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

Célia Chenebault, Roman Moscoviz, Eric Trably, Renaud Escudié, Benjamin Percheron

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

Célia Chenebault [a], Roman Moscoviz [a], Eric Trably [b], Renaud Escudié [b], Benjamin Percheron [a]*

[a] Suez, CIRSEE, 38 rue du Président Wilson, 78230 Le Pecq, France

[b] LBE, INRAE, Univ Montpellier, 102 Avenue des Etangs, Narbonne, F-11100, France

* **Corresponding author**: benjamin.percheron@suez.com

Abstract

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

Keywords

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

1. Introduction

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

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

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

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

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

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

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

2. Materials and Methods

2.1. Substrate and inoculum

A model FW was prepared according to Capson-Tojo *et al.* (2017) to ensure the relative stability and reproducibility of the substrate properties during the experiments. This substrate, composed of fruits and vegetables (25.9% apple and 25.9% lettuce), carbohydrates (25.9% potato, 4.8% wheat meal and 6.2% bread), meat (4.1% chicken and 4.1% beef), dairy products (1.9% yoghurt) and pastries (1.5% cookies), is representative of real FW collected in Europe from households or canteens and has already been used as substrate in previous research on anaerobic digestion (Capson-Tojo *et al.*, 2017). The measured TS and VS content of this model FW is $25.13\%_{TS}$ and $89.53\%_{VS/TS}$ (Table 1). The total carbohydrates content of the model FW was estimated at 0.69 g/gTS, its lipids content at 0.07 g/gTS and its crude protein content at 0.17 g/gTS. The C/N ratio is 16.3 g/g and the pH of this model FW was estimated to be 5.6 (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. bulgaricu*s 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 (CO_2 and H_2) was also
148 regularly quantified. After each opening, the flasks were purged with N_2 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 TELEDO 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_2SO_4 at 4 mM
185 was used at mobile phase. A pre-column (Micro guard cation H refill cartbridges, 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

Elmer) equipped with a thermal conductivity detector. The columns used were a RtQbond column (for H_2, O_2, N_2 and CH_4) and a RtMolsieve column (for CO_2). Argon was used as mobile phase.

2.6. Calculation

Four performance indicators (see equation 1 to 4) were monitored: LA concentration in the reaction medium (g/L), the yield of LA produced depending on the substrate introduced (g/gTS), the productivity (g/L/h) and the selectivity for LA production over other organics acids ($\%_{molOA}$).

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

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

Eq. (3) Productivity = Δ concentration / Δ hours

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

2.7. Microbial community analysis

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

3. Results and Discussion

3.1. Identification of key parameters for industrially relevant food waste

fermentation

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

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

At 35°C and without pH control, the maximal concentration of LA (36.3 ± 1.3 g/L *vs* 15.9 ± 0.5 g/L) and its selectivity (77 ± 2%$_{molOA}$ *vs* 66 ± 2%$_{molOA;}$ see supplementary material) were higher when an inoculum consisting of a mixture of yoghurt and compost was added compared to a fermentation without exogeneous inoculation. First, addition of compost induced a higher initial pH with a value of 6.3 ± 0.2 compared to 5.1 ± 0.1 without inoculation. In addition, 16S rRNA gene sequencing at the beginning of the experiment indicated that the initial microbial community structure was similar with or without addition of inoculum (main bacterial class are *Clostridia*: the genus *MBA03_ge* representing 25.2 ± 2% of the total microorganism relative abundance; *Sphingobacteriia*: an unidentified genus from the *Lentimicrobiaceae family* representing

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

Temperature is known to play an important role in LA fermentation (Song *et al.*, 2021) due to the higher hydrolysis rates of FW at high temperature and to a modification of the microbial community structure and enzymatic activities (Tang *et al.*, 2016). In this study, maximal LA concentration was higher at 35°C (36.3 ± 1.3 g/L) than at 24°C (29.9 ± 0.2 g/L) or 55°C (10.5 ± 0.8 g/L). LA selectivity was similar at 24°C and 35°C (78 ± 1%$_{molOA}$ and 77 ± 2%$_{molOA}$ respectively) and lower at 55°C (61 ± 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 H_2 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 unknow (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

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

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

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

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

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

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

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

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

relevant conditions

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

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

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

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

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

The final concentration, yield and productivity of LA obtained in this study were higher than the ones reported by Farah *et al.* (2009) at pilot scale operating in similar conditions (57.6 g/L, 0.8 g/L/h and 0.32 g/gTS respectively). However, Pleissner *et al.* (2017) reported an higher productivity of 2 g/L/h related to the use of a specific inoculation with *Streptococcus sp.* and Sakai *et al.* (2003) achieved a higher concentration of 80 g/L by applying an enzymatic pretreatment and an inoculation with *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 ($77\%_{molOA}$ for Fed-batch 1, $71\%_{molOA}$ for Fed-batch 2 and $70\%_{molOA}$ 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

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

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

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

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

After 35 days, the methane potential of fermented FW reached 365 ± 4 NmL CH_4 per g of FW initial volatile solids (*i.e.*, before fermentation), which was similar to the methane potential of non-fermented FW (366 ± 7 NmL CH_4 per gVS(FW)) indicating that fermentation did not induced a loss of the methane potential. Moreover, the methane produced after 17 days was even higher when using fermented FW (346 ± 3 NmL CH_4 per gVS(FW) *vs* 333 ± 6 NmL CH_4 per gVS(FW)) most likely because

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

By extrapolating the results obtained in this study at pilot scale and assuming an extraction of 75% of the produced LA (using a pre-purification step followed by an ion exchange and vacuum distillation as performed in Alvarado-Morales *et al.*, 2021), the final products would theoretically be 0.31 gLA/gVS(FW) and 250 NmL CH_4/gVS(FW) (Fig. 3). Therefore, 58 kg LA and 47 Nm^3 CH_4 can be produced per ton FW (at 20%$_{TS}$), using a two-stage process with FW fermentation in LA followed by AD of the remaining residues.

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

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

A lower maximal LA concentration (39.2 ± 0.4 g/L), yield (0.21 ± 0.0 g/gTS), productivity (0.23 ± 0.0 g/L/h) and selectivity (70 ± 0.0%$_{molOA}$) was achieved using depackaging pulp (Fig. 4a-b) compared to the performance obtained with model FW (68.5 g/L; 0.38 g/gTS; 0.95 g/L/h; 77%$_{molOA}$). This can be partially explained by the fact that there is slightly less volatile matter in depackaging pulp (86%$_{TS}$ *vs* 89.5%$_{TS}$ in model FW) and that a part of depackaging pulps volatile matter is composed of non-biodegradable plastics. Moreover, the soluble sugars content (especially fructose) in 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 abondance 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 $NmLCH_4/gVS(FW)$) of the remaining fermentation residues strengthen
478 the process ~~promising~~ industrial potential. Lastly, further optimization is required to

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

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

CRediT authorship contribution statement

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

Acknowledgements

Authors want to express their gratitude to Antoine Valentin and Bintou Diakite for their participation in the laboratory work and their help for data collection. This work was supported by SUEZ through internal funding.

References

1. Abdel-Rahman, M.A., Sonomoto, K., 2016. Opportunities to overcome the current limitations and challenges for efficient microbial production of optically pure lactic acid. J. Biotechnol. 236, 176–192. https://doi.org/10.1016/j.jbiotec.2016.08.008
2. Abdel-Rahman, M.A., Tashiro, Y., Sonomoto, K., 2013. Recent advances in lactic acid production by microbial fermentation processes. Biotechnol. Adv., “Bioenergy

503 and Biorefinery from Biomass" through innovative technology development 31,
504 877–902. https://doi.org/10.1016/j.biotechadv.2013.04.002
505 3. Alexandri, M., Blanco-Catalá, J., Schneider, R., Turon, X., Venus, J., 2020. High
506 L(+)-lactic acid productivity in continuous fermentations using bakery waste and
507 lucerne green juice as renewable substrates. Bioresour. Technol. 316, 123949.
508 https://doi.org/10.1016/j.biortech.2020.123949
509 4. Alvarado-Morales, M., Kuglarz, M., Tsapekos, P., Angelidaki, I., 2021. Municipal
510 biopulp as substrate for lactic acid production focusing on downstream processing.
511 J. Environ. Chem. Eng. 9, 105136. https://doi.org/10.1016/j.jece.2021.105136
512 5. Alves de Oliveira, R., Komesu, A., Vaz Rossell, C.E., Maciel Filho, R., 2018.
513 Challenges and opportunities in lactic acid bioprocess design—From economic to
514 production aspects. Biochem. Eng. J. 133, 219–239.
515 https://doi.org/10.1016/j.bej.2018.03.003
516 6. Arras, W., Hussain, A., Hausler, R., Guiot, S.R., 2019. Mesophilic, thermophilic
517 and hyperthermophilic acidogenic fermentation of food waste in batch: Effect of
518 inoculum source. Waste Manag. 87, 279–287.
519 https://doi.org/10.1016/j.wasman.2019.02.011
520 7. Bonk, F., Bastidas-Oyanedel, J.-R., Yousef, A.F., Schmidt, J.E., 2017. Exploring the
521 selective lactic acid production from food waste in uncontrolled pH mixed culture
522 fermentations using different reactor configurations. Bioresour. Technol. 238, 416–
523 424. https://doi.org/10.1016/j.biortech.2017.04.057
524 8. Capson-Tojo, G., Rouez, M., Crest, M., Steyer, J.-P., Delgenès, J.-P., Escudié, R.,
525 2016. Food waste valorization via anaerobic processes: a review. Rev. Environ. Sci.
526 Biotechnol. 15, 499–547. https://doi.org/10.1007/s11157-016-9405-y

9. Capson-Tojo, G., Trably, E., Rouez, M., Crest, M., Steyer, J.-P., Delgenès, J.-P., Escudie, R., 2017. Dry anaerobic digestion of food waste and cardboard at different substrate loads, solid contents and co-digestion proportions. Bioresour. Technol. 233. https://doi.org/10.1016/j.biortech.2017.02.126
10. Chen, Y., Geever, L.M., Killion, J.A., Lyons, J.G., Higginbotham, C.L., Devine, D.M., 2016. Review of Multifarious Applications of Poly (Lactic Acid). Polym.-Plast. Technol. Eng. 55, 1057–1075. https://doi.org/10.1080/03602559.2015.1132465
11. Demichelis, F., Pleissner, D., Fiore, S., Mariano, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J., 2017. Investigation of food waste valorization through sequential lactic acid fermentative production and anaerobic digestion of fermentation residues. Bioresour. Technol. 241, 508–516. https://doi.org/10.1016/j.biortech.2017.05.174
12. Dou, Z., Toth, J.D., 2021. Global primary data on consumer food waste: Rate and characteristics – A review. Resour. Conserv. Recycl. 168, 105332. https://doi.org/10.1016/j.resconrec.2020.105332
13. Dreschke, G., Probst, M., Walter, A., Pümpel, T., Walde, J., Insam, H., 2015. Lactic acid and methane: Improved exploitation of biowaste potential. Bioresour. Technol. 176, 47–55. https://doi.org/10.1016/j.biortech.2014.10.136
14. Farah, N.O., Norrsquo Aini, A.R., Halimatun, S.H., Phang, L.Y., Mohd, A.H., 2009. Separation and recovery of organic acids from fermented kitchen waste by an integrated process. Afr. J. Biotechnol. 8, 5807–5813. https://doi.org/10.5897/AJB09.992

15. Feng, K., Li, H., Zheng, C., 2018. Shifting product spectrum by pH adjustment during long-term continuous anaerobic fermentation of food waste. Bioresour. Technol. 270, 180–188. https://doi.org/10.1016/j.biortech.2018.09.035
16. Hussain, A., Filiatrault, M., Guiot, S.R., 2017. Acidogenic digestion of food waste in a thermophilic leach bed reactor: Effect of pH and leachate recirculation rate on hydrolysis and volatile fatty acid production. Bioresour. Technol. 245, 1–9. https://doi.org/10.1016/j.biortech.2017.08.130
17. Kim, K.I., Kim, W.K., Seo, D.K., Yoo, I.S., Kim, E.K., Yoon, H.H., 2003. Production of Lactic Acid from Food Wastes. Appl. Biochem. Biotechnol. 107, 637–648. https://doi.org/10.1385/ABAB:107:1-3:637
18. Kim, M.-S., Na, J.-G., Lee, M.-K., Ryu, H., Chang, Y.-K., Triolo, J.M., Yun, Y.-M., Kim, D.-H., 2016. More value from food waste: Lactic acid and biogas recovery. Water Res. 96, 208–216. https://doi.org/10.1016/j.watres.2016.03.064
19. Kitpreechavanich, V., Hayami, A., Talek, A., Chin, C.F.S., Tashiro, Y., Sakai, K., 2016. Simultaneous production of l-lactic acid with high optical activity and a soil amendment with food waste that demonstrates plant growth promoting activity. J. Biosci. Bioeng. 122, 105–110. https://doi.org/10.1016/j.jbiosc.2015.12.017
20. Kwan, T.H., Hu, Y., Lin, C.S.K., 2016. Valorisation of food waste via fungal hydrolysis and lactic acid fermentation with Lactobacillus casei Shirota. Bioresour. Technol. 217, 129–136. https://doi.org/10.1016/j.biortech.2016.01.134
21. Li, X., Chen, Y., Zhao, S., Chen, H., Zheng, X., Luo, J., Liu, Y., 2015. Efficient production of optically pure l-lactic acid from food waste at ambient temperature by regulating key enzyme activity. Water Res. 70, 148–157. https://doi.org/10.1016/j.watres.2014.11.049

22. Li, X., Sadiq, S., Zhang, W., Chen, Y., Xu, X., Abbas, A., Chen, S., Zhang, R., Xue, G., Sobotka, D., Makinia, J., 2021. Salinity enhances high optically active L-lactate production from co-fermentation of food waste and waste activated sludge: Unveiling the response of microbial community shift and functional profiling. Bioresour. Technol. 319, 124124. https://doi.org/10.1016/j.biortech.2020.124124
23. López-Garzón, C.S., Straathof, A.J.J., 2014. Recovery of carboxylic acids produced by fermentation. Biotechnol. Adv. 32, 873–904. https://doi.org/10.1016/j.biotechadv.2014.04.002
24. López-Gómez, J.P., Alexandri, M., Schneider, R., Latorre-Sánchez, M., Coll Lozano, C., Venus, J., 2020. Organic fraction of municipal solid waste for the production of L-lactic acid with high optical purity. J. Clean. Prod. 247, 119165. https://doi.org/10.1016/j.jclepro.2019.119165
25. Moscoviz, R., Trably, E., Bernet, N., 2016. Consistent 1,3-propanediol production from glycerol in mixed culture fermentation over a wide range of pH. Biotechnol. Biofuels 9, 32. https://doi.org/10.1186/s13068-016-0447-8
26. Motte, J.-C., Escudié, R., Beaufils, N., Steyer, J.-P., Bernet, N., Delgenès, J.-P., Dumas, C., 2014. Morphological structures of wheat straw strongly impacts its anaerobic digestion. Ind. Crops Prod. 52, 695–701. https://doi.org/10.1016/j.indcrop.2013.11.038
27. Nagaoka, S., 2019. Yogurt Production. Methods Mol. Biol. Clifton NJ 1887, 45–54. https://doi.org/10.1007/978-1-4939-8907-2_5
28. Nayak, A., Bhushan, B., 2019. An overview of the recent trends on the waste valorization techniques for food wastes. J. Environ. Manage. 233, 352–370. https://doi.org/10.1016/j.jenvman.2018.12.041

29. Ohkouchi, Y., Inoue, Y., 2006. Direct production of l(+)-lactic acid from starch and food wastes using Lactobacillus manihotivorans LMG18011. Bioresour. Technol. 97, 1554–1562. https://doi.org/10.1016/j.biortech.2005.06.004
30. Pleissner, D., Demichelis, F., Mariano, S., Fiore, S., Navarro Gutiérrez, I.M., Schneider, R., Venus, J., 2017. Direct production of lactic acid based on simultaneous saccharification and fermentation of mixed restaurant food waste. J. Clean. Prod. 143, 615–623. https://doi.org/10.1016/j.jclepro.2016.12.065
31. Probst, M., Walde, J., Pümpel, T., Wagner, A.O., Insam, H., 2015. A closed loop for municipal organic solid waste by lactic acid fermentation. Bioresour. Technol. 175, 142–151. https://doi.org/10.1016/j.biortech.2014.10.034
32. RedCorn, R., Engelberth, A.S., 2016. Identifying conditions to optimize lactic acid production from food waste co-digested with primary sludge. Biochem. Eng. J. 105, 205–213. https://doi.org/10.1016/j.bej.2015.09.014
33. Sakai, K., Taniguchi, M., Miura, S., Ohara, H., Matsumoto, T., Shirai, Y., 2003. Making Plastics from Garbage.: A Novel Process for Poly-L-Lactate Production from Municipal Food Waste. J. Ind. Ecol. 7, 63–74. https://doi.org/10.1162/108819803323059406
34. Sakai, K., Yamanami, T., 2006. Thermotolerant Bacillus licheniformis TY7 produces optically active l-lactic acid from kitchen refuse under open condition. J. Biosci. Bioeng. 102, 132–134. https://doi.org/10.1263/jbb.102.132
35. Song, L., Yang, D., Liu, R., Liu, S., Dai, L., Dai, X., 2021. Microbial production of lactic acid from food waste: latest advances, limits, and perspectives. Bioresour. Technol. 126052. https://doi.org/10.1016/j.biortech.2021.126052

621 36. Tang, J., Wang, X., Hu, Y., Zhang, Y., Li, Y., 2016. Lactic acid fermentation from
622 food waste with indigenous microbiota: Effects of pH, temperature and high OLR.
623 Waste Manag. 52, 278–285. https://doi.org/10.1016/j.wasman.2016.03.034
624 37. Tang, J., Wang, X.C., Hu, Y., Ngo, H.H., Li, Y., 2017. Dynamic membrane-assisted
625 fermentation of food wastes for enhancing lactic acid production. Bioresour.
626 Technol. 234, 40–47. https://doi.org/10.1016/j.biortech.2017.03.019
627 38. Tashiro, Y., Inokuchi, S., Poudel, P., Okugawa, Y., Miyamoto, H., Miayamoto, H.,
628 Sakai, K., 2016. Novel pH control strategy for efficient production of optically
629 active l -lactic acid from kitchen refuse using a mixed culture system. Bioresour.
630 Technol. 216, 52–59. https://doi.org/10.1016/j.biortech.2016.05.031
631 39. Wang, M., Xu, Z.J., Qiu, T.L., Sun, X.H., Han, M.L., Wang, X.M., 2010. Kinetics
632 of Lactic Acid Fermentation on Food Waste by *Lactobacillus bulgaricus*. Adv.
633 Mater. Res. 113–116, 1235–1238.
634 https://doi.org/10.4028/www.scientific.net/AMR.113-116.1235
635 40. Wang, Q., Feng, K., Li, H., 2020a. Nano iron materials enhance food waste
636 fermentation. Bioresour. Technol. 315, 123804.
637 https://doi.org/10.1016/j.biortech.2020.123804
638 41. Wang, Q., Li, H., Feng, K., 2021. Effect of honeycomb, granular, and powder
639 activated carbon additives on continuous lactic acid fermentation of complex food
640 waste with mixed inoculation. J. Biosci. Bioeng. 131, 655–662.
641 https://doi.org/10.1016/j.jbiosc.2021.02.009
642 42. Wang, Q., Li, H., Feng, K., Liu, J., 2020b. Oriented Fermentation of Food Waste
643 towards High-Value Products: A Review. Energies 13, 5638.
644 https://doi.org/10.3390/en13215638

645 43. Xu, X., Zhang, W., Gu, X., Guo, Z., Song, J., Zhu, D., Liu, Yanbiao, Liu, Yanan,
646 Xue, G., Li, X., Makinia, J., 2020. Stabilizing lactate production through repeated
647 batch fermentation of food waste and waste activated sludge. Bioresour. Technol.
648 300, 122709. https://doi.org/10.1016/j.biortech.2019.122709
649 44. Yang, X., Zhu, M., Huang, X., Lin, C.S.K., Wang, J., Li, S., 2015. Valorisation of
650 mixed bakery waste in non-sterilized fermentation for l -lactic acid production by an
651 evolved Thermoanaerobacterium sp. strain. Bioresour. Technol. 198, 47–54.
652 https://doi.org/10.1016/j.biortech.2015.08.108
653 45. Ye, T., Li, X., Zhang, T., Su, Y., Zhang, W., Li, J., Gan, Y., Zhang, A., Liu, Y.,
654 Xue, G., 2018. Copper (II) addition to accelerate lactic acid production from co-
655 fermentation of food waste and waste activated sludge: Understanding of the
656 corresponding metabolisms, microbial community and predictive functional
657 profiling. Waste Manag. 76, 414–422.
658 https://doi.org/10.1016/j.wasman.2018.03.028
659 46. Yousuf, A., Bastidas-Oyanedel, J.-R., Schmidt, J.E., 2018. Effect of total solid
660 content and pretreatment on the production of lactic acid from mixed culture dark
661 fermentation of food waste. Waste Manag. 77, 516–521.
662 https://doi.org/10.1016/j.wasman.2018.04.035
663 47. Zhang, Z., Tsapekos, P., Alvarado-Morales, M., Angelidaki, I., 2021. Bio-
664 augmentation to improve lactic acid production from source-sorted organic
665 household waste. J. Clean. Prod. 279, 123714.
666 https://doi.org/10.1016/j.jclepro.2020.123714
667 48. Zhao, B., Wang, L., Ma, C., Yang, C., Xu, P., Ma, Y., 2010. Repeated open
668 fermentative production of optically pure L-lactic acid using a thermophilic Bacillus

sp. strain. Bioresour. Technol. 101, 6494–6498. https://doi.org/10.1016/j.biortech.2010.03.051

Web references

49. UNEP Food Waste Index Report 2021 published in March 2021 and available on https://www.unep.org/resources/report/unep-food-waste-index-report-2021

50. Lactic Acid Market Size, Share & Trends Analysis Report By Raw Material (Sugarcane, Corn, Cassava), By Application (PLA, Food & Beverages), By Region, And Segment Forecasts, 2021 – 2028 published in April 2021 by grand view research, report number 978-1-68038-126-9 available on https://www.grandviewresearch.com/industry-analysis/lactic-acid-and-poly-lactic-acid-market

Tables and figures captions

Table 1. Characteristics of the substrates and inocula

Table 2. Model food waste fermentation performance depending on the key operational conditions (inoculum, temperature, pH). Error bars represent standard deviation from n ≥ 3 experimental replicates. ND = Not determined

Fig. 1. Model food waste fermentation depending on the pH regulation mode (uncontrolled or regulated at pH 5 or 6 every two days). Evolution of the LA concentration (a.), selectivity over other OA (b.) and microbial community (c.). Error bars represent standard deviation from 3 experimental replicates.
LA = Lactic Acid; AA = Acetic Acid; PA= Propionic Acid; FA= Formic Acid; BA = Butyric and isobutyric Acid

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

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

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

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

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

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

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 ($\%_{molOA}$)	Hetero-fermentation (mol Ethanol produced per mol of lactacte)
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

Fig. 1

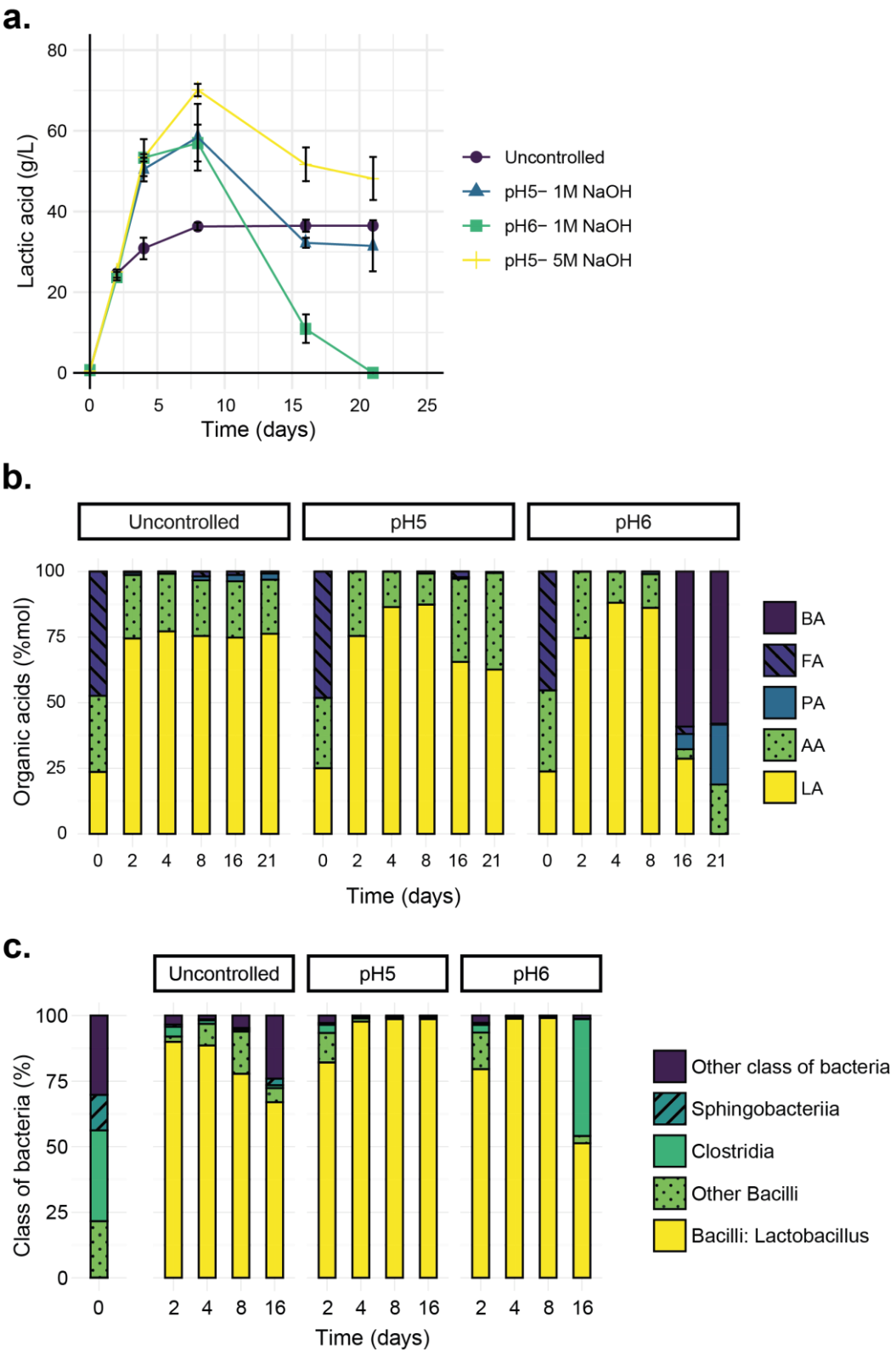
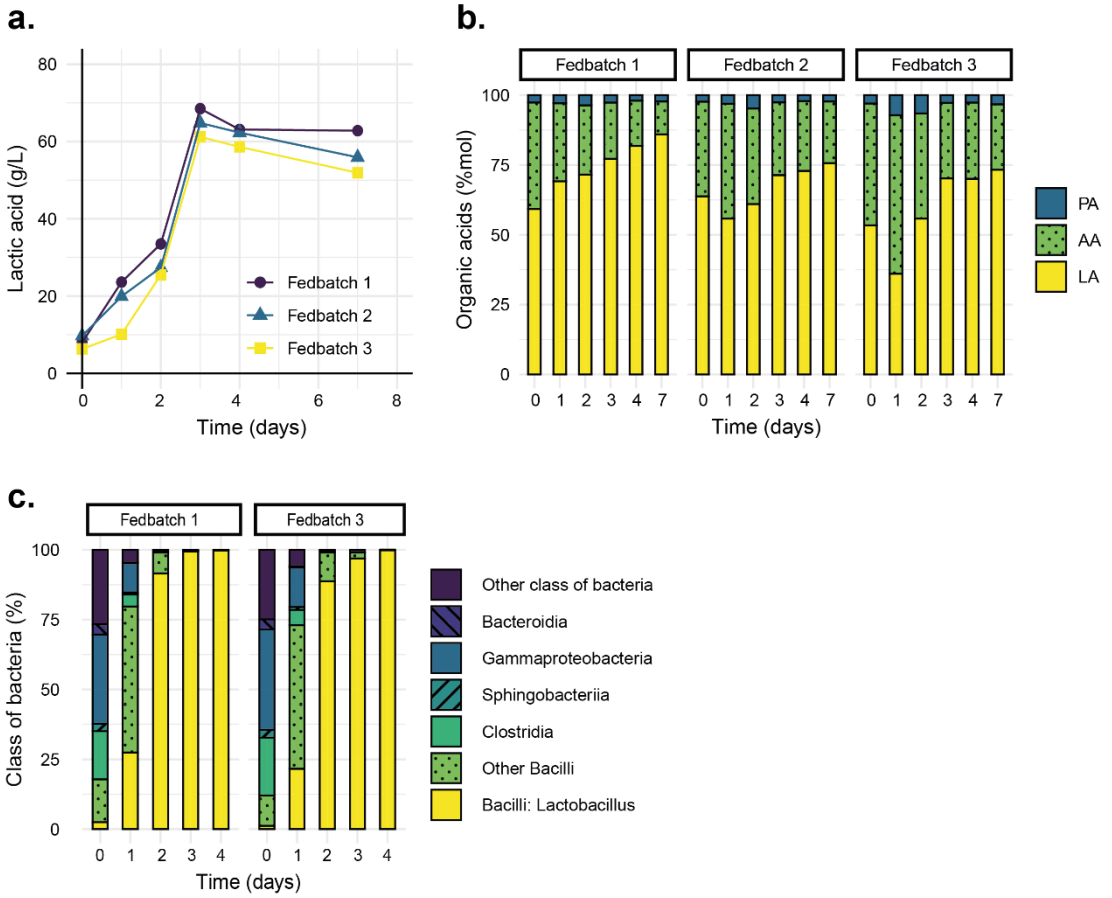
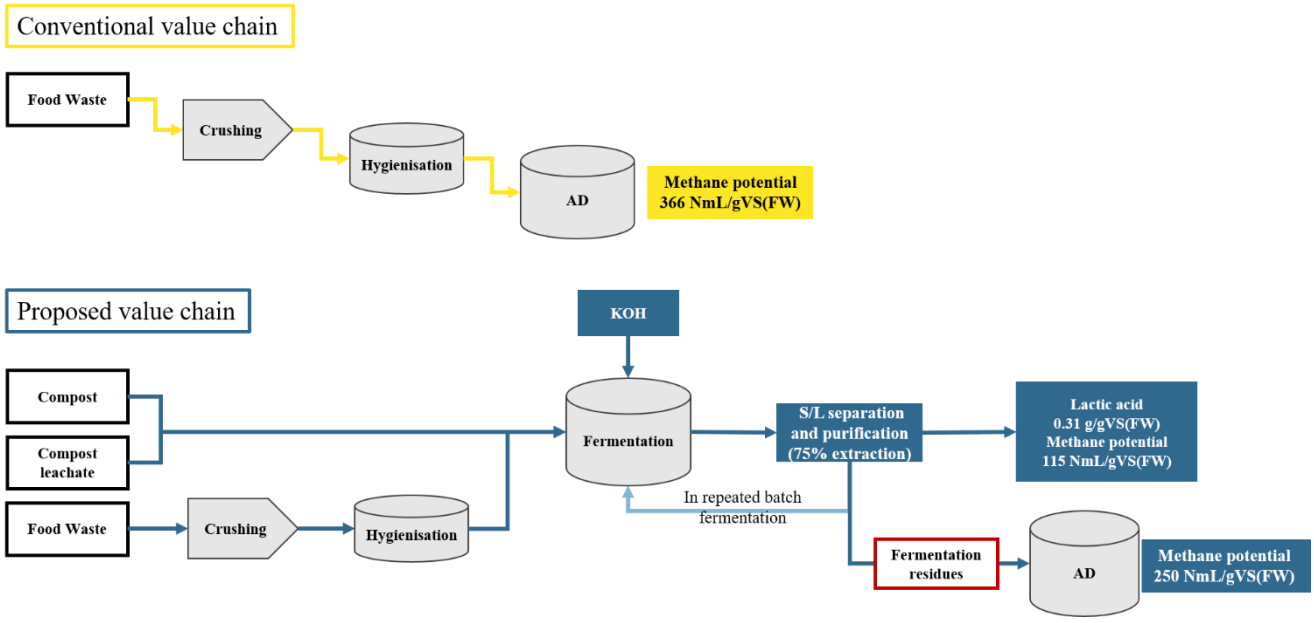

a.
Lactic acid (g/L)
Time (days)
Uncontrolled
pH5– 1M NaOH
pH6– 1M NaOH
pH5– 5M NaOH
b.
Uncontrolled
pH5
pH6
Organic acids (%mol)
Time (days)
BA
FA
PA
AA
LA
c.
Uncontrolled
pH5
pH6
Class of bacteria (%)
Time (days)
Other class of bacteria
Sphingobacteriia
Clostridia
Other Bacilli
Bacilli: Lactobacillus

Fig. 2


a.
Lactic acid (g/L)
Time (days)
Fedbatch 1
Fedbatch 2
Fedbatch 3
b.
Fedbatch 1
Fedbatch 2
Fedbatch 3
Organic acids (%mol)
Time (days)
PA
AA
LA
c.
Fedbatch 1
Fedbatch 3
Class of bacteria (%)
Time (days)
Other class of bacteria
Bacteroidia
Gammaproteobacteria
Sphingobacteriia
Clostridia
Other Bacilli
Bacilli: Lactobacillus

722 **Fig. 3**

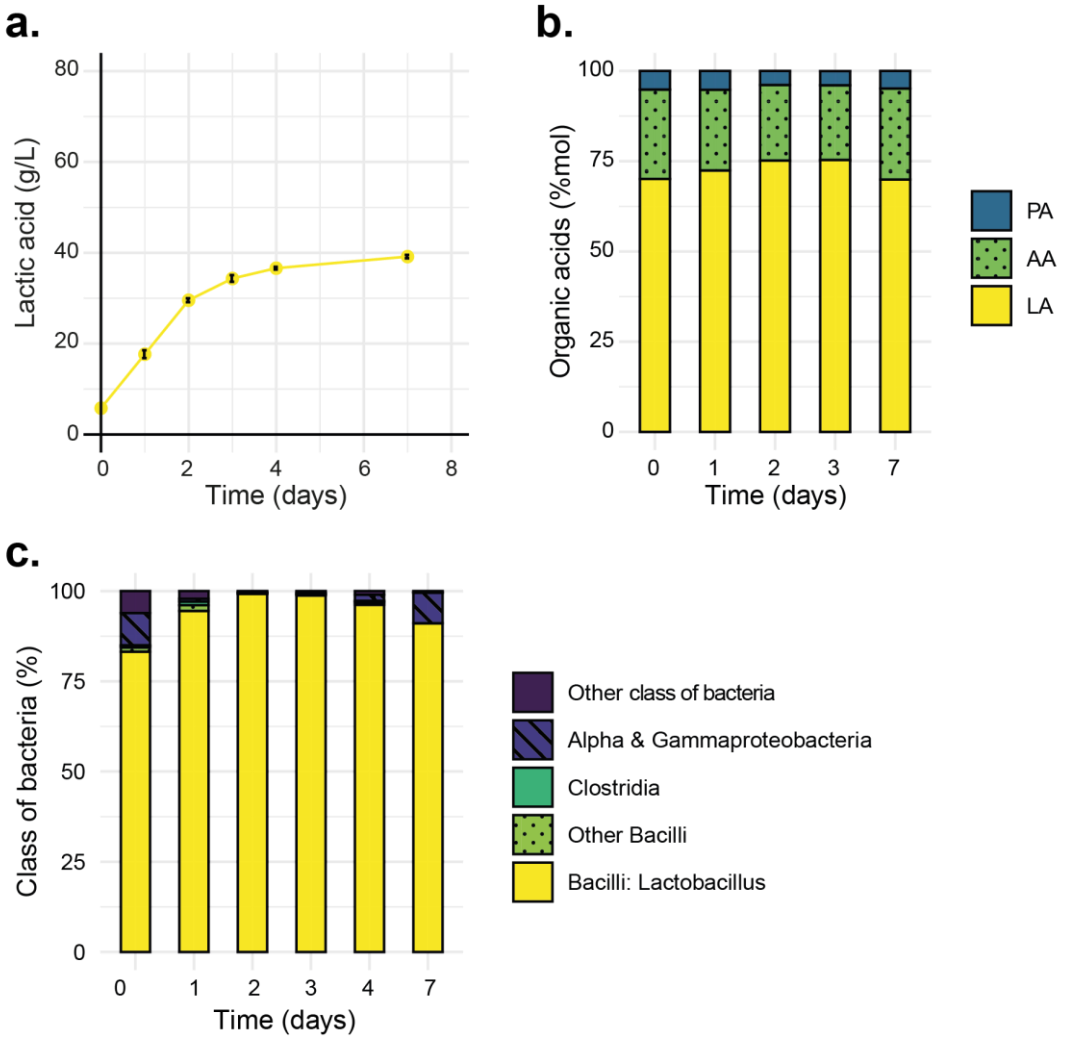
723



724

725

726 **Fig 4.**


a.
Lactic acid (g/L)
80
60
40
20
0
0
2
4
6
8
Time (days)
b.
Organic acids (%mol)
100
75
50
25
0
0
1
2
3
7
Time (days)
PA
AA
LA
c.
Class of bacteria (%)
100
75
50
25
0
0
1
2
3
4
7
Time (days)
Other class of bacteria
Alpha & Gammaproteobacteria
Clostridia
Other Bacilli
Bacilli: Lactobacillus

727