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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%_{TS} 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%_{TS} and
116 89.53%_{VS/TS} (Table 1). The total carbohydrates content of the model FW was estimated
117 at 0.69 g/g_{TS}, its lipids content at 0.07 g/g_{TS} and its crude protein content at 0.17
118 g/g_{TS}. 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 NH_4^+ and 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%_{TS}. 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 (CO₂ and H₂) was also
148 regularly quantified. After each opening, the flasks were purged with N₂ 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. H₂SO₄ 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 H₂, O₂, N₂ and CH₄) and a RtMolsieve column (for CO₂). 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 (%_{molOA}).

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 = Δ concentration / Δ 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 ± 1.3 g/L
231 vs 15.9 ± 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 ± 0.2 compared to
235 5.1 ± 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_i = 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%_{molOA} and 77 ± 2%_{molOA} respectively) and lower at 55°C (61 ±
263 3.6%_{molOA}). 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 H₂ 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 ± 2.6 g/L
289 LA after 4 days and 36.3 ± 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 ± 6.6 g/L vs 57.0 ± 4.8 g/L; Table 2 and Fig. 1a). However, as the
292 fermentation continued, an important decrease in LA concentration (from 57.0 ± 4.8 g/L
293 at day 8 to 0 g/L at day 21; Fig. 1a) and specificity (from $86 \pm 1\%$ _{molOA} to 0% _{molOA}; 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 ± 1.5 g/L),
307 productivity (0.37 ± 0.01 g/L/h) and yield (0.39 ± 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 ± 0.0 mol
310 per mol of LA; Table 2) and high selectivity ($89 \pm 2\%$ _{molOA}) 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%_{molOA}) 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 ± 1.1 g/L for real vs 70.1 ± 1.5 g/L for model FW), productivity
325 (0.35 ± 0.01 g/L/h for real vs 0.37 ± 0.01 g/L/h for model FW), yield (0.37 ± 0.01 g/gTS
326 for real vs 0.39 ± 0.01 g/gTS for model FW) and a good selectivity (over 90%_{molOA} for
327 real vs 89%_{molOA} 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 ± 10.8
352 g/L with NaOH and 55.8 ± 9.2 g/L with KOH), yield (0.31 ± 0.06 g/gTS with NaOH
353 and 0.29 ± 0.05 g/gTS with KOH), productivity (0.28 ± 0.05 g/L/h with NaOH and 0.26
354 ± 0.04 g/L/h with KOH) and selectivity ($80 \pm 6.6\%$ _{molOA} with NaOH vs $79 \pm 0.4\%$ _{molOA}
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%_{TS}). 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%_{molOA})
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%_{molOA}). 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 3rd 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 ± 4 NmL CH₄
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 ± 7 NmL CH₄ 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 ± 3
431 NmL CH₄ per gVS(FW) *vs* 333 ± 6 NmL CH₄ 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 CH₄/gVS(FW)
438 (Fig. 3). Therefore, 58 kg LA and 47 Nm³ CH₄ can be produced per ton FW (at 20%_{TS}),
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 ± 0.4 g/L), yield (0.21 ± 0.0 g/gTS),
449 productivity (0.23 ± 0.0 g/L/h) and selectivity ($70 \pm 0.0\%$ _{molOA}) 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% _{molOA}). This can be partially explained by the fact
452 that there is slightly less volatile matter in depackaging pulp (86% _{TS} vs 89.5% _{TS} 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%_{molOA}) and the high methane
477 potential (250 NmLCH₄/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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496

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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.**

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

708

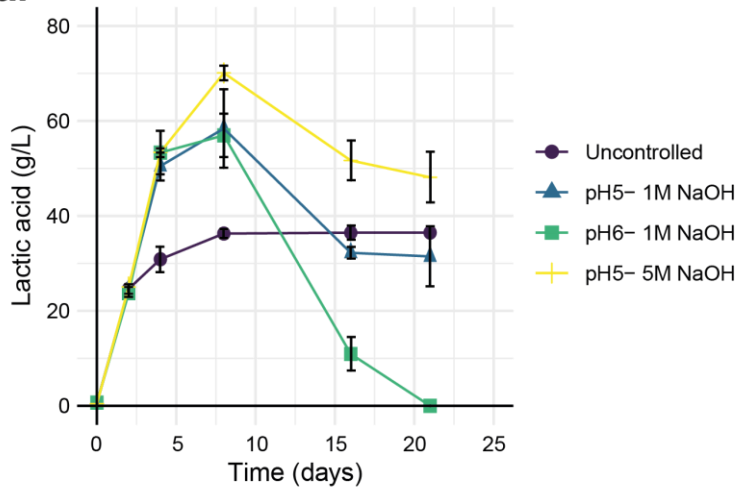
709 **Table 2.**

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 (% _{mol^{OA}})	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

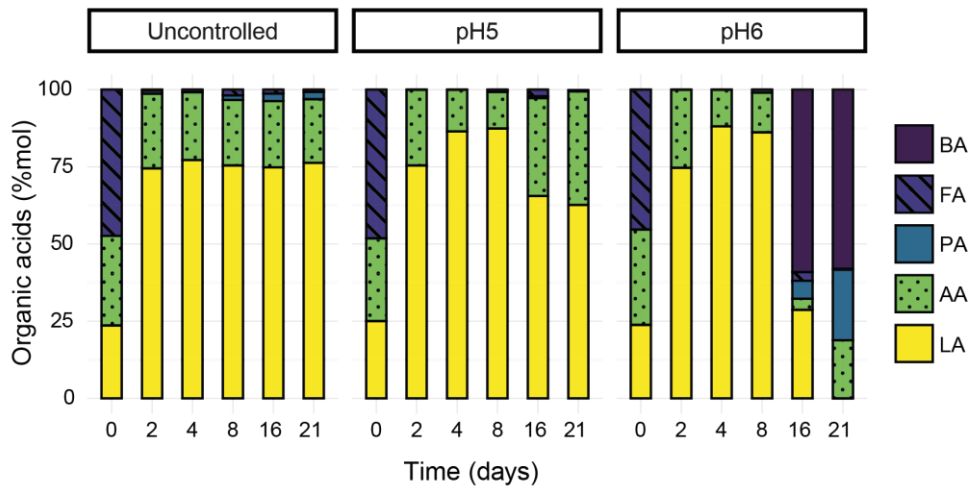
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712 **Fig. 1**

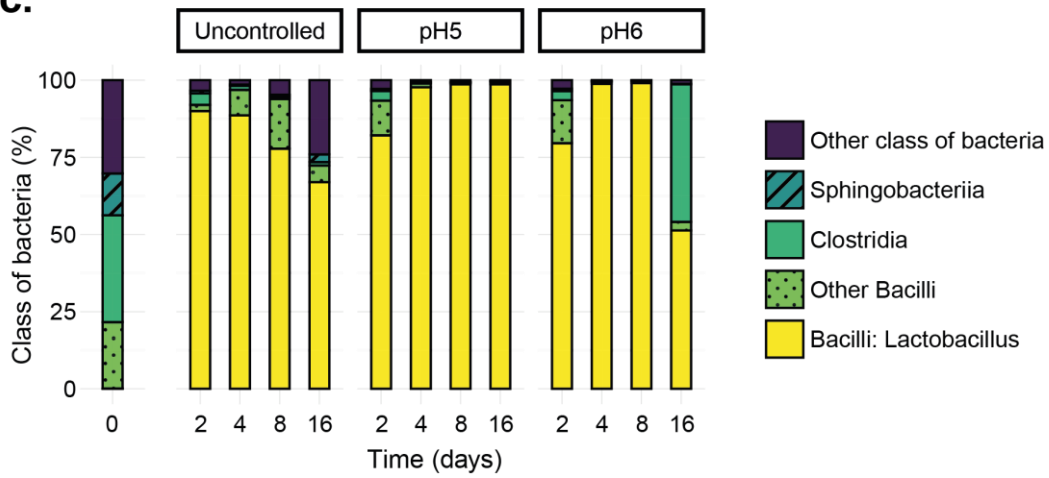
a.



b.



c.

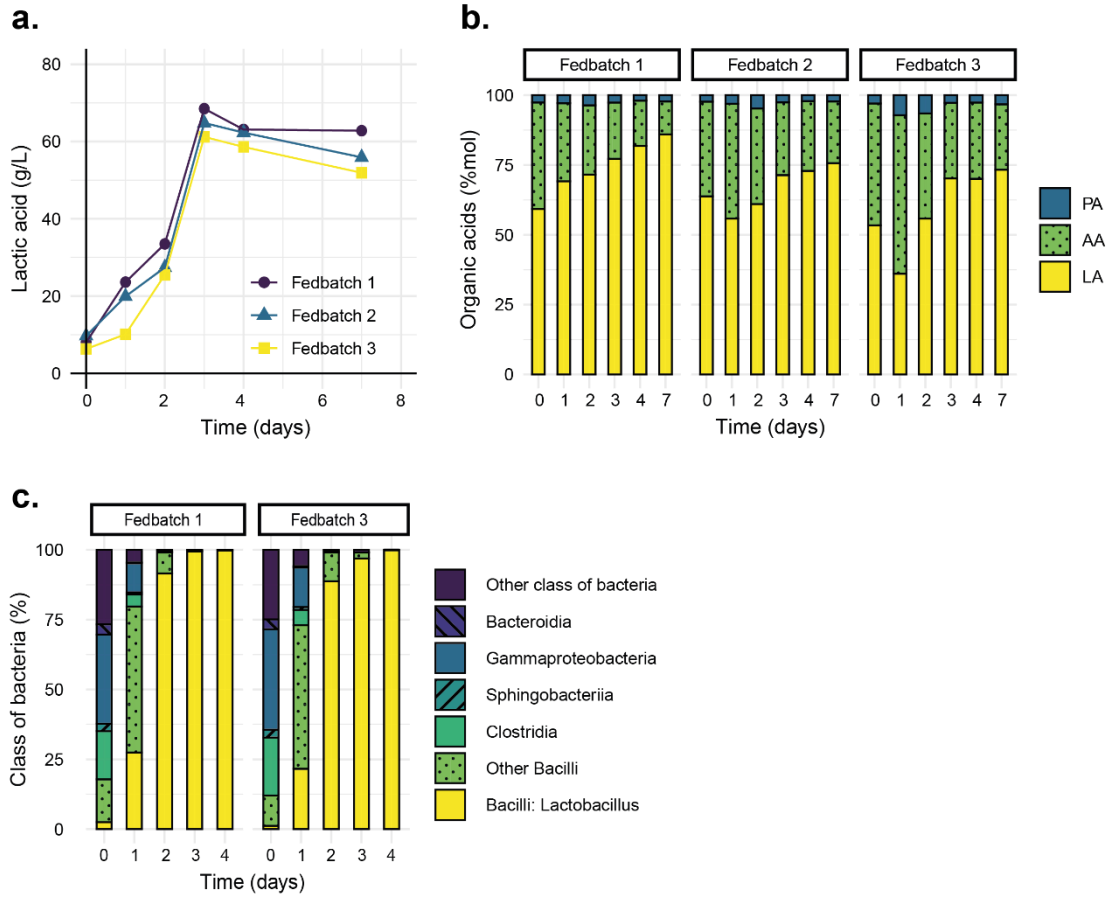


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715 **Fig. 2**

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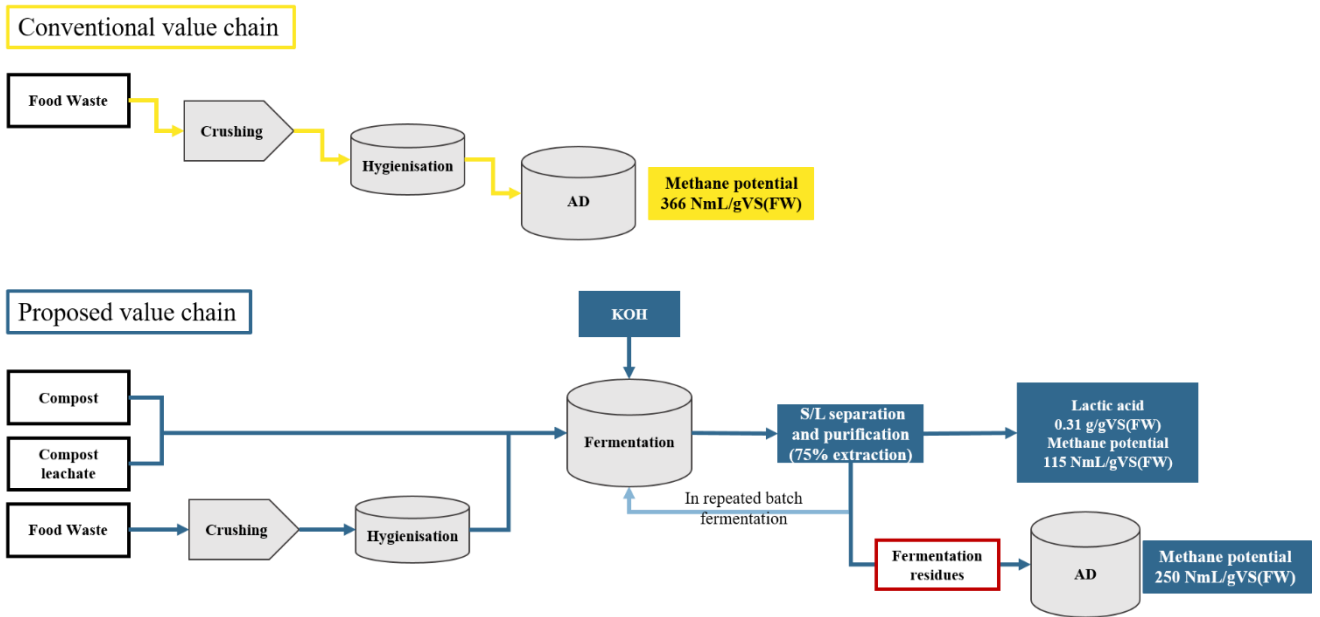
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722 **Fig. 3**

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Fig 4.

