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

Célia Chenebault <sup>a</sup>, Roman Moscoviz <sup>a</sup>, Eric Trably <sup>b</sup>, Renaud Escudie <sup>b</sup>, Benjamin Percheron <sup>a\*</sup>

<sup>a</sup> Suez, CIRSEE, 38 rue du Président Wilson, 78230 Le Pecq, France

<sup>b</sup> LBE, INRAE, Univ Montpellier, 102 Avenue des Etangs, Narbonne, F-11100, France

\* **Corresponding author:** [benjamin.percheron@suez.com](mailto: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 pharmaceuticals 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%<sub>TS</sub> 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%<sub>TS</sub> and 89.53%<sub>VS/TS</sub> (Table 1). The total carbohydrates content of the model FW was estimated at 0.69 g/g<sub>TS</sub>, its lipids content at 0.07 g/g<sub>TS</sub> and its crude protein content at 0.17 g/g<sub>TS</sub>. The C/N ratio is 16.3 g/g and the pH of this model FW was estimated to be 5.6 (Capson-Tojo *et al.*, 2017). This FW mixture was roughly milled (shredder BLICK

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

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

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

## 2.2. Batch fermentation (0.5 L)

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

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

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

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

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

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

### 2.4. *Potential for methane production*

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



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

## 2.5. Analytical methods

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

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

### 2.5.2. High Performance Liquid Chromatography

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

### 2.5.3. Gas Chromatography

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<sub>2</sub>, O<sub>2</sub>, N<sub>2</sub> and CH<sub>4</sub>) and a RtMolsieve column (for CO<sub>2</sub>). Argon 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 (%<sub>molOA</sub>).

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

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

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

216 *fermentation*

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

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

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

11.1  $\pm$  2.1% of the total microorganism relative abundance; and *Bacilli* with the genus *Streptococcus* representing 6.0  $\pm$  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  $\pm$  0.5 g/L) was lower than in the condition using 18% of yoghurt which is likely related to a lower initial pH (pH<sub>i</sub> = 5.6  $\pm$  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  $\pm$  1.3 g/L) than at 24°C (29.9  $\pm$  0.2 g/L) or 55°C (10.5  $\pm$  0.8 g/L). LA selectivity was similar at 24°C and 35°C (78  $\pm$  1%<sub>molOA</sub> and 77  $\pm$  2%<sub>molOA</sub> respectively) and lower at 55°C (61  $\pm$  3.6%<sub>molOA</sub>). At 24°C, the LAB dominated the microbial community but grew more

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

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

The final concentration of LA, its productivity and yield were improved by pH regulation at 5 or 6 (regulated every two days allowing a dynamic evolution of pH; Table 2 and Fig.1a). Indeed when pH was not regulated, it rapidly decreased as LA was accumulating, preventing the growth and activity of many micro-organisms (including LAB (Alves de Oliveira *et al.*, 2018; Farah *et al.*, 2009)). As a consequence, the quantity of LA produced reached a plateau after 4 days of fermentation (Fig. 1a). This 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  $30.9 \pm 2.6$  g/L LA after 4 days and  $36.3 \pm 1.3$  g/L after 8 days).

LA fermentation performances were similar by regulating pH at 5 or 6 after 8 days of fermentation ( $58.4 \pm 6.6$  g/L vs  $57.0 \pm 4.8$  g/L; Table 2 and Fig. 1a). However, as the fermentation continued, an important decrease in LA concentration (from  $57.0 \pm 4.8$  g/L at day 8 to 0 g/L at day 21; Fig. 1a) and specificity (from  $86 \pm 1\%$ <sub>molOA</sub> to  $0\%$ <sub>molOA</sub>; Fig. 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 ( $70.1 \pm 1.5$  g/L), productivity ( $0.37 \pm 0.01$  g/L/h) and yield ( $0.39 \pm 0.01$  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 ( $0.18 \pm 0.0$  mol per mol of LA; Table 2) and high selectivity ( $89 \pm 2\%$ <sub>molOA</sub>) 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%<sub>molOA</sub>) 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 ( $66.3 \pm 1.1$  g/L for real vs  $70.1 \pm 1.5$  g/L for model FW), productivity ( $0.35 \pm 0.01$  g/L/h for real vs  $0.37 \pm 0.01$  g/L/h for model FW), yield ( $0.37 \pm 0.01$  g/gTS for real vs  $0.39 \pm 0.01$  g/gTS for model FW) and a good selectivity (over 90%<sub>molOA</sub> for real vs 89%<sub>molOA</sub> 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*

336                    *relevant conditions*

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

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



### 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%<sub>TS</sub>). 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%<sub>molOA</sub>) 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%<sub>molOA</sub>). 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

strategy to enhance LA production from FW.

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

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

The fermentation profile, maximal LA concentration (68.5 g/L for Fed-batch 1, 64.8 g/L for Fed-batch 2 and 61.2 g/L for Fed-batch 3; Fig. 2a) and selectivity (77%<sub>molOA</sub> for Fed-batch 1, 71%<sub>molOA</sub> for Fed-batch 2 and 70%<sub>molOA</sub> for Fed-batch 3; Fig. 2b) were similar for every run, indicating that LA fermentation is stable in a repeated fed-batch configuration although a minor decrease in fermentation performance can be observed at the beginning of the 3<sup>rd</sup> fermentation run. The initial microbial community was slightly richer (2.5% vs 1%) in *Lactobacillus* when the reactor was reinoculated with fermentation residues from a previous run (Fig. 2c). In every run, the microbial community was rapidly dominated by *Lactobacillus* with more than 99% of *Lactobacillus* observed after 4 days of fermentation (Fig. 2c). In a previous study conducted using waste activate sludge as an inoculum, authors have shown the 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  $365 \pm 4$  NmL CH<sub>4</sub> per g of FW initial volatile solids (*i.e.*, before fermentation), which was similar to the methane potential of non-fermented FW ( $366 \pm 7$  NmL CH<sub>4</sub> 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 ( $346 \pm 3$  NmL CH<sub>4</sub> per gVS(FW) *vs*  $333 \pm 6$  NmL CH<sub>4</sub> 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<sub>4</sub>/gVS(FW) (Fig. 3). Therefore, 58 kg LA and 47 Nm<sup>3</sup> CH<sub>4</sub> can be produced per ton FW (at 20%<sub>TS</sub>), 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 ( $39.2 \pm 0.4$  g/L), yield ( $0.21 \pm 0.0$  g/gTS), productivity ( $0.23 \pm 0.0$  g/L/h) and selectivity ( $70 \pm 0.0\%$ <sub>molOA</sub>) 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%<sub>molOA</sub>). This can be partially explained by the fact that there is slightly less volatile matter in depackaging pulp (86%<sub>TS</sub> vs 89.5%<sub>TS</sub> 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

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

#### **4. Conclusions**

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

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### **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  $n \geq 3$  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.**

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

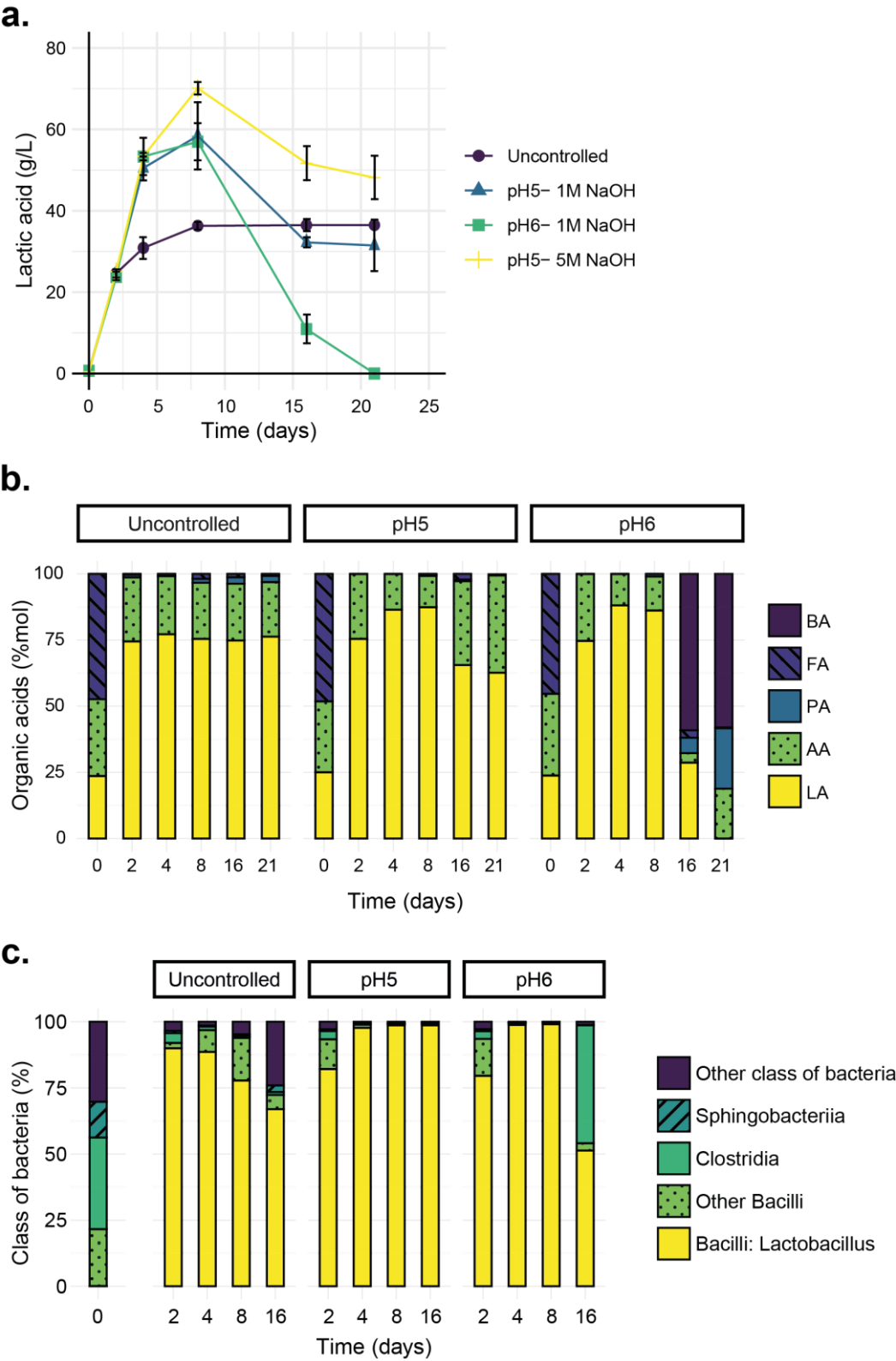
709 **Table 2.**

Number of experiments * replicates	Inoculum	Temperature	pH	Fermentation time	Maximum LA concentration (g/L)	Average productivity (g/L/h)	Yield (g/g TS FW)	Selectivity (% <sub>mol</sub> 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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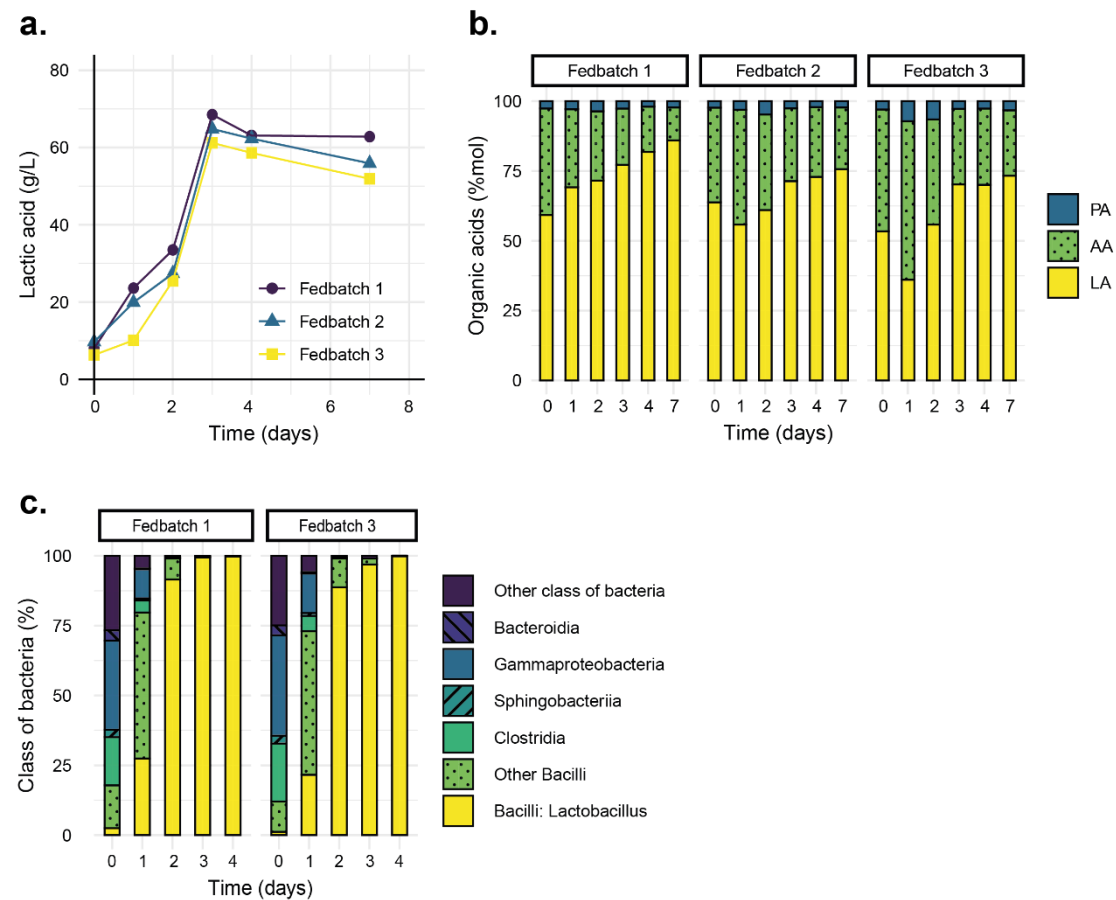
712 **Fig. 1**



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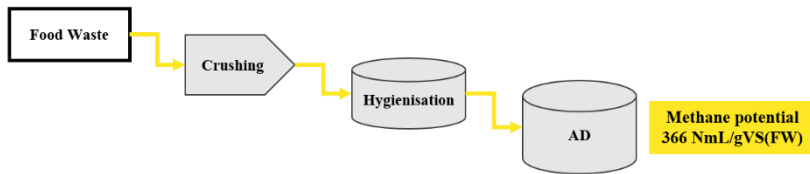
714

**Fig. 2**

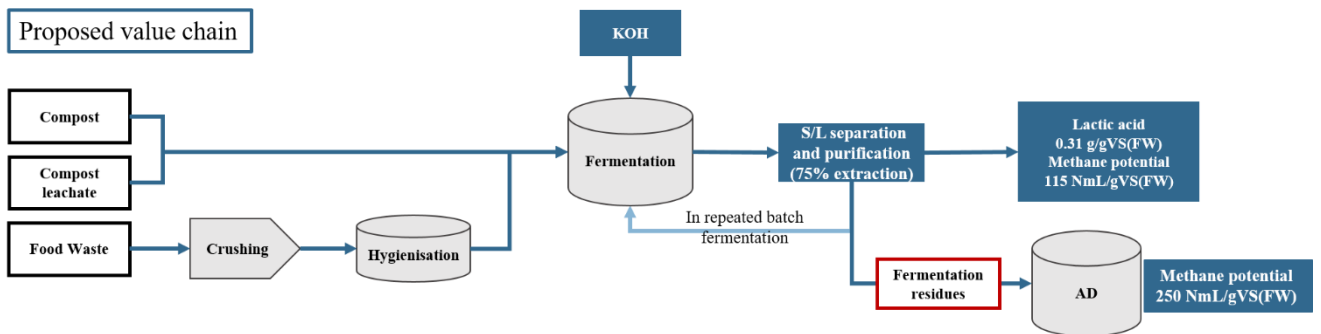


**Fig. 3**

Conventional value chain



Proposed value chain



**Fig 4.**

