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1 **Lactic acid production from food waste using a microbial consortium: Focus on**  
2 **key parameters for process upscaling and fermentation residues valorization**

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9  
10 **Abstract**

11 In this study, the production of lactic acid from food waste in industrially relevant  
12 conditions was investigated. Laboratory assays were first performed in batch conditions  
13 to determine the suitable operational parameters for an efficient lactic acid production.  
14 The use of compost as inoculum, the regulation of the temperature at 35°C and pH at 5  
15 enhanced the development of *Lactobacillus sp.* resulting in the production of 70 g/L of  
16 lactic acid with a selectivity of 89% over the other carboxylic acids. Those parameters  
17 were then applied at pilot scale in successive fed-batch fermentations. The subsequent  
18 high concentration (68 g/L), yield (0.38 g/gTS) and selectivity (77%) in lactic acid  
19 demonstrated the applicability of the process. To integrate the process into a complete  
20 value chain, fermentation residues were then converted into biogas through anaerobic  
21 digestion. Lastly, the experiment was successfully replicated using commercial and  
22 municipal waste collected in France.

25        **Keywords**

26    Lactic acid; Food waste; Mixed culture fermentation; Biogas; Microbial community  
27    analysis

28

29        **1. Introduction**

30        Lactic acid (LA) is an important platform chemical that has a wide range of  
31    application. It is commonly used in the food and beverage sector as a preservative and  
32    pH adjusting agent but also in the cosmetics and pharmaceuticals industries. LA can also  
33    be converted into lactate ester or poly-lactic-acid (PLA), a nontoxic, biocompatible,  
34    thermo-tolerant and biodegradable plastic (Chen *et al.*, 2016). The market value of LA  
35    was 2.7 billion dollars in 2020 and is predicted to increase at a compound annual  
36    growth rate of 8.0% from 2021 to 2028 (see Lactic Acid Market Share, Industry Report,  
37    2021-2028).

38        Nowadays, most of LA is produced by homolactic microorganisms such as  
39    *Lactobacillus delbrueckii* using sugars extracted from agricultural resources as a carbon  
40    source (mainly cassava, sugarcane and corn; see Lactic Acid Market Share, Industry  
41    Report, 2021-2028 and Alves de Oliveira *et al.*, 2018). However, this biological  
42    production route competes with food and feed and is often expensive due to the high  
43    price of raw materials, which represents 40-70% of the total production cost (Abdel-  
44    Rahman and Sonomoto, 2016). Thus, studies have been conducted to produce LA from  
45    inexpensive and more renewable resources such as lignocellulosic biomass and food  
46    waste (Abdel-Rahman *et al.*, 2013; Alves de Oliveira *et al.*, 2018; Wang *et al.*, 2020b).  
47    The industrial feasibility and rentability of those processes are still to be demonstrated.

48 According to the UNEP (United Nations Environment Programme) Food Waste  
49 Index Report, around 931 million tons of food waste were generated in the world in  
50 2019 coming from households, food services and retails (UNEP Food Waste Index  
51 Report 2021). Food wastes (FW) represent a large part of the organic fraction of  
52 municipal solid waste (OFMSW) and still often end-up in landfill or incinerated.  
53 Anaerobic digestion (AD) is seen as a good strategy to valorize complex organic wastes  
54 such as FW into biogas and fertilizers (Capson-Tojo *et al.*, 2016). However, this  
55 solution is not always the best choice in terms of economic and environmental impact  
56 due to its long digestion cycle (low organic loading rate and long retention time) and its  
57 instability related to high ammonia concentration and volatile fatty acids accumulation  
58 (Nayak and Bhushan, 2019). Recently, numerous regulations have been implemented to  
59 better valorize FW. The European Union develops a policy of household biowaste  
60 selective sorting that will lead to the implementation of novel strategies for FW  
61 conversion into more valuable products (such as LA). Indeed, FW is an attractive  
62 feedstock due to its high biodegradability, its high sugars and proteins content and its  
63 availability throughout the year (Dou and Toth, 2021; Wang *et al.*, 2020b).

64 LA fermentation performance is known to be strongly dependent on the quality of  
65 the substrate and its pretreatment, on the inoculum used and on operational conditions,  
66 such as pH, temperature, and content of total solids (TS) during the bioconversion.  
67 Several strategies have been implemented to maximize LA production including  
68 substrate pretreatment (Demichelis *et al.*, 2017; Yousuf *et al.*, 2018), bioaugmentation  
69 with lactic acid bacteria (LAB: *Streptococcus sp.*, *Bacillus coagulans*, *Pediococcus*  
70 *acidilactici* or *Lactobacillus sp.* (López-Gómez *et al.*, 2020; Ohkouchi and Inoue, 2006;  
71 Pleissner *et al.*, 2017; Wang *et al.*, 2010; Zhang *et al.*, 2021)) and pH control (Feng *et*

72 *al.*, 2018; Li *et al.*, 2015; Wang *et al.*, 2020b). By combining different strategies (*e.g.*,  
73 fungal hydrolysis, bioaugmentation with *Lactobacillus casei* Shirota and pH regulation  
74 to 6), Kwan *et al.*, (2016) reached a maximal LA concentration of 94 g/L with a  
75 productivity of 2.61 g/L/h and a yield of 0.31 g/gTS. Other strategies including co-  
76 fermentation with other substrates (Alexandri *et al.*, 2020; Li *et al.*, 2015; Tang *et al.*,  
77 2016), different reactor configurations (Bonk *et al.*, 2017; Tang *et al.*, 2017), activated  
78 carbon addition (Wang *et al.*, 2021), supplementation with copper and nano iron (Wang  
79 *et al.*, 2020a; Ye *et al.*, 2018) and modification of the osmotic pressure (Li *et al.*, 2021)  
80 were also evaluated as efficient solutions to improve LA production. However, no  
81 consensus has been achieved on a preferential strategy for industrialization.

82       Several studies highlighted that final LA concentration and fermentation yield were  
83 respectively positively and negatively correlated with the TS concentration of the  
84 feedstock (Kim *et al.*, 2003; Pleissner *et al.*, 2017; Yousuf *et al.*, 2018). To avoid  
85 excessive substrate costs and the generation of significant amounts of fermentation  
86 residues, studies often focused on maximizing fermentation yields (*i.e.*, g of LA per g of  
87 initial feedstock) by operating the process at low TS content. However, from a techno-  
88 economic point of view, a high final LA concentration combined with a high selectivity  
89 over other organic acids is mandatory to limit the costs of downstream processing  
90 (Abdel-Rahman and Sonomoto, 2016; López-Garzón and Straathof, 2014). The value of  
91 fermentation residues can still be upgraded through two-stage processes in which LA is  
92 produced from FW fermentation while the remaining solid residues are extracted and  
93 valorized into biomethane and/or compost (Demichelis *et al.*, 2017; Dreschke *et al.*,  
94 2015; Kim *et al.*, 2016). In such process setting, the overall process performance and  
95 costs can be optimized (Demichelis *et al.*, 2017; Kim *et al.*, 2016).

96 This study focused on the identification of key parameters for industrially relevant  
97 FW fermentation at high concentration (20%<sub>TS</sub> is representative of non-diluted FW). No  
98 substrate pretreatment was applied to minimize the process complexity and costs. The  
99 effects of inoculum, temperature and pH regulation were assessed on both LA  
100 fermentation performance and the evolution of the microbial community structure.  
101 Then, the best operational conditions were assessed at pilot scale and the fermentation  
102 residues were valorized into biogas through anaerobic digestion. Lastly, replication  
103 assays were performed with an industrial waste stream consisting of FW pulps collected  
104 from a commercial depackaging unit.

105

## 106 **2. Materials and Methods**

### 107 *2.1. Substrate and inoculum*

108 A model FW was prepared according to Capson-Tojo *et al.* (2017) to ensure the  
109 relative stability and reproducibility of the substrate properties during the experiments.  
110 This substrate, composed of fruits and vegetables (25.9% apple and 25.9% lettuce),  
111 carbohydrates (25.9% potato, 4.8% wheat meal and 6.2% bread), meat (4.1% chicken  
112 and 4.1% beef), dairy products (1.9% yoghurt) and pastries (1.5% cookies), is  
113 representative of real FW collected in Europe from households or canteens and has  
114 already been used as substrate in previous research on anaerobic digestion (Capson-Tojo  
115 *et al.*, 2017). The measured TS and VS content of this model FW is 25.13%<sub>TS</sub> and  
116 89.53%<sub>VS/TS</sub> (Table 1). The total carbohydrates content of the model FW was estimated  
117 at 0.69 g/g<sub>TS</sub>, its lipids content at 0.07 g/g<sub>TS</sub> and its crude protein content at 0.17  
118 g/g<sub>TS</sub>. The C/N ratio is 16.3 g/g and the pH of this model FW was estimated to be 5.6  
119 (Capson-Tojo *et al.*, 2017). This FW mixture was roughly milled (shredder BLICK

120 BB230) and blended (Hachoir Reber 9603) to ensure its homogeneity, and then stored at  
121 -20°C before use.

122 When indicated, real FW collected in France from either a high-school canteen or a  
123 commercial depackaging unit (a unit in which wrapped food are crushed and plastics  
124 from packaging are then separated from the organic matter) were tested.

125 In some conditions, a microbial inoculum, composed of a mixture of commercial  
126 yoghurt and/or leachate and solid compost from an industrial platform, was used. The  
127 compost and leachate have a buffering capacity (presence of  $\text{NH}_4^+$  and  $\text{HCO}_3^-$  ions) and  
128 bring a diversified microbial consortium while yoghurt was tested as an input of LAB  
129 (especially *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*  
130 (Nagaoka, 2019)). Each inoculum was characterized by measuring its total and volatile  
131 solids content (Table 1).

132

## 133 2.2. *Batch fermentation (0.5 L)*

134 Batch fermentations in 500 mL flasks were first performed to maximize LA  
135 production through operational parameters optimization. The total substrate mass feed  
136 was 200 g at 20%<sub>TS</sub>. This total solid content is consistent with previously reported FW  
137 solid contents (Capson-Tojo *et al.*, 2016) and may allow to reach the reasonable  
138 production of 50 g/L lactic acid, enabling a cost-effective downstream processing  
139 (López-Garzón and Straathof, 2014). Substrate to inoculum ratio (S/X) was set to 10 (on  
140 a VS basis) as a starting point. Fermentations were carried out during 21 days at several  
141 temperature (24, 35 or 55°C).

142 Depending on the condition, pH was either left uncontrolled or corrected at 5 or 6  
143 by manual addition of NaOH (1 M or 5 M) or KOH (1 M) every 2 days. This pH

144 regulation mode allows for a swing of pH that oscillates between 3.5 and 5 or 6,  
145 improving the development of LAB (Tashiro *et al.*, 2016). The culture medium was  
146 regularly sampled in order to monitor pH and to measure the quantity of lactic acid,  
147 ethanol and other organic acids produced. Gas production (CO<sub>2</sub> and H<sub>2</sub>) was also  
148 regularly quantified. After each opening, the flasks were purged with N<sub>2</sub> gas to restore  
149 anaerobic conditions.

150

### 151 2.3. *Fed-batch fermentation at a pilot scale (12 L)*

152 Fed-batch fermentations were carried out in a laboratory scale pilot (12 L working  
153 volume) designed for high TS anaerobic digestion (Garaud, France). The temperature  
154 was automatically controlled at 35°C using a water bath circulator and a built-in water  
155 jacket. A pH electrode (METTLER TELEDIO InPro® 42XX) allowed for continuous pH  
156 monitoring which was then manually adjusted once a day using 1 M KOH. In pilot scale  
157 fermentation, compost and leachate were used as an inoculum. Fermentation residues  
158 from previous experiments were also tested as an input for LAB.

159 Those reactors were fed once a day without digestate withdrawal (fed-batch  
160 fermentation). The initial TS content in the reactor was 20% with an initial S/X ratio of  
161 20 g VS of substrate per g VS of inoculum. The Organic Loading Rates (OLR) was 25  
162 gTS/L/d for the first four days.

163

### 164 2.4. *Potential for methane production*

165 LA-fermented residues using model FW were recovered to perform AD tests.  
166 Those residues had a TS and VS contents equal to 18% and 16.5%. Non fermented FW  
167 was also used as a control. Methane production assays were realized as described



168 previously (Motte *et al.*, 2014). Batch assays (400 mL) were carried out in anaerobic  
169 conditions at 35 °C for 35 days. To correct the endogenous contribution to the biogas  
170 from the inoculum, blank assays were conducted. Each condition was performed in  
171 triplicate.

172

## 173 2.5. Analytical methods

### 174 2.5.1. Determination of total solids and volatile solids contents

175 The Total Solids (TS) and Volatile Solids (VS) contents of substrates and inocula  
176 were obtained by drying samples at 105°C (Memmert) for 24h and then at 550°C for 3h  
177 (Nabertherm). The differences of mass at each step indicates the percentage of TS and  
178 VS.

179

### 180 2.5.2. High Performance Liquid Chromatography

181 Concentrations of organic acids, sugars and alcohols were measured by High  
182 Performance Liquid Chromatography (HPLC) with a refractive index detector (Waters  
183 R410). HPLC analysis were performed at a flow rate of 0.3 mL/min on an Aminex  
184 HPX-87H, 300\*7.8 mm (Bio-Rad) column at a temperature of 35°C. H<sub>2</sub>SO<sub>4</sub> at 4 mM  
185 was used at mobile phase. A pre-column (Micro guard cation H refill cartridges, Bio-  
186 Rad) was disposed before the main column. Lactate, ethanol, and organic acids  
187 concentration, given in this study, are uncorrected for dilution due to NaOH or KOH  
188 addition.

189

### 190 2.5.3. Gas Chromatography

191 Biogas composition was determined using a gas chromatograph (Clarus 580, Perkin

192 Elmer) equipped with a thermal conductivity detector. The columns used were a  
193 RtQbond column (for H<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> and CH<sub>4</sub>) and a RtMolsieve column (for CO<sub>2</sub>). Argon  
194 was used as mobile phase.

195

## 196 2.6. Calculation

197 Four performance indicators (see equation 1 to 4) were monitored: LA  
198 concentration in the reaction medium (g/L), the yield of LA produced depending on the  
199 substrate introduced (g/gTS), the productivity (g/L/h) and the selectivity for LA  
200 production over other organics acids (%<sub>molOA</sub>).

201 Eq. (1) Concentration = g of LA / L of medium

202 Eq. (2) Yield = g of LA / g of TS of FW introduced

203 Eq. (3) Productivity =  $\Delta$  concentration /  $\Delta$  hours

204 Eq. (4) Selectivity = mol LA / mol total organic acids x 100

205

## 206 2.7. Microbial community analysis

207 Samples of the initial inoculum and from the batch reactors were analyzed by 16S  
208 rRNA gene sequencing to determine the structure of the microbial community and to  
209 evaluate the effect of operating conditions on the evolution of this community. DNA  
210 was extracted from the samples using the QIAamp fast DNA stool mini kit (Qiagen),  
211 amplified by PCR and sequenced. Precise description of the methodology employed can  
212 be found in the literature (Moscoviz *et al.*, 2016).

213

## 214 **3. Results and Discussion**

215 3.1. Identification of key parameters for industrially relevant food waste

216 *fermentation*

217 *3.1.1. Laboratory scale fermentation of model food waste: optimization of*  
218 *fermentation performance*

219 Fermentation experiments were conducted at high TS (20%) using unsterilized  
220 model FW. To determine the most suitable conditions to achieve a high LA  
221 concentration with a high selectivity, several operational parameters were tested. Hence,  
222 FW was incubated with or without addition of compost and yoghurt, at several  
223 temperature (24°C, 35°C and 55°C) and with (pH = 5 or pH = 6) or without  
224 (uncontrolled) pH regulation. The resulting fermentation performances (concentration,  
225 productivity, yield and selectivity, summarized in Table 2) are given for the day at  
226 which LA concentration is maximal (most of the time after 8 days). Typical time  
227 courses for FW fermentation with or without pH regulation are shown in Fig.1a and  
228 typical time courses for FW fermentation at several temperatures and with different  
229 inoculums can be found in supplementary material.

230 At 35°C and without pH control, the maximal concentration of LA ( $36.3 \pm 1.3$  g/L  
231 vs  $15.9 \pm 0.5$  g/L) and its selectivity ( $77 \pm 2\%_{\text{molOA}}$  vs  $66 \pm 2\%_{\text{molOA}}$ ; see supplementary  
232 material) were higher when an inoculum consisting of a mixture of yoghurt and  
233 compost was added compared to a fermentation without exogeneous inoculation. First,  
234 addition of compost induced a higher initial pH with a value of  $6.3 \pm 0.2$  compared to  
235  $5.1 \pm 0.1$  without inoculation. In addition, 16S rRNA gene sequencing at the beginning  
236 of the experiment indicated that the initial microbial community structure was similar  
237 with or without addition of inoculum (main bacterial class are *Clostridia*: the genus  
238 *MBA03\_ge* representing  $25.2 \pm 2\%$  of the total microorganism relative abundance;  
239 *Sphingobacteriia*: an unidentified genus from the *Lentimicrobiaceae* family representing

240 11.1 ± 2.1% of the total microorganism relative abundance; and *Bacilli* with the genus  
241 *Streptococcus* representing 6.0 ± 2.7% of the total microorganism relative abundance;  
242 see supplementary material). This may be explained by the fact that there are many  
243 endogenous microorganisms in the model FW (micro-organisms coming from the raw  
244 materials used and the natural contamination during its preparation) and therefore, the  
245 LAB coming from yaourt were not detected. Sequencing results also indicated that the  
246 *Lactobacillus* species that dominated the microbial community at the end of the  
247 fermentation were similar with or without inoculum addition. The increase in final LA  
248 concentration and selectivity with inoculum addition might therefore be correlated to  
249 the higher initial pH. Indeed, it would allow for more LA to be produced before  
250 reaching pH values below 3.5 that would prevent *Lactobacillus* growth and more  
251 globally all biological activities. These results are consistent with the experiments  
252 carried out when the inoculum was made of 72% of yoghurt. In this condition, the final  
253 LA concentration (33.6 ± 0.5 g/L) was lower than in the condition using 18% of yoghurt  
254 which is likely related to a lower initial pH (pH<sub>i</sub> = 5.6 ± 0.1). The inoculum composed  
255 of compost and 18% of yoghurt was therefore retained for further screening  
256 experiments.

257 Temperature is known to play an important role in LA fermentation (Song *et al.*,  
258 2021) due to the higher hydrolysis rates of FW at high temperature and to a  
259 modification of the microbial community structure and enzymatic activities (Tang *et al.*,  
260 2016). In this study, maximal LA concentration was higher at 35°C (36.3 ± 1.3 g/L)  
261 than at 24°C (29.9 ± 0.2 g/L) or 55°C (10.5 ± 0.8 g/L). LA selectivity was similar at  
262 24°C and 35°C (78 ± 1%<sub>molOA</sub> and 77 ± 2%<sub>molOA</sub> respectively) and lower at 55°C (61 ±  
263 3.6%<sub>molOA</sub>). At 24°C, the LAB dominated the microbial community but grew more

264 slowly and therefore more time was required to reach a similar LA concentration (Table  
265 2: 8 days at 35°C vs 16 days at 24°C). At 55°C, butyric acid and H<sub>2</sub> production were  
266 observed ([see supplementary material](#)) indicating that the microbial community was not  
267 dominated by LAB and resulting in lower LA production, as reported previously (Tang  
268 *et al.*, 2016; Zhang *et al.*, 2021). In further studies, an adapted bacterial consortium  
269 (such as thermophilic anaerobic sludge (Arras *et al.*, 2019)) or a specific thermotolerant  
270 LAB (Sakai and Yamanami, 2006; Wang *et al.*, 2010; Yang *et al.*, 2015) could be used  
271 to operate in thermophilic conditions but the strength of this selected microbial  
272 community remains to be proven overtime when facing a rich endogenous microbial  
273 diversity.

274 Lastly, pH has been demonstrated to have an important effect on LA fermentation  
275 performance (Feng *et al.*, 2018; Li *et al.*, 2015; Wang *et al.*, 2020b) but the optimal pH  
276 and its ideal regulation type (*i.e.*, continuous or at regular interval) for LA fermentation  
277 is still unknown (Song *et al.*, 2021). In this study, only acidic pH values were considered  
278 because alkaline pH would require an excessive quantity of reagents for pH buffering  
279 (*i.e.*, increasing process costs) and because it has been proven that acidic pH allowed for  
280 the selection of LAB among all the microorganism present in FW.

281 The final concentration of LA, its productivity and yield were improved by pH  
282 regulation at 5 or 6 (regulated every two days allowing a dynamic evolution of pH;  
283 Table 2 and Fig. 1a). Indeed when pH was not regulated, it rapidly decreased as LA was  
284 accumulating, preventing the growth and activity of many micro-organisms (including  
285 LAB (Alves de Oliveira *et al.*, 2018; Farah *et al.*, 2009)). As a consequence, the  
286 quantity of LA produced reached a plateau after 4 days of fermentation (Fig. 1a). This  
287 mode of fermentation is not efficient to reach high LA concentration, but it is robust and

288 repeatable (5 fermentations realized in triplicates led to a production of  $30.9 \pm 2.6$  g/L  
289 LA after 4 days and  $36.3 \pm 1.3$  g/L after 8 days).

290 LA fermentation performances were similar by regulating pH at 5 or 6 after 8 days  
291 of fermentation ( $58.4 \pm 6.6$  g/L vs  $57.0 \pm 4.8$  g/L; Table 2 and Fig. 1a). However, as the  
292 fermentation continued, an important decrease in LA concentration (from  $57.0 \pm 4.8$  g/L  
293 at day 8 to 0 g/L at day 21; Fig. 1a) and specificity (from  $86 \pm 1\%$ <sub>molOA</sub> to  $0\%$ <sub>molOA</sub>; Fig.  
294 1b) occurred at pH 6 mainly due LA consumption for butyric, propionic, and acetic acid  
295 production. Sequencing results indicated a change in the microorganism community that  
296 shifted from a *Lactobacillus* dominated consortium towards a *Clostridium* dominated  
297 consortium (acetate-butyrate producing bacteria; Fig. 1c). LA only being an  
298 intermediate fermentation product have already been observed in previous studies (Feng  
299 *et al.*, 2018; Hussain *et al.*, 2017; Kim *et al.*, 2003; Ohkouchi and Inoue, 2006; Probst *et*  
300 *al.*, 2015; Tang *et al.*, 2016). At pH 5, a decrease in LA concentration was also observed  
301 after 8 days of fermentation but it was less intense than at pH 6. This result suggests a  
302 growth inhibition of microorganisms other than *Lactobacillus* at this pH, as observed in  
303 the final microbial community (Fig. 1c).

304 Lastly, by regulating pH with a more concentrated NaOH solution (5 M instead of 1  
305 M), the fermentation broth was less diluted resulting in a higher LA concentration  
306 (Table 2 and Fig. 1a). Hence, the maximal LA concentration ( $70.1 \pm 1.5$  g/L),  
307 productivity ( $0.37 \pm 0.01$  g/L/h) and yield ( $0.39 \pm 0.01$  g/gTS) was obtained after 8 days  
308 of fermentation at 35°C, by regulating pH at 5 with 5 M NaOH and with an inoculum  
309 composed of yaourt and compost. The low quantity of ethanol produced ( $0.18 \pm 0.0$  mol  
310 per mol of LA; Table 2) and high selectivity ( $89 \pm 2\%$ <sub>molOA</sub>) for LA achieved using  
311 those parameters also strengthen the great industrial potential of this process with a

312 minimization of downstream processing cost for LA separation from ethanol and others  
313 organic acids. The fermentation performance (except for productivity) reported in this  
314 study were higher than those previously achieved in batch (19.6 to 58.4 g/L; 0.12 to  
315 2.38 g/L/h, 0.10 to 0.24 g/gTS, 63 to 71%<sub>molOA</sub>) in studies operating at a high TS  
316 without substrate pretreatment or inoculation with a specific micro-organisms (RedCorn  
317 and Engelberth, 2016; Yousuf *et al.*, 2018).

318

319 *3.1.2. Validation of the retained parameters for efficient fermentation of*  
320 *canteen food waste*

321 As LA fermentation was efficient at 35°C by regulating pH at 5 with 5 M NaOH  
322 and with compost and yoghurt as an initial seed, those optimal operational conditions  
323 were tested for LA production from canteen FW. As for model FW, a high LA  
324 concentration ( $66.3 \pm 1.1$  g/L for real *vs*  $70.1 \pm 1.5$  g/L for model FW), productivity  
325 ( $0.35 \pm 0.01$  g/L/h for real *vs*  $0.37 \pm 0.01$  g/L/h for model FW), yield ( $0.37 \pm 0.01$  g/gTS  
326 for real *vs*  $0.39 \pm 0.01$  g/gTS for model FW) and a good selectivity (over 90%<sub>molOA</sub> for  
327 real *vs* 89%<sub>molOA</sub> for model FW, see supplementary material) were achieved after 8 days  
328 when using canteen FW. Those results indicated that the model FW used for process  
329 parameters optimization was representative of real FW and that the retained parameters  
330 were suitable for efficient canteen FW fermentation. Fed-batch pilot scale test were  
331 therefore performed to provide further insights onto process industrial feasibility.

332

333 *3.2. Model food waste fermentation at pilot scale in industrially relevant*  
334 *conditions*

335 *3.2.1. Adaptation of the operational parameters to get closer to industrially*

336 *relevant conditions*

337 Pilot scale experiments were conducted under mesophilic conditions (35°C) and by  
338 regulating pH at 5. To get closer to industrially relevant conditions and because  
339 preliminary experiments have shown that addition of yoghurt as a LAB input in a pH-  
340 regulated condition did not improve final LA concentration, the inoculum was only  
341 composed of compost and leachate. Compost was added as a buffering agent but the  
342 substrate to inoculum ratio was increased to 20 (instead of 10 in flasks experiments) to  
343 maximize the quantity of FW processed and because no difference in fermentation  
344 efficiencies have been noticed when operating at those two ratios (data not shown).  
345 Furthermore, in the perspective of developing an industrially relevant process, the  
346 question of the valorization of remaining solids residues was addressed. Since it has  
347 previously been demonstrated that anaerobically fermented model kitchen refuse (*i.e.*,  
348 residues after LA fermentation by *B. subtilis* KBKU21 and LA removal) can be used as  
349 soil amendment to promote plants (*Brassica rapa*) growth (Kitpreechavanich *et al.*,  
350 2016), the use of KOH instead of NaOH for pH regulation was assessed to increase the  
351 agronomical value of the fermentation residues. Similar LA concentration ( $60.4 \pm 10.8$   
352 g/L with NaOH and  $55.8 \pm 9.2$  g/L with KOH), yield ( $0.31 \pm 0.06$  g/gTS with NaOH  
353 and  $0.29 \pm 0.05$  g/gTS with KOH), productivity ( $0.28 \pm 0.05$  g/L/h with NaOH and  $0.26$   
354  $\pm 0.04$  g/L/h with KOH) and selectivity ( $80 \pm 6.6\%$ <sub>molOA</sub> with NaOH vs  $79 \pm 0.4\%$ <sub>molOA</sub>  
355 with KOH) were achieved with the two pH regulating chemicals. Those two alkaline  
356 agents allowed for suitable pH regulation and favored *Lactobacillus* growth (98.8%  
357 and 98.9% of *Lactobacillus* with NaOH and KOH, respectively).

358 Hence, pilot experiments were conducted with compost and leachate (S/X = 20) as  
359 a pH buffering agent and by regulating pH at 5 with KOH instead of NaOH.



360

361           3.2.2. *Validation of the optimized operational parameters at pilot scale using a*  
362                           *fed-batch feeding strategy*

363           The operational parameters (use of compost, 35°C, pH regulated to 5 with KOH)  
364           were evaluated at pilot scale (12 L working volume) using model FW (20%<sub>TS</sub>). A fed-  
365           batch feeding strategy was preferred to alleviate the decrease in fermentation efficiency  
366           due to lower substrate availability in late batch fermentation.

367           The final concentration (68.5 g/L), yield (0.38 g/gTS) and selectivity (77%<sub>molOA</sub>)  
368           obtained at pilot scale after 3 days of fermentation (Fig. 2a-c) were slightly lower than  
369           the performance obtained after 7 days of fermentation at laboratory scale (83.0 ± 8.2  
370           g/L, 0.43 ± 0.04 g/gTS, 88 ± 0.6%<sub>molOA</sub>). Yet, the global productivity in fed-batch pilot  
371           (0.95 g/L/h) was twice better than in flask batch assays (0.49 ± 0.05 g/L/h). The  
372           productivity was similar for the first two days of fermentation using a batch or fed-batch  
373           strategy, but it increased rapidly afterwards in the fed-batch mode. This can be  
374           explained by the addition fresh substrate in a reactor already dominated by the  
375           *Lactobacillus* community and therefore the rapid conversion of newly added sugars into  
376           LA.

377           The final concentration, yield and productivity of LA obtained in this study were  
378           higher than the ones reported by Farah *et al.* (2009) at pilot scale operating in similar  
379           conditions (57.6 g/L, 0.8 g/L/h and 0.32 g/gTS respectively). However, Pleissner *et al.*  
380           (2017) reported an higher productivity of 2 g/L/h related to the use of a specific  
381           inoculation with *Streptococcus sp.* and Sakai *et al.* (2003) achieved a higher  
382           concentration of 80 g/L by applying an enzymatic pretreatment and an inoculation with  
383           *L. rhamnosus*. In conclusion, the fed-batch feeding strategy seems to be an appropriate

384 strategy to enhance LA production from FW.

385

### 386 3.2.3. Assessment of the process robustness in repeated fed-batch fermentation

387 Repeated batch or fed-batch fermentation, which involves the inoculation of a  
388 reactor with fermentation residues from the previous one (Zhao *et al.*, 2010), has been  
389 described as a good strategy to improve LA fermentation while reducing operational  
390 cost (no cleaning of the fermenter and no seed purchase or preparation ; as summarized  
391 in Abdel-Rahman *et al.*, 2013). Hence, 3 repeated fed-batch runs were carried out using  
392 compost and leachate as a seed for the first one (Fed-batch 1) and then fermentation  
393 residues from the previous run for the two others (Fed-batch 2 and 3). These  
394 fermentation residues had a TS content of 18%, a VS/TS content of 79% and their  
395 microbial community was rich in *Lactobacillus* (over 99% in relative abundance).

396 The fermentation profile, maximal LA concentration (68.5 g/L for Fed-batch 1,  
397 64.8 g/L for Fed-batch 2 and 61.2 g/L for Fed-batch 3; Fig. 2a) and selectivity  
398 ( $77\%_{\text{molOA}}$  for Fed-batch 1,  $71\%_{\text{molOA}}$  for Fed-batch 2 and  $70\%_{\text{molOA}}$  for Fed-batch 3;  
399 Fig. 2b) were similar for every run, indicating that LA fermentation is stable in a  
400 repeated fed-batch configuration although a minor decrease in fermentation  
401 performance can be observed at the beginning of the 3<sup>rd</sup> fermentation run. The initial  
402 microbial community was slightly richer (2.5% vs 1%) in *Lactobacillus* when the  
403 reactor was reinoculated with fermentation residues from a previous run (Fig. 2c). In  
404 every run, the microbial community was rapidly dominated by *Lactobacillus* with more  
405 than 99% of *Lactobacillus* observed after 4 days of fermentation (Fig. 2c). In a previous  
406 study conducted using waste activate sludge as an inoculum, authors have shown the  
407 stability of LA production for nine repeated batch cycles. They reported an increase

408 (from 26% in batch 1 to 44.5% in repeated cycle 6) of the relative abundance of LAB  
409 genera (*i.e.*, *Alkaliphilus*, *Dysgonomonas*, *Enterococcus* and *Bifidobacterium*) but also  
410 of propionic acid producing microorganisms (Xu *et al.*, 2020).

411 To conclude, the recirculation of fermentation residues rich in *Lactobacillus* from  
412 one batch to the next did not increased *Lactobacillus* propagation kinetics nor LA  
413 production efficiency. This could be due to the high concentration of endogenous  
414 micro-organisms in FW. However, operating in successive batches will allow for cost  
415 reduction and process stabilization especially when operating using FW with variable  
416 endogenous microbial community. To be truly meaningful, this experiment should be  
417 further continued to determine the maximum number of cycles that can be performed.

418

419 3.3. *Integration of the process into a complete value chain: biogas production*  
420 *using fermentation residues*

421 Coupling LA fermentation to anaerobic digestion of solid residues has been  
422 proposed as a suitable strategy to improve the process rentability. However, only a few  
423 studies demonstrated the possibility to recover both LA and biogas from FW  
424 (Demichelis *et al.*, 2017; Kim *et al.*, 2016). Therefore, in this study, the methane  
425 production potential of LA-fermented residues was determined.

426 After 35 days, the methane potential of fermented FW reached  $365 \pm 4$  NmL CH<sub>4</sub>  
427 per g of FW initial volatile solids (*i.e.*, before fermentation), which was similar to the  
428 methane potential of non-fermented FW ( $366 \pm 7$  NmL CH<sub>4</sub> per gVS(FW)) indicating  
429 that fermentation did not induced a loss of the methane potential. Moreover, the  
430 methane produced after 17 days was even higher when using fermented FW ( $346 \pm 3$   
431 NmL CH<sub>4</sub> per gVS(FW) *vs*  $333 \pm 6$  NmL CH<sub>4</sub> per gVS(FW)) most likely because

432 fermented substrate has already been hydrolyzed during fermentation. This result is  
433 consistent with a previous study (Demichelis *et al.*, 2017).

434 By extrapolating the results obtained in this study at pilot scale and assuming an  
435 extraction of 75% of the produced LA (using a pre-purification step followed by an ion  
436 exchange and vacuum distillation as performed in Alvarado-Morales *et al.*, 2021), the  
437 final products would theoretically be 0.31 gLA/gVS(FW) and 250 NmL CH<sub>4</sub>/gVS(FW)  
438 (Fig. 3). Therefore, 58 kg LA and 47 Nm<sup>3</sup> CH<sub>4</sub> can be produced per ton FW (at 20%<sub>TS</sub>),  
439 using a two-stage process with FW fermentation in LA followed by AD of the  
440 remaining residues.

441

442 3.4. *Efficiency of the developed process for the fermentation of an industrial*  
443 *food waste stream: depackaging pulp*

444 Most of the work carried out in this study was realized using model FW for  
445 simplicity and repeatability reasons. However, once the best operational parameters  
446 have been identified, it is important to transpose them to available industrial FW  
447 streams. Hence, fermentation assays were performed using undiluted depackaging pulp.

448 A lower maximal LA concentration ( $39.2 \pm 0.4$  g/L), yield ( $0.21 \pm 0.0$  g/gTS),  
449 productivity ( $0.23 \pm 0.0$  g/L/h) and selectivity ( $70 \pm 0.0\%$ <sub>molOA</sub>) was achieved using  
450 depackaging pulp (Fig. 4a-b) compared to the performance obtained with model FW  
451 ( $68.5$  g/L;  $0.38$  g/gTS;  $0.95$  g/L/h;  $77\%$ <sub>molOA</sub>). This can be partially explained by the fact  
452 that there is slightly less volatile matter in depackaging pulp ( $86\%$ <sub>TS</sub> vs  $89.5\%$ <sub>TS</sub> in  
453 model FW) and that a part of depackaging pulps volatile matter is composed of non-  
454 biodegradable plastics. Moreover, the soluble sugars content (especially fructose) in  
455 model FW was twice higher than in the depackaging pulp. Interestingly, the relative

456 abundance of *Lactobacillus* at the beginning of the depackaging pulp fermentation was  
457 80% but this was not correlated with a high initial amount of LA. This higher relative  
458 abundance can then be explained by the lower amount of microbial biomass in  
459 deconditioning pulp compared to FW. LA fermentation from depackaging pulp was  
460 driven by *Lactobacillus* which dominated the microbial community (Fig. 4c). Hence,  
461 the lower LA concentration achieved with depackaging pulp is more likely correlated to  
462 the lower sugars content of this substrate than to an unsuited process, since a high  
463 selectivity for LA and a *Lactobacillus* dominated microorganism community were  
464 observed. In conclusion, the developed process is suitable for depackaging pulp  
465 fermentation into LA but the valorization of the high quantity of fermentation residues  
466 should be considered (not address in this study). To our knowledge, this is the first pilot  
467 scale fermentation of a low quality and already industrially available food waste  
468 substrate.

469

#### 470 **4. Conclusions**

471 Operating fermentation conditions were tested and validated in this study to drive the  
472 development of a *Lactobacillus* dominated microbial community selected from  
473 endogenous bacterial consortia. Among key parameters, pH was the most critical factor  
474 enhancing LA production from model and industrial FW. At pilot scale, 68 g/L of LA  
475 were produced in successive fed-batch fermentations showing the efficiency and  
476 robustness of the process. The high LA selectivity (77%<sub>molOA</sub>) and the high methane  
477 potential (250 NmLCH<sub>4</sub>/gVS(FW)) of the remaining fermentation residues strengthen  
478 the process promising industrial potential. Lastly, further optimization is required to

479 increase yields when applied to industrial waste such as depackaging pulps, for which  
480 lower LA concentration (39.2 g/L) were produced in the current study.

481

482 **E-supplementary data for this work can be found in e-version of this paper**  
483 **online**

484

#### 485 **CRediT authorship contribution statement**

486 **Célia Chenebault:** Data Curation, Writing - original draft, Writing - review &  
487 editing; **Roman Moscoviz:** Conceptualization, Writing - review & editing; **Eric**  
488 **Trably:** Conceptualization, Supervision, Writing - review & editing; **Renaud Escudié:**  
489 Conceptualization, Supervision, Writing - review & editing; **Benjamin Percheron:**  
490 Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

491

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680

681 **Tables and figures captions**

682 **Table 1. Characteristics of the substrates and inocula**

683 **Table 2. Model food waste fermentation performance depending on the key**

684 **operational conditions (inoculum, temperature, pH).** Error bars represent standard

685 deviation from  $n \geq 3$  experimental replicates. ND = Not determined

686

687 **Fig. 1. Model food waste fermentation depending on the pH regulation mode**

688 **(uncontrolled or regulated at pH 5 or 6 every two days).** Evolution of the LA

689 concentration (a.), selectivity over other OA (b.) and microbial community (c.). Error

690 bars represent standard deviation from 3 experimental replicates.

691 LA = Lactic Acid; AA = Acetic Acid; PA= Propionic Acid; FA= Formic Acid; BA =

692 Butyric and isobutyric Acid

693

694 **Fig. 2. Fed batch fermentation of model food waste at pilot scale (12 L) during 3**  
695 **consecutive cycles.** Evolution of the LA concentration (a.), selectivity over other OA  
696 (b.) and microbial community (c.).

697 LA = Lactic Acid; AA = Acetic Acid; PA= Propionic Acid

698

699 **Fig. 3. Mass balance of conventional food waste valorization chain through**  
700 **anaerobic digestion compared to the proposed two stages valorization chain**

701

702 **Fig. 4. Fed batch fermentation of a commercial and industrial food waste stream**  
703 **(depackaging pulp) at pilot scale (12 L).** Evolution of the LA concentration (a.),  
704 selectivity over other OA (b.) and microbial community (c.). Error bars represent the  
705 minimum and maximum of the 2 experimental replicates.

706 LA = Lactic Acid; AA = Acetic Acid; PA= Propionic Acid

707 **Table 1.**

	% <sub>TS</sub> [gTS/100 g product]	% <sub>VS/TS</sub>	Methane production potential (NmL/gVS)
<b>Model FW</b>	25.13%	89.53%	366 ± 7
<b>Canteen FW</b>	25.62%	94.66%	Not measured
<b>Depackaging pulp</b>	19.8%	86%	Not measured
<b>Yoghurt</b>	14.51%	94.43%	Not measured
<b>Leachate</b>	3.02%	59.65%	205
<b>Compost</b>	55.54%	46.43%	Not measured

708

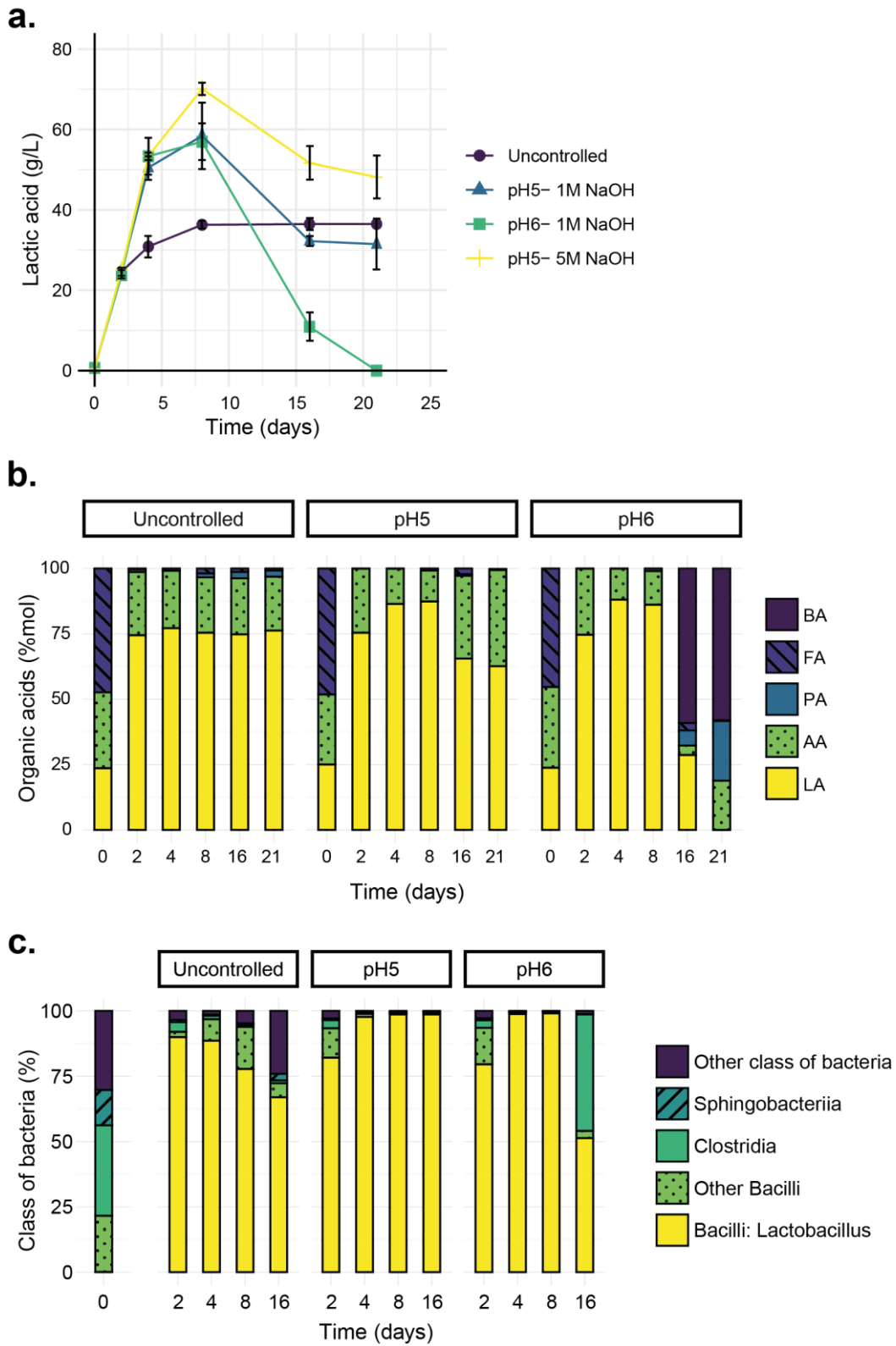
709 **Table 2.**

Number of experiments * replicates	Inoculum	Temperature	pH	Fermentation time	Maximum LA concentration (g/L)	Average productivity (g/L/h)	Yield (g/g TS FW)	Selectivity (% <sub>mol<sup>OA</sup></sub> )	Hetero-fermentation (mol Ethanol produced per mol of lactate)
N = 1 * 3	No inoculum	35	Uncontrolled	8 days	15.9 ± 0.5	0.08 ± 0.0	0.09 ± 0.0	66% ± 2%	ND
N = 5 * 3	Compost + 18% yoghurt	35	Uncontrolled	8 days	36.3 ± 1.3	0.19 ± 0.01	0.20 ± 0.01	77% ± 2%	0.18 ± 0.03
N = 1 * 3	Compost + 72% yoghurt	35	Uncontrolled	8 days	33.5 ± 0.5	0.17 ± 0.0	0.19 ± 0.0	79% ± 0.5%	0.13 ± 0.02
N = 1 * 3	Compost + 18% yoghurt	24	Uncontrolled	15 days	29.9 ± 0.2	0.08 ± 0.0	0.17 ± 0.0	78% ± 1%	0.14 ± 0.02
N = 2 * 3	Compost + 18% yoghurt	55	Uncontrolled	4 days	10.5 ± 0.8	0.11 ± 0.01	0.06 ± 0.0	61% ± 3.6	0.0 ± 0.0
N = 1 * 3	Compost + 18% yoghurt	35	pH 6 NaOH = 1 M	8 days	57.0 ± 4.8	0.30 ± 0.02	0.32 ± 0.03	86% ± 1%	0.21 ± 0.01
N = 2 * 3	Compost + 18% yoghurt	35	pH 5 NaOH = 1 M	8 days	58.4 ± 6.6	0.30 ± 0.03	0.33 ± 0.04	87% ± 2%	0.20 ± 0.02
N = 1 * 3	Compost + 18% yoghurt	35	pH 5 NaOH = 5 M	8 days	70.1 ± 1.5	0.37 ± 0.01	0.39 ± 0.01	89% ± 2%	0.18 ± 0.0

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712 **Fig. 1**

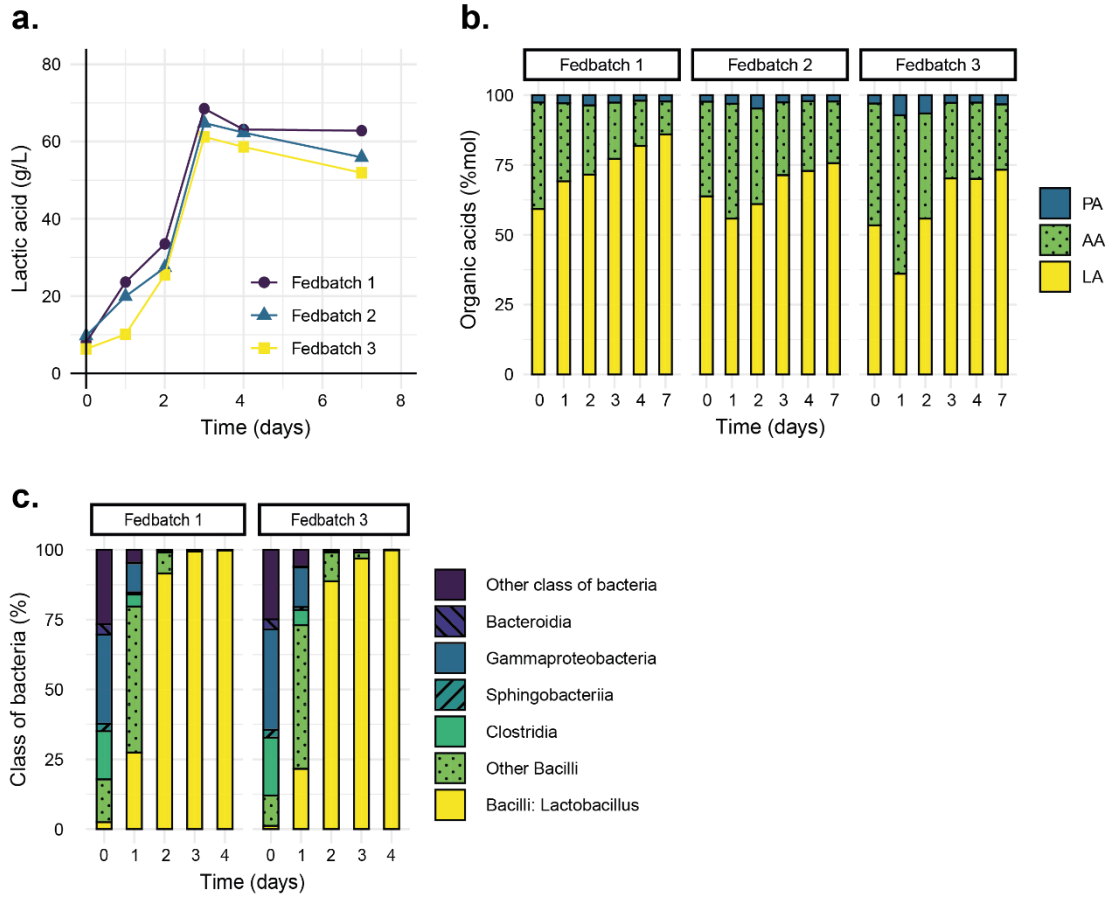


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715 **Fig. 2**

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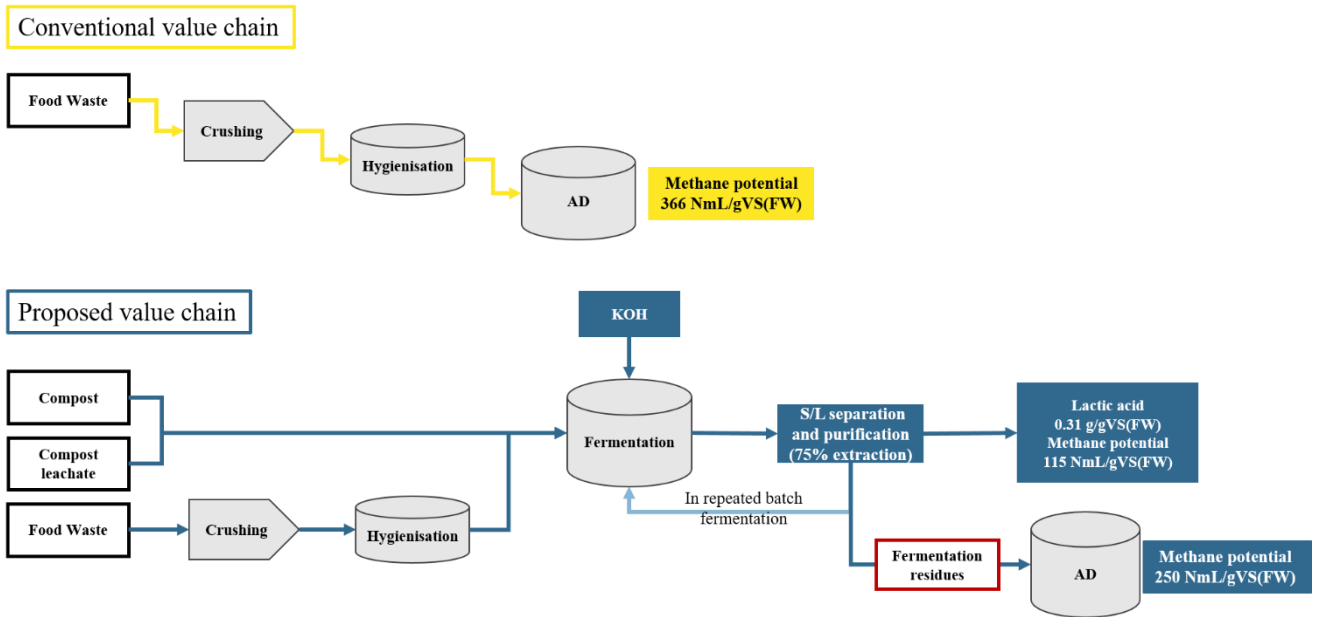
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722 **Fig. 3**

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**Fig 4.**

