high concentration (C_s) of food waste and
21 waste activated sludge (WAS) via lactic acid pathway by using of *Propionibacterium*
22 *acidipropionici*. It was observed that production of L-lactate overwhelmed to
23 D-lactate at first stage, which improved from 3.21 to 35.45 g COD/L with increase of
24 substrate C_s . However, kinetic model analysis indicated that *P. acidipropionici*
25 growth rate $\mu_{\max}$ was decreased with increase of L-lactate concentration, which
26 explained second stage free cell fermentation of propionic acid was inhibited when fed
27 by first stage liquid from R-40, R-55 and R-70. Then, the fibrous bed bioreactor was
28 employed to eliminate the feed inhibition. The maximal percentage of propionic acid
29 (68.3%) and production (16.31 g COD/L) was obtained by feeding liquid of R-55,
30 which was improved by 3.33 folds compared to the free cell fermentation.

31 **Key Word:** propionic acid; L-lactic acid; feed inhibition, kinetic model, fibrous bed
32 bioreactor.

33

34 1. Introduction

35 Propionic acid-enriched volatile fatty acid (VFA) derived from waste activated
36 sludge (WAS) fermentation is a preferable carbon for biological nutrient removal (BNR)
37 (Ji & Chen, 2010; Li et al., 2011). The bioconversion from organic waste is a
38 promising alternative way due to its renewable raw sources and the overall increasing
39 consumer demand in the future (Lin et al., 2013; Okamura et al., 2016; Sambusiti et al.,
40 2016). Although it has been extensively studied that the acetic acid-containing VFA
41 could be produced from organic wastes (Karthikeyan et al., 2016; Yuan et al., 2016),
42 few method, till now, was available to improve propionic acid fraction in VFA by
43 fermentation organic waste, and the percentage of propionic acid to VFA was only
44 approximately 48% (Chen et al., 2013b; Luo et al., 2015).

45 Previously, we had introduced the two-stage fermentation method for significantly
46 improving the propionic acid fraction in VFA (68.4%) derived from WAS by using of
47 food waste and *Propionibacterium acidipropionici* (Chen et al., 2013a): in the first stage
48 mixture of WAS and food waste was fermented for lactic acid accumulation, and then in
49 the second stage *P. acidipropionici* was inoculated to the first stage liquid containing
50 lactic acid to produce propionic acid-enriched VFA. Lactic acid was a crucial
51 intermediate in the first stage liquid, which was utilized for propionic acid production in
52 the second stage (Li et al., 2014). However, in previous work, organic waste substrate
53 was diluted by tap water to make up concentration around 25 g/L (Chen et al., 2013a; Li
54 et al., 2014; Li et al., 2013), which inevitably limited the final concentration of
55 propionic acid. Since high organic loading rate (C_s) has advantages of reducing

56 working volume and increasing amount of fermentative substrate (Liu et al., 2016), it is
57 unclear the impact of high C_s substrate on two-stage fermentation performance.

58 Lately, we found that large amount of lactic acid with two optical isomers, i.e. L-
59 and D-lactic acid, was generated in the first stage (Li et al., 2015). It can be speculated
60 from the proposed metabolism pathway that improving initial C_s could enhance the
61 generation of L- and D-lactic acid, which might impact on the second stage propionic
62 acid production. However, no any reference is available presently regarding the effect
63 of high C_s in the first stage on optical purity of lactic acid. And the effect of two
64 enantiomers ratio of L- and D- lactic acid on *P. acidipropionici* fermentation remained
65 largely unknown.

66 It was observed that improperly increasing the substrate concentration normally
67 resulted in negatively influence on fermentation efficiency (Liu et al., 2016; Zhang et al.,
68 2015). Fibrous bed bioreactor (FBB), packed in a fibrous matrix, was reported to
69 immobilize high cell density, and therefore can enhance the endurance capability of
70 strain to high concentration of substrate (such as glucose and glycerol) as well as the
71 end product (Bradfield & Nicol, 2016; Tsai et al., 2016; Xu et al., 2014). However,
72 there are free reports on the use of FBB to convert high C_s of food waste and sludge
73 hydrolyzed liquid to propionic acid.

74 Therefore, this paper aims on investigating the feasibility of improved production
75 propionic acid-enriched VFA from high concentration of initial substrate. First, the
76 effect of various C_s on the L- and D-lactic acid generation in the first stage was first
77 explored with mechanism study. Then, the performance of the second stage driven by

78 first stage hydrolyzed liquid was evaluated. The impact of L-lactic acid concentration
79 on propionic acid production was studied via kinetic model analysis. Furthermore,
80 influence of synthetic optical activity (OA) of L-lactic acid on propionic acid
81 fermentation was elaborated. Finally, the feasibility of using FBB for propionic acid
82 production from hydrolyzed liquid was testified.

83 2. Material and methods

84 2.1. First stage fermentation at various initial C_s

85 To evaluate the impact of initial C_s on two-stage fermentation, it is essential to first
86 unveil its influence on first stage fermentation results. Five anaerobic fermentation
87 reactors (working volume of 3.0 L and mechanically stirred at 100 rpm) containing
88 different initial C_s of mixture food waste and sludge in volatile solids ratio of $5.45 \pm$
89 $0.11:1$. The mixture were prepared as: R-10 (10.5 ± 0.7 g COD/L), R-25 (25.7 ± 1.1),
90 R-40 (38.9 ± 1.6), R-55 (54.2 ± 2.3), R-70 (68.3 ± 2.4). The above reactors were
91 mechanically stirred for 7 d at 120 rpm and maintained at ambient temperature (21 ± 1
92 $^{\circ}\text{C}$). The pH was adjusted to 8.2 ± 0.4 using 5 M NaOH or 5 M HCl according to the
93 optimal parameter (Chen et al., 2013a). L- and D-lactic acid were recorded daily. To
94 evaluate the reason of overwhelming production of L-lactate, the enzymes responsible
95 for L- and D-lactic acid production (i.e., NAD-dependent L-lactate dehydrogenase
96 (L-LDH) and NAD-dependent D-lactate dehydrogenases (D-LDH) were respectively
97 assayed accordingly (Li et al., 2015). Meanwhile, protein, polysaccharide and key
98 hydrolysis enzymes (protease and α -glucosidase) were tested to evaluate the efficiency
99 of hydrolysis. Other fermentative intermediates, like ethanol, monosaccharide, VFA,

100 ammonium and soluble ortho-phosphate (SOP) were also measured. The fluorescence
101 in situ hybridization (FISH) technique with 16S rRNA-targeted oligonucleotide probes
102 was employed to disclose the L- and D-lactic acid bacteria community.

103 2.2. Second stage fermentation for propionic acid production

104 *Propionibacterium acidipropionici* ATCC (American Type Culture Collection) 4875
105 used in the second stage was purchased from ATCC collection. Five batch
106 experiments for second stage free cell fermentation using *P. acidipropionici* for
107 propionic acid production were conducted accordingly. Batch Exp.1 was fed with first
108 stage hydrolyzed liquid from R-10 to R-70. To reveal the inhibitory intermediates
109 effect of the first stage hydrolyzed liquid on propionic acid production, Batch Exp.2 was
110 conducted in several serum bottles containing individual synthetic intermediate
111 including bovine serum albumin (BAS), dextran (D), ethanol (E), VFA, lactic acid (LA),
112 NH₄⁺-N (N⁺) and SOP (P⁺). Batch Exp.3 was to explain the influence of lactic acid
113 concentration on propionic acid fermentation, which was further analyzed by kinetic
114 model. Batch Exp.4 was to assess the influence of various OA of L-lactic acid on
115 propionic acid fermentation. To evaluate cells density on high concentration of feed
116 lactic acid tolerance, Batch Exp.5 was conducted using different concentrated cell
117 inoculums.

118 2.4. Immobilization fermentation by fibrous bed bioreactor

119 Based on the prototype of two-stage fermentation, fibrous bed bioreactor (FBB) was
120 introduced to replace the free cell fermentation in the second stage. As shown in Fig.
121 1, the FBB comprises of a jacketed glass column (0.5 m ×Φ 8 cm, working volume 2.5

122 L) packed with a spiral wound fibrous matrix (~4 mm thickness) and stainless steel mesh,
123 which could provide large void volume for cell attachment. A recycle loop to the
124 buffer reactor (working volume of 0.5 L) was made to allow pH adjustment, sampling,
125 initial inoculation, draw out and feed stock loading. After startup FBB, 3 L of
126 hydrolyzed liquid obtained from R-40, R-55 and R-70 were pumped to FBB in
127 succession, each of which was operated for six fed-batches respectively. And each
128 batch last for 2 d. L- and D-lactic acid and VFA were recorded and the productivity
129 and yield was calculated.

130 2.5. Chemical analysis

131 L- and D-lactic acid were assayed by high-performance liquid chromatography
132 according to our method (Li et al., 2015). VFA (including acetic, propionic, butyric
133 and valeric acid), protein, polysaccharide, ethanol, SOP, ammonia, COD were
134 previously introduced (Huang et al., 2016; Li et al., 2011). The analyses of
135 α -glucosidase and protease were according to the reported method (Goel et al., 1998).
136 The pre-treatment of the samples for intracellular enzymes assay, monosaccharide and
137 dry cell weight (DCW) of *P. acidipropionici* measurement were conducted accordingly.
138 The enzymes activities were determined according to the reference (Feng et al., 2009).

139 2.6. Calculation and kinetic model

140 The optical activity (OA%) of L-lactic acid was defined as enantiomeric excess
141 value according to the following equation (eq 1)

$$142 \quad OA(\%) = \frac{[L] - [D]}{[L] + [D]} \times 100\% \quad (eq\ 1)$$

143 Where [L] and [D] were the concentration of L- and D-lactic acid, respectively.

The kinetic study was frequently employed for the evaluation of fermentation efficiency (Kavitha et al., 2015; Kavitha et al., 2016). In this study, kinetic model was also developed to described the cell growth, lactate consumption, propionic acid production during the Batch Exp.3. Monod equation is modified concerning the products and substrate inhibition and specific production rate of propionic acid Q_p can be expressed by Leudeking-Piret (1959) equation:

$$\frac{dX}{Xdt} = \mu(h^{-1}) = \mu_{\max} \frac{S_L}{K_S + S_L} \frac{K_P}{K_P + P_P} \quad (eq\ 1)$$

$$\frac{dP_P}{Xdt} = r_P (gg^{-1}h^{-1}) = \alpha\mu + \beta \quad (eq\ 2)$$

$$\frac{dS_L}{Xdt} = Q_L (gg^{-1}h^{-1}) = -(\frac{\mu}{\gamma} + m) \quad (eq\ 3)$$

Where X is the dry cell weight (DCW) from *P. acidipropionici*; μ is specific growth rate; $\mu_{\max}$ is the maximal specific growth rate; S_L is substrate concentration; K_S is the half-saturation constant of substrate; K_P is the inhibition constant from propionic acid; P_P is the concentration of propionic acid; r_P is the specific propionic acid production rate; α is the growth coefficient associated propionic acid production; β is the non-growth coefficient associated propionic acid production; Q_L is the specific lactic acid consumption rate; γ is the cell yield coefficient based on lactate; m is the non-growth coefficient of constant for cell energy maintenance. MATLAB (V.2012a 7.14) software was used to find suitable settings of the parameters.

3. Results and discussion

3.1. First stage fermentation at various C_S

3.1.1. L- and D-lactic acid production

165 **Fig. 2** shows the time curves of L- and D-lactic acid production at various initial C_s
166 at 7 d fermentation. In R-10 and R-25, concentration of L- and D-lactic acid was only
167 3.21 and 1.43 g COD/L (R-10), 9.35 and 3.68 g COD/L (R-25) but soon consumed up in
168 Day 4. The depletion of L-lactic acid was lessened with increase of C_s from R-40 to
169 R-70 g/L, and the maximal concentration of L-lactic acid were respectively (g COD/L)
170 17.32 at 3 d in R-40, 29.21 at 4 d in R-55, 35.45 at 6 d in R-70. D-lactic acid was
171 continuously accumulated during the whole fermentation time, and the final
172 concentrations (g COD/L) reached to 4.64 in R-40, 6.56 in R-55 and 8.76 in R-70
173 respectively.

174 The relationship between the initial substrate concentration (C_s) and the maximal
175 concentration of L-lactic acid (C_L), or D-lactic acid (C_D) can be linearly described by:
176 $C_L = 0.562 \times C_s - 3.583$ ($R^2=0.988$), and $C_D = 0.117 \times C_s + 0.337$ ($R^2=0.987$). The
177 coefficient related to L-lactic acid (0.562) was much higher than that to D-isomer
178 (0.117), which implied that production of L-lactate was significantly affected by initial
179 substrate. The maximal OA (82.2%) and yield of L-lactic acid (0.53 g/g) were both
180 achieved at R-55 at 4 d of fermentation. The yields of D-lactic acid in all reactors
181 were similar with each other (0.12-0.14 g/g).

182 3.1.2. Mechanism study for the first stage

183 It has been reported that the hydrolysis was the rate-limiting step, and protein and
184 polysaccharide are the two main fermentative substances (Kavitha et al., 2014; Luo et
185 al., 2014). The activities of hydrolysis enzymes related to protein and polysaccharide,
186 i.e., protease and α -glucosidase, were observed slightly enhancing with an increase of

187 *C_s*. Additionally, soluble ammonia and SOP were released with sludge hydrolysis
188 (Zhang & Chen, 2009), which was improved with the increase of *C_s*. Herein, it can be
189 concluded that a higher *C_s* was beneficial for first stage hydrolysis. The enzymes
190 activity of L-LDH responsible for L-lactate generation was the highest at R-55
191 compared to other reactors, whereas that of D-lactate exhibited the lowest in R-55,
192 which explained the maximal OA of L-lactic acid obtained from R-55. FISH assays
193 from R-10 to R-70 were also conducted in this study, and the images were analyzed by
194 Image-Pro Plus V6.0. The abundances of L-lactic acid bacteria in total lactic acid
195 from R-55 reached the highest 85.5% among other reactors, which was in good
196 conformity with the OA observations.

197 3.2. Propionic acid production in the second stage

198 In the first stage, hydrolyzed liquid containing high concentration of L-lactic acid
199 was obtained from the co-fermentation of WAS and food waste. Then, in the second
200 stage, Batch exp.1 was carried out inoculating *P. acidipropionici* to the hydrolyzed
201 liquid. It could be noted that the maximal concentration of propionic acid (6.70 g
202 COD/L) and productivity (0.068 g/L.h) was obtained at 4 d using hydrolyzed liquid
203 from R-25. Although high content of lactic acid presented in hydrolyzed liquid at
204 R-40, R-55 and R-70, long lag phases in the second stage around 6 and 8 d for propionic
205 acid production were observed in R-40 and R-55, while propionic acid production was
206 completely halted in R-70. Previous finding demonstrated that lactic acid in the first
207 stage liquid was the only effective substrate for propionic acid production in the second
208 stage (Chen et al., 2013a). The inconsistent observations of negative relationship

209 between lactic acid and propionic acid might be due to uncertain inhibition effect from
210 hydrolyzed liquid.

211 3.3. Investigation of inhibit effect on propionic acid production

212 3.3.1. Determination inhibitory intermediate

213 Literature reported that some compounds released from hydrolysis step were toxic
214 for microbial metabolism, which caused low yield and productivity of fermentation
215 (Landaeta et al., 2013). In present study, the concentration of protein was enhanced
216 with the increase of C_s when the maximal L-lactic acid was achieved: 0.85 g COD/L
217 (R-10 at 2 d), 1.85 (R-25 at 2 d), 4.27 (R-40 at 3 d), 5.82 (R-55 at 4 d) and 7.52 (R-70 at
218 6 d), while the remaining polysaccharide was decreased from R-10 to R-70 respectively.
219 Meanwhile, concentration of VFA, ethanol, ammonia and SOP were all improved with
220 the increase of C_s . Therefore, it is necessary to disclose the impact of the high
221 concentration of individual intermediate on propionic acid fermentation. Batch exp.2
222 showed that propionic acid production was significantly inhibited by high content of
223 lactic acid compared to others, and partially undermined by high content of VFA. In
224 the literature, end-product inhibition of propionic acid was observed in the free cell
225 fermentation (Liang et al., 2012), but very small fraction of propionic acid (~0.3 g
226 COD/L) was presenting in the first stage liquid. Therefore, the significant inhibition
227 was mainly due to the feed substrate of lactic acid.

228 3.3.2. Effect of L-lactic acid concentration

229 The experimental results and coefficient for kinetic model are shown in Table 1.

230 The developed model successfully predicted the experimental data, which are

231 illustrated in Fig. 3. The yield and productivity of propionic acid were improved with
232 increase of C_s from 6 to 18 g COD/L, but was decreased rapidly with continuously C_s
233 improving to 30 g COD/L. The maximal percentage of propionic acid (around 75%)
234 in total VFA was similar around lactate 6-18 g/L, but decreased rapidly to 15%
235 providing lactate to 30 g/L. The maximal yield and productivity of propionic acid
236 were respectively 0.62 g/g and 0.075 g/L·h at B-18.

237 From kinetic model analysis, α and $1/\gamma$ was similar at B-6, B-12 and B-18, but
238 negligible at B-24 and B-30, which indicated that the significant inhibition of propionic
239 acid production was occurred at high concentration of lactate. $\mu_{\max}$ was decreased
240 with increase of lactate concentration, which implied that *P. acidipropionici* growth
241 potential was undermined with improved lactate provision. In the literature, lactate
242 was a preferred substrate over other organic acids, like glucose, glycerol and lactose,
243 for it giving a higher propionic acid yield, but lower cell yield, and lower specific
244 growth rate (Coral et al., 2008; Liu et al., 2012; Zhu et al., 2010). This could be
245 verified by improved K_s along with lactate concentration, which indicated that lactate
246 assimilation is more difficult with higher concentration. One probable reason was
247 that cells referred to convert lactate to propionate rather than cell maintenance. This
248 assumption is in good agreement with negligible m , an index of non-growth coefficient
249 for cell energy maintenance, in most of the bottles. The maximal K_P was obtained at
250 B-18, but decreased rapidly at B-24 and B-30. This implied that fermentation
251 inhibition was margin at lower substrate concentration, but became more serious at
252 higher substrate concentration. One reason was that DCW with low growth rate at

253 B-24 and B-30 was the lowest which became more sensitive to both feed and product
254 inhibition. Therefore, to adapt higher substrate concentration, it is essential to
255 increase DCW in the fermentation system.

256 3.3.3. Effect of L-lactic acid optical activity

257 In the literature, lactate promotes the formation of pyruvate and directs the
258 proportion of the flow to propionate in *P. acidipropionici* (Guan et al., 2015; Coral et al.,
259 2008). However, no information is available regarding the production of propionic
260 acid from different isomer of lactate. Therefore, Batch exp.4 was carried out fed by
261 different OA of L-lactic acid. Fig. 4 exhibited the maximal concentration and
262 percentage of propionic acid at various OA of L-lactic acid as substrate. Apparently,
263 the increase of D-isomer ratio led to a decrease of propionic acid production from 6.85
264 to 3.42 g COD/L. There were no significant differences in the concentration and
265 percentage of propionic acid between OA 100% and 80% with statistical analysis ($p >$
266 0.05). But significant difference ($p < 0.05$) was observed if OA was below 50%. The
267 consumption of D-lactic acid was much lower than that of L-lactic acid in all runs,
268 which indicated that D-lactic acid was unfavorable substrate for *P. acidipropionici*.
269 Obviously, to achieve the best performance of propionic acid production, the OA of
270 L-lactic acid in the fed substrate should remain above 80%.

271 3.4 Improved production of propionic acid by using FBB

272 Free cell fermentation was usually encounter substrate inhibition (Shi et al., 2014).
273 To eliminate the inhibition from feed substrate, repeated-batch fermentation using FBB
274 was therefore conducted. As it can be seen from Fig. 5, propionic acid fermentation

275 was stabilized after the third batch by adding the hydrolyzed liquid from R-40. No
276 lag phase was observed during the batch circles, indicating that *P. acidipropionici*
277 successfully attached in the fibrous matrix. With the increase of fermentative
278 substrate from R-55 to R-70, the average concentration of VFA (g COD/L) was
279 increased respectively 15.90 ± 0.13 (R-40), 23.87 ± 0.13 (R-55) and 28.93 ± 1.0 (R-70).
280 Meanwhile, production of propionic acid (g COD/L) was also observed enhanced to
281 10.86 ± 0.16 (R-40), 16.30 ± 0.39 (R-55) and 17.49 ± 0.79 (R-70). The maximal
282 percentage of propionic acid (68.3 ± 1.0 %) and the yield of propionic acid from total
283 lactic acid (0.49 g/g) were both observed fed by the hydrolyzed liquid from R-55,
284 which was attributed to high OA of L-lactic acid (82%). It was enhanced by 3.33
285 folds (16.31 versus 3.76 g COD/L) compared to the free cell fermentation.

286 In the literature, FBB was a promising cell immobilizing process for organic acid
287 production, which was reported to hold high cell density, and therefore could enhance
288 the endurance capability of strain to high concentration of substrate as well as the end
289 product (Landaeta et al., 2013). Jerusalem artichoke hydrolysate, containing 40 g/L
290 fructose and 20 g/L glucose, could be stably converted to propionic acid with high yield
291 of 0.483 g/g by fed-batch of FBB (Liang et al., 2012). If an adapted acid-tolerant *P.*
292 *acidipropionici* strain was utilized in the FBB, sugarcane bagasse hydrolysate, consist of
293 25.4 g/L glucose and 32.1 g/L xylose, could be efficiently fermented to propionic acid
294 with high yield of 0.51 g/g (Zhu et al., 2012). Further investigation revealed that the
295 increase of cell density could eliminate substrate inhibition, and the lag phase was
296 shortened from 4 to 2 d when *P. acidipropionici* inoculums were increased from 100%

297 to 200%. The concentration and percentage of propionic acid were also improved in a
298 high cell density. It explained that the high cell density capacity of FBB led to the
299 improvement of propionic acid production from hydrolyzed liquid containing high
300 concentration of L-lactic acid.

301 **4. Conclusion**

302 In this study, two-stage propionic acid fermentation driven by high substrate
303 concentration of food waste and sludge was evaluated. High OA of L-lactic acid
304 (>80%) was achieved at the first stage, but propionic acid fermentation at second stage
305 inhibited severely. Kinetic model analysis revealed that propionic acid production was
306 severely inhibited at high concentration of L-lactic acid due to low yield of cell density.
307 Therefore, FBB process was utilized in the second stage to immobilize *P.*
308 *acidipropionici* strain, which improved propionic acid production by 3.33 folds.

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314 **Appendix. supplementary information**

315 Supplementary data associated with this article contains additional details of 6 tables
316 and 10 figures, which can be found in the online version.

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415

416 **Figure captions**

417 **Fig. 1.** Configuration of the modified two-stage fermentation coupling FBB.

418 **Fig. 2.** Effect of C_S on production of L-lactic acid (A) and D-lactic acid (B) in the first
419 stage.

420 **Fig. 3.** Comparison of the measured data and simulated values in batch cultivations at
421 various feed lactic acid concentration.

422 **Fig. 4.** The maximal propionic acid concentration and percentage fed by various OA of
423 L-lactic acid.

424 **Fig. 5.** Performance of propionic acid production in FBB driven by different hydrolyzed
425 liquid. During 0-13 d FBB was fed by hydrolyzed liquid from R-40, R-55 during
426 14-25 d and R-70 during 26-37 d..

427 **Table captions**

428 Table 1. Effect of L-lactic acid concentration on *P. acidipropionici* fermentation

429

1

2 **Fig. 1.** Configuration of the modified two-stage fermentation coupling FBB.

Fig. 2. Effect of C_S on production of L-lactic acid (A) and D-lactic acid (B) in the first stage.

Fig. 3. Comparison of the measured data and simulated values in batch cultivations at various feed lactic acid concentration.

1

2 **Fig. 4.** The maximal propionic acid concentration and percentage fed by various

3 **OA of L-lactic acid.**

Fig. 5. Performance of propionic acid production in FBB driven by different hydrolyzed liquid. During 0-13 d FBB was fed by hydrolyzed liquid from R-40, R-55 during 14-25 d and R-70 during 26-37 d.

1 **Table 1**

2 Effect of L-lactic acid concentration on *P. acidipropionici* fermentation ^a

Batch serum bottles	B-6	B-12	B-18	B-24	B-30
Lactic acid (g COD/L)	5.76	12.36	17.40	25.25	31.56
C_p (g COD/L)	3.4	7.3	11.1	2.7	1.3
Percentage (%)	79.5	76.5	73.8	51.5	12.5
Y_p (g/g)	0.57	0.61	0.62	0.11	0.04
P_p (g/L·h)	0.048	0.061	0.075	0.019	0.011
DCW(g/L)	1.04	2.12	2.33	0.91	0.67
$\mu_{\max}$ (1/h)	4.1465	3.136	2.1919	1.4734	0.3014
K_S (g/L)	2.5151	2.1739	4.522	12.7481	-
K_P (g/L)	1.3572	1.8974	5.0951	0.2440	0.1708
α (g/g)	2.3175	2.8375	2.9488	-	-
β (1/h)	0.118	0.1732	0.3203	0.5082	-
I/γ (g/g)	6.7523	6.288	6.8157	-	-
m (1/h)	-	-	-	0.8673	-

3 ^a Data was recorded when the maximal concentration was obtained: B-6 at 3d, B-12 at 5 d, B-18,

4 B-24 and 30 at 7 d; C_p is the maximal concentration of propionic acid; Y_p , yield of propionic acid;

5 P_p , productivity of propionic acid. – negligible