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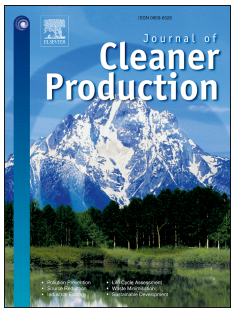


Organic fraction of municipal solid waste for the production of L-lactic acid with high optical purity

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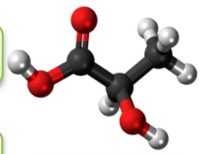
OFMSW

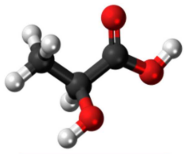
Enzymatic hydrolysis

Sugars

Nutrients



50%D- LA



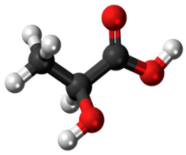
50% L- LA

Pre-treatment

Sugars

Nutrients

Fermentation



L-LA>98%

# *Organic fraction of municipal solid waste for the production of L-lactic acid with high optical purity*

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**Abstract:** The organic fraction of municipal solid waste (OFMSW) is an abundant biowaste with great potential in the bioeconomy model. Previous reports have demonstrated that OFMSW hydrolysates are good substrates for lactic acid (LA) production. However, LA can exist in two enantiomeric forms (L- and D-) and most commercial LA applications require a high enantiomeric purity, typically of the L- isomer. Due to natural occurring bacteria in the waste, a mixture of D- and L- LA can form in the substrate before the fermentation; reducing the final enantiomeric purity of the product and limiting its commercial application. In the research reported in this article, hydrolysates from OFMSW were evaluated for the production L-LA with high enantiomeric purity. Firstly, a pre-treatment with monopolar electrodialysis membranes was implemented to remove the unfavourable D-LA in the hydrolysate. This step allowed the reduction in LA concentration and subsequent fermentations of the hydrolysate resulted in enantiomeric purities over 98 %. At the pilot scale, a fermentation of the pre-treated hydrolysate, by *B. coagulans* A166, resulted in a final LA concentration of 61.1 $g \cdot L^{-1}$ and a yield 0.94 $g \cdot g^{-1}$. The downstream of the process resulted on a LA recovery of 51.5 % and a purity of L-LA of 98.7 %.

**Keywords**: lactic acid; organic fraction of municipal solid waste; *B. coagulans*; electrodialysis; downstream; pilot scale; enantiomeric purity

## 1 Introduction

The term 'organic fraction of municipal solid waste' (OFMSW) describes the heterogeneous organic wastes derived from urban areas (Abudi et al., 2016). Although their composition can vary, in general, these biowastes contain high levels of carbohydrates, proteins and lipids, making them an interesting substrate for biotechnological applications (Pleissner and Lin, 2013; Uçkun Kiran et al., 2014). Therefore, instead of being incinerated, composted or used in biogas production (Burnley et al., 2011; Grosso et al., 2010), the residues have been utilized, solely or in combination to other waste streams and/or nutrients, for hydrogen, ethanol, butanol and other bio-based products (Abudi et al., 2016; Kannengiesser et al., 2018; Matsakas et al., 2017).

In our previous work, we explored the production of lactic acid (LA) from OFMSW (López-Gómez et al., 2019). LA is an important building block, with a wide range of applications (Alves de Oliveira et al., 2018), that can exist in the L- and D- enantiomeric forms. However, a high enantiomeric purity is crucial when LA is intended to be used for high-value applications, such as in the production of the biopolymer polylactic acid (PLA), which typically requires purities over 98% (Alves de Oliveira et al., 2018; Jem et al., 2010; Klotz et al., 2016). Furthermore, since D-LA can cause metabolic problems its utilization is restricted in the food, pharmaceutical and agrochemical industries. Unlike in its chemical synthesis, which results in a racemic mixture of the D- and L-enantiomers, various microorganisms possess homofermentative pathways that synthesize only one isomer and thus, biochemical production of LA is widely preferred (Alves de Oliveira et al., 2018).

Abbreviations: D-LA, dextro- lactic acid ; H1, coarse filtrated hydrolysate ; H2, microfiltrated hydrolysate; H3, hydrolysate after electrodialysis; HMF, hydroxymethylfurfural; LA, lactic acid; L-LA, levo- lactic acid; $N_{Total}$, total nitrogen; OFMSW, organic fraction of municipal solid waste; P, productivity; $P_{exp}$, productivity during the exponential phase; PLA, polylactic acid; $P_{max}$, maximum productivity; $P_{Total}$, total phosphorus.

The need for a cost-efficient and more sustainable production of L-LA with high enantiomeric purity has pushed research towards the utilization of inexpensive renewable resources. Many studies have already shown that L-LA with high optical purity can be produced from various waste and by-product streams like coffee pulp and mucilage (Neu et al., 2016; Pleissner et al., 2016), food waste streams (Demichelis et al., 2017; Pleissner et al., 2017a), defatted rice bran (Alexandri et al., 2018a) and sugarcane bagasse hemicellulosic hydrolysate (Alves de Oliveira et al., 2019) with purity higher than 99%, using *Bacillus coagulans*. *B. coagulans* strains present many advantages over other LA producing bacteria, due to their ability to grow at high temperatures (optimum 52°C), on different carbon sources and while having low nutrient requirements. To this end, fermentation of OFMSW using *B. coagulans* for high optical purity L-LA would be a promising alternative for the sustainable exploitation of this waste stream.

Although studies available in the literature regarding the conversion of OFMSW into LA are scanty, they have shown that OFMSW contain a mixture of mainly LA producing bacteria able to proliferate and produce LA (Probst et al., 2013). Nonetheless, being a mixed culture, the production of LA is prone to low yields and yields racemic mixtures of D- and L-LA (Demichelis et al., 2017). As explained by Probst et al. (2015), although there is a good potential for the production of LA, the purification and synthesis of products with high optical purity still needs to be addressed. This was confirmed by López-Gómez et al. (2019) who determined that depending on the collection system of OFMSW, hydrolysates contained a racemic mixture of D- and L-LA in concentrations from 5 to 20 $g \cdot L^{-1}$ approximately. As a result the maximum LA optical purity achieved was only 93 % which would hinder its use for some specific

applications. Therefore, the research reported in this article aimed to explore the possibility of utilising OFMSW for the fermentation of LA with high enantiomeric purity and, additionally, to carry out the required steps for its purification. After its chemical characterization, OFMSW hydrolysate samples were pre-treated for the removal of LA produced by natural occurring bacteria. Following that, the hydrolysates were used in lab scale experiments and a screening was carried out for the selection of the most appropriate microorganism. Results obtained at the lab scale were used as the basis to conduct fermentations at the pilot scale to provide further insights on the performance of the fermentation. Following that, a downstream process based on electrodialysis was carried out for the purification of LA.

## 2 Materials and methods

### 2.1 Substrate: OFMSW hydrolysates

OFMSW hydrolysates were kindly provided by IMECAL SA company (L'Alcúdia, Valencia, Spain). Based on previous reported results (López-Gómez et al., 2019), the hydrolysates used in this study were produced with batches of separately collected OFMWS from a municipal solid waste treatment plant in Valencia, Spain. Before the hydrolysis, the samples were screened to manually remove inert materials such as glass, plastics, stones, textiles, etc. Following that, a pilot hammer mill was used for homogenisation and finally the wastes were sterilized at 121ºC for 1 h in an autoclave. The solids load for the enzymatic hydrolysis was 20% and it was carried out for 72 h at 50ºC, 150 rpm. The pH of was controlled at 5 by the addition of NaOH (20 % $w \cdot w^{-1}$). The cocktail of enzymes, provided by Novozymes, is based on a mixture of cellulases and amylases and was developed particularly for OFMSW substrates. Samples were taken during the hydrolysis to quantify the liberation of sugars and the production of

growth inhibitors. A total of 13 hydrolysate batches were prepared and High Pressure Liquid Chromatography (HPLC) (Coregel 87H3 7.8mm x 300mm column) was used for the quantification of sugars and organic acids.

## 2.2 Microorganisms and inoculum

All the strains used in this study were gram-positive, thermophilic and homofermentative L-LA producing bacteria, obtained from the strain bank of the Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB) in Potsdam, Germany. MALDI-TOF was used for the identification of the strains. Pre-cultures were carried out in MRS broth (Merck, Germany) supplemented with dolomite EVERZIT Dol 0.5-2.5 mm (Evers, Germany) as a buffer. Flasks containing the seed culture were placed in an orbital shaker at 150 rpm and 52 °C for 12-16 h.

## 2.3 Hydrolysate purification

A series of purification steps were implemented to removed the LA (racemic mixture of D- and L-) from the hydrolysate. In the first step i.e. coarse filtration, a filter bag with a pore size of 150 µm was used for the removal of larger solid particles. Following that a microfiltration was carried out with a cross-flow micro-filtration system (UFI-TEC, Germany), pore size 0.2 µm, at 1.5 bar and 15°C, equipped with 4 TAMI membranes (TAMI Industries, France). Finally, LA was removed by monopolar electrodialysis, at 35°C, 20 V and 3 A, using 11 cation exchange membranes and 10 anion exchange membranes Type IV (Fujifilm, The Netherlands).

## 2.4 Fermentations

### *2.4.1 Evaluation of the effects of the purification steps*

Experiments in small scale bioreactors were performed to evaluate how the purification steps impact the fermentation potential of the OFMSW hydrolysate. Thus, 3 sets of

fermentations were carried out, one with the coarse filtrated hydrolysate (H1), one with the microfiltrated hydrolysate (H2) and one with the hydrolysate after electrodialysis (H3). Fermentations were carried out, in duplicate, with 250 mL working volume, at 52°C and 400 rpm, using an Eloferm multifermentation system (Biotronix GmbH, Germany). The pH was controlled at 6.0 with a solution of NaOH 20% (w/v). The bioreactors were inoculated with *B. coagulans* A166 (3% v/v) and samples were withdrawn every few hours (in most cases after 2 h intervals) for the quantification of sugars and LA.

### *2.4.2 Evaluation of various strains for the production of L-LA with high optical purity*

A total of 13 strains were evaluated for the production of LA with high optical purity using the OFMSW hydrolysate after electrodialysis. Experiments were carried out under the same conditions as described in section 2.4.1. After the selection of the strain, an experiment was carried out to compare its performance when yeast extract (5 $g \cdot L^{-1}$) was added to the hydrolysate after electrodialysis.

### *2.4.3 Fermentations in 1 L bioreactor and pilot scale bioreactor*

The OFMSW hydrolysate after electrodialysis, supplemented with yeast extract (5 $g \cdot L^{-1}$), was used for lab scale fermentation (1 L working volume) and pilot scale fermentation (23 L working volume) with the strain A166. The lab scale fermentation was carried out in a 2 L BIOSTAT bioreactor (Sartorius AG, Germany) whereas the pilot scale fermentation was carried out in a 72 L BIOSTAT UD bioreactor (B-Braun Biotech, Germany). Inoculum for the fermentation in pilot scale was prepared in a 1 L BIOSTAT bioreactor (Sartorius AG, Germany) with 700 mL working volume of the same hydrolysate medium. The fermentations were carried out at 52°C, with constant agitation of 200 rpm and a pH of 6.0 regulated by the addition of NaOH 20% (w/v).

Samples were withdrawn from the fermenters at various times, inactivated at 95 °C for 20 min and stored at -20°C until the quantification of sugars and LA was performed.

**2.5 Downstream and purification**

Figure 1 shows the flow diagram of the process. The units (*b*),(*c*),and (*d*) correspond to the pre-treatment of the hydrolysate carried out as described in section 2.3. After fermentation (*e*) the broth was inactivated and afterwards microfiltrated (*f*) as described in section 2.3 but at 5°C. Following that, a nanofiltration step (*g*) was performed (150-300 Da cut-off) at 30 bar with an UFI-TEC crossflow nanofiltration system (UFI-TEC, Germany). Calcium and magnesium ions were then removed in a column (*h*) packed with a PUROLITE S950 acid chelating resin (Purolite, Germany). After softening, the electrodialysis was carried out in 2 steps using 11 cation and 10 anion exchange membranes Type II (Fujifilm, The Netherlands). The monopolar step (*i*) was finished when the conductivity of the filtrate was below 1 $mS \cdot cm^2$, whereas the bipolar step (*j*) was stopped when the conductivity of the filtrate was below 2 $mS \cdot cm^2$. The acid stream obtained was then decolorized (*k*) using an adsorbent resin MACRONET$^{TM}$ MN-502 (Purolite, Germany). Following that cation (*l*) and anion (*m*) exchange chromatography were performed using resins RELITE EXA 133 and RELITE EXC 08 (Resindion S. R. L., Italy), respectively. Finally, vacuum distillation (*n*) was used to concentrate the product at 55°C, -1 bar and 350rpm (Büchi Labortechnik, Germany). A detailed description of the downstream process can be found in Neu et al. (2016).

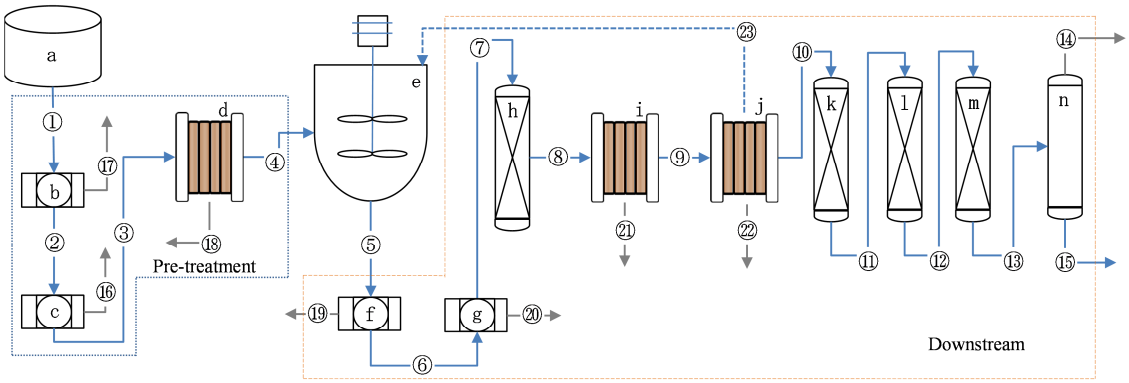



Figure 1: Process flow diagram of the production of LA with high enantiomeric purity from OMSW hydrolysate. (a) hydrolysate tank, (b) coarse filtration, (c) microfiltration, (d) monopolar electrodialysis, (e) fermentation, (f) microfiltration, (g) nanofiltration, (h) softening column, (i) monopolar electrodialysis, (j) bipolar electrodialysis, (k) decolourisation column, (l) anion exchange column, (m) cation exchange column, (n) distillation.

## 2.6 Analytical assays

A detailed description of the methods for the quantification of sugars, LA, ions, total nitrogen, total biomass and total cells can be found in Neu et al. (2016). Analytical essays for the quantification of sugars and LA concentration and LA enantiomeric purity were carried out as detailed in Alexandri et al. (2018b).

During the hydrolysis, high Pressure Liquid Chromatography (HPLC) (Coregel 87H3 7.8mm x 300mm column, Chrom Tech, USA) was implemented to measure the release of sugars and the formation of furfural and 5-HMF. During the fermentations, quantification of sugars and LA was carried out via HPLC (Dionex, USA) and a Eurokat H column (300mm x 8 mm x 10 µm, Knauer, Germany). An aqueous solution of 5 mM $H_2SO_4$ was the mobile phase at a flow rate of 0.8 mL·min$^{-1}$. The detection of the components was achieved using a refractive index detector (RI-71, Shodex, Japan). Likewise, optical purity of the samples was performed using HPLC (Dionex, USA) with a Phenomenex Chirex 3126 column (150 x 4.6mm ID, Phenomenex, USA) at 30°C coupled to an ultraviolet detector. In this case, $Cu_2SO_4$ flowing at 1 mL·min$^{-1}$ was the mobile phase.

188 The total number of cells was determined with a THOMA cell chamber
189 (Glaswarenfabrik Karl Hecht GmbH & Co KG, Germany) whereas the number of
190 colony forming units was used to determine the number of living cells as described in
191 Alexandri et al. (2018b).

192 **3 Results and discussion**

193 **3.1 Composition of the hydrolysate**

194 An initial characterisation of the substrate was fundamental to determine the amount of
195 sugars available and the total concentration of LA. Results for the analysis of the 13
196 batches of OFMSW hydrolysate have been reported in López-Gómez et al. (2019). On
197 average, hydrolysates showed a sugar content above 70 $g \cdot L^{-1}$. Glucose was the
198 predominant sugar with an average value of 55.41±2.01 $g \cdot L^{-1}$, followed by xylose with
199 10.13±1.2 $g \cdot L^{-1}$. As indicated by Nwobi et al. (2014), the mild conditions of enzymatic
200 pre-treatments avoid the formation of inhibitory compounds which are typically
201 produced in harsh thermochemical pre-treatments. This was confirmed by the samples
202 analysed in this study which did not show the presence of inhibitory compounds such as
203 furfural and 5-HMF.

204 Nonetheless, LA was detected in every sample with an average concentration of
205 5.69±0.88 $g \cdot L^{-1}$. The presence of LA is the result of a variety of naturally occurring
206 bacteria, mostly *Lactobacillus* spp., in the OFMSW (Probst et al., 2013). Analysis of the
207 LA enantiomeric purity revealed a racemic mixture of D- and L-LA at a ratio of 1:1.
208 Thus, an average sample of OFMSW hydrolysate would contain approximately 2.55
209 $g \cdot L^{-1}$ of D- LA which represents a problem if a product with high optical purity is
210 required. The vast majority of industrially produced PLA is obtained from L-LA with
211 optical purities above 98% (Jem et al., 2010; Kunasundari et al., 2013). Considering an

OFMSW hydrolysate sample, initially containing 70 $g \cdot L^{-1}$ of sugars, fermented by a homofermentative LA producer, even with a 100% sugar conversion and a yield of 1 $g \cdot g^{-1}$, a maximum final LA concentration of 75.11 $g \cdot L^{-1}$ would be achieved. From that, 2.55 $g \cdot L^{-1}$ correspond to the D- LA initially present in the sample and to a final enantiomeric purity of around 97%. Hence, even in a scenario with optimal conversions the optical purity of the product would be below the desired threshold for the production of PLA with high optical purity (Inkinen et al., 2011; Jem et al., 2010).

### 3.2 Hydrolysate purification

The separation of LA enantiomers has been a topic of research for some time (Boonpan et al., 2013; Huang et al., 2018), however, an economically feasible method to achieve it is still unavailable. Therefore, in this study a series of purification steps were carried out to remove the total LA initially present in the OFMSW hydrolysate. The separation was carried out in three main steps: coarse filtration, microfiltration and electrodialysis. Table 1 shows the average composition (from 2 batches) of the hydrolysates before and after the purification steps. As seen in the table, the concentrations for the sugars remained stable after every step. LA concentration was successfully pulled down from 5.70±0.01 to 0.89±0.89 $g \cdot L^{-1}$. Naturally, there was also a reduction in the content of nutrients most prominently after electrodialysis. The total nitrogen content went down from a concentration of 3953±34 $mg \cdot L^{-1}$ in the raw hydrolysate to a concentration of 1731±04 $mg \cdot L^{-1}$ in the hydrolysate samples after electrodialysis. Furthermore, all the other ions saw a reduction in their concentration of more than 95%. The effect in this decrease is clearly demonstrated in the fermentation profiles shown in Figure 2.

Table 1: Variation in the composition of the OFMSW hydrolysate after the purification steps. As seen, LA was completely removed in one experiment and partially removed (85%) in the second one.

| Step | Concentration ($g \cdot L^{-1}$) |
|---|---|

| | Glucose | Disaccharides | Xylose | Arabinose | Lactic acid | Acetic acid |
|---|---|---|---|---|---|---|
| Raw hydrolysate | 52.66±0.2 | 6.51±0.0 | 10.16±0.0 | 1.13±0.0 | 5.70±0.0 | 2.28±0.2 |
| Coarse filtration (H1) | 50.45±1.6 | 7.79±0.4 | 09.99±0.3 | 1.28±0.0 | 6.54±0.5 | 4.91±2.0 |
| Microfiltration (H2) | 52.68±1.8 | 7.42±0.7 | 10.18±0.4 | 1.30±0.0 | 6.68±0.5 | 4.71±2.2 |
| Electrodialysis (H3) | 55.07±0.7 | 7.43±0.5 | 10.62±0.0 | 1.36±0.1 | 0.89±0.9 | n.d. |

| Step | Concentration (mg·L$^{-1}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $N_{Total}$ | $P_{Total}$ | $Cl^-$ | $SO_4^{2-}$ | $Na^+$ | $K^+$ | $Mg^{2+}$ | $Ca^{2+}$ | $NH^{4+}$-N |
| Raw hydrolysate | 3953±03 | 459±20 | 1853±01 | 322±2.9 | 16±0.1 | 1354±12 | 2114±02 | 171±03 | 894±0.1 |
| Coarse filtration (H1) | 3298±20 | 437±12 | 1840±26 | 320±14 | 17±0.1 | 907±6.8 | 2151±20 | 167±01 | 595±3.4 |
| Microfiltration (H2) | 2016±18 | 427±14 | 1803±30 | 297±09 | 17±0.1 | 850±4.7 | 2071±20 | 159±01 | 565±4.2 |
| Electrodialysis (H3) | 1731±04 | 23±2.6 | 10±2.6 | 10±1.7 | 1±0 | 84±2.3 | 90±5.8 | 1±0.1 | 6±0 |

** n.d.: not detected*

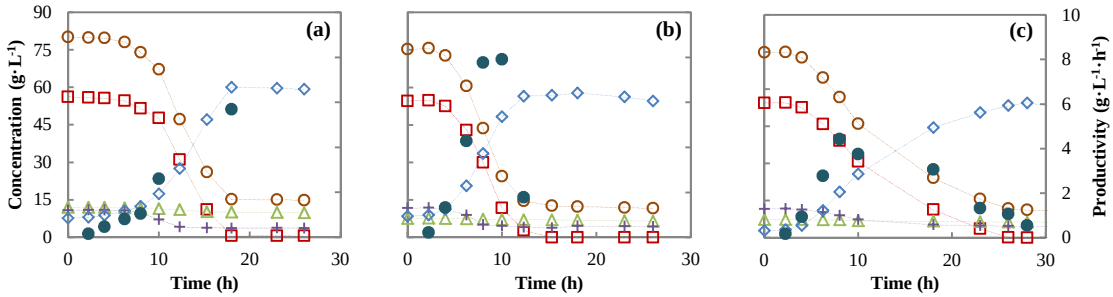



Figure 2: Variation in the concentration of total sugars (○), glucose (□), disaccharides (Δ), xylose (+), lactic acid (◊) and productivity (●) for the fermentations of OFMSW hydrolysate samples after (a) coarse filtration-H1, (b) microfiltration-H2 (both from López-Gómez et al. (2019), published in https://doi.org/10.1016/j.bej.2019.107251, licensed under CC BY NC ND (https://creativecommons.org/licenses/by-nc-nd/4.0/) and (c) electrodialysis-H3, using *B. coagulans* A166.

Final LA concentrations were approximately 60, 56 and 54 g·L$^{-1}$ as shown in Figure 2a, b and c, respectively. Though, it may appear that H3 had a lower titre, it is important to consider that the initial concentration of LA was only about 2.8 g·L$^{-1}$ compared to 7.6 and 8.5 g·L$^{-1}$·h$^{-1}$ for H1 and H2. Glucose was completely consumed in all the experiments and final values for the residual sugars were 14.8, 11.6 and 10.9 g·L$^{-1}$ for H1, H2 and H3 respectively. Additionally, it is apparent that there was a reduction of around 4h in the lag phase after the microfiltration step. However, there was an evident difference in the fermentation rate between H3 and the other two fermentations. While there is a clear exponential phase in the curves for H1 and H2, lasting approximately 10 h in both cases, it took H3 about 22 h (from the time the reduction in sugars was firstly noticeable) until the value of residual sugars was again stable. Congruently, the value for maximum productivity ($P_{max}$) was only 4.40±0.00 g·L$^{-1}$·h$^{-1}$ for H3 compared to

255 5.67±0.32 and 7.89±0.02 $g \cdot L^{-1} \cdot h^{-1}$ for H1 and H2 respectively. Values for global
256 productivities (P) were calculated from the inoculation time until the beginning of the
257 stationary phase. P values were 2.95, 2.98 and 2.09 $g \cdot L^{-1} \cdot h^{-1}$ and yields 0.91, 0.97 and
258 0.89 $g \cdot g^{-1}$ for H1, H2 and H3, respectively. It is reasonable to assume that the reduction
259 in the concentrations of nitrogen and other nutrients, resulting from the electrodialysis
260 step, was responsible for the slowdown in H3. Nonetheless, the experiments proved that
261 the hydrolysate was able to support the growth of bacteria even without the addition of
262 any extra nutrients. As expected, the final LA enantiomeric purity was only 92.75 and
263 92.50 % for the fermentations with H1 and H2. On the other hand, the pre-treatment
264 carried out to produce H3 allowed achieving a final L-LA of 98.25% after the
265 fermentation.

266 **3.3 Screening of the hydrolysate with various L-LA bacteria**

267 A screening of various isolates was carried out using the hydrolysate after
268 electrodialysis. Table 2 shows the strains tested, their internal code, from where they
269 were obtained/isolated and summarizes the results of yield, productivities, LA titre,
270 residual sugars fraction and final enantiomeric purity for the fermentations. The value of
271 productivity during the exponential phase ($P_{exp}$) was calculated using the values of LA
272 only during the exponential period of growth. Graphs for the fermentation profiles can
273 be found in the supplementary material.

274 Table 2: Results for yield, maximum productivity, overall productivity, LA final concentration and optical purity for
275 the screening of 13 LA producing strains.

| ID | Isolated from | Yield* ($g \cdot g^{-1}$) | $P_{max}$ ($g \cdot L^{-1} \cdot h^{-1}$) | P ($g \cdot L^{-1} \cdot h^{-1}$) | $P_{exp}$ ($g \cdot L^{-1} \cdot h^{-1}$) | LA ($g \cdot L^{-1}$) | Residual sugars fraction | L-LA% |
|---|---|---|---|---|---|---|---|---|
| A20 | DSM 2314 | 0.93 | 4.34 | 1.85 | 2.32 | 56.5 | 0.16 | 98.6 |
| A116 | Mulberry | 0.90 | 3.56 | 1.63 | 2.00 | 55.3 | 0.14 | 98.0 |
| A120 | Mulberry | 0.96 | 3.66 | 1.47 | 2.09 | 54.5 | 0.09 | 98.7 |
| A166 | Fresh hemp mass | 0.94 | 4.00 | 1.92 | 2.01 | 53.8 | 0.12 | 98.6 |
| A183 | Grass silage | 0.88 | 3.60 | 1.66 | 1.65 | 55.4 | 0.11 | 97.7 |
| A300 | Foliage (rotted) | 0.84 | 3.44 | 1.77 | 1.89 | 49.6 | 0.15 | 97.8 |
| A432 | Press juice alfalfa + dandelion | 0.92 | 3.63 | 1.31 | 1.71 | 56.2 | 0.06 | 98.5 |
| A516 | Horse manure | 0.92 | 3.47 | 1.54 | 1.72 | 51.8 | 0.14 | 98.3 |
| A541 | Olive remains Israel | 0.91 | 3.84 | 1.63 | 2.02 | 54.4 | 0.13 | 98.1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A547 | Ground sunflower seeds | 0.92 | 4.43 | 1.87 | 2.00 | 54.6 | 0.17 | 98.4 |
| A562 | Sugar beet | 0.87 | 4.76 | 1.61 | 1.74 | 52.3 | 0.10 | 95.7 |
| A585 | Algae | 0.84 | 4.00 | 1.65 | 1.85 | 52.0 | 0.14 | 97.2 |

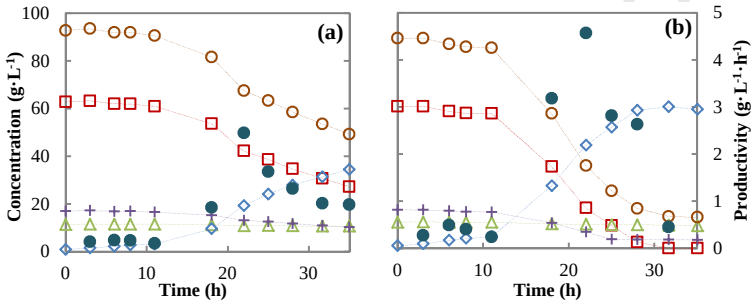
*Calculated from the fraction of sugars consumed.

Yields higher than 0.90 $g \cdot g^{-1}$ and final LA concentrations over 50 $g \cdot L^{-1}$ were observed for most of the strains. In general, the strain A166 was amongst the best in terms of productivities and yields and it also showed the maximum value for L-LA purity at 98.6%.

### 3.4 Effect of the addition of yeast extract in the fermentations

Likewise, in the fermentation with H3, during the screening, the consumption of sugars and production of LA occurred at a slower rate than in the fermentations in which the hydrolysate was only microfiltrated, with $P_{max}$ values around 75-80% lower. Therefore, prior the experiments in the lab and pilot scale bioreactors, an experiment was carried out to evaluate how the addition of yeast extract could enhance the productivities. Parallel fermentations were carried out in duplicate, one with the hydrolysate after electrodialysis (Figure 3a) and one with the same hydrolysate but supplemented with yeast extract (Figure 3b). In a previous report, Neu et al. (2016) showed that the performances of *B. coagulans* could be enhanced 4-5 times by the addition of 5 $g \cdot L^{-1}$ of yeast extract. The same pre-culture was used for both fermentations and as seen, a similar lag phase of around 11 h could be observed. Nonetheless, the positive effect of the addition of yeast extract is evident after the 11 h mark. The fermentation with the supplementation of yeast extract exhibited a sharper variation in the sugars and LA concentrations. Glucose concentration went from 60 $g \cdot L^{-1}$ at t=12 h to only 3.7 $g \cdot L^{-1}$ at t=28 h and the LA concentration increased from 4.9 to 58.6 $g \cdot L^{-1}$ during the same period. In essence, the addition of yeast extract brought a reduction in the fermentation time of around 14 h compared to Figure 2c. By contrast, the fermentation without yeast

299 extract showed a decrease in the glucose concentration from 60.9 to only 34.7 $g \cdot L^{-1}$
300 during the same period. Naturally, the addition of yeast extract also resulted in an
301 increase in both $P_{max}$ and P with corresponding values of 4.56 $g \cdot L^{-1} \cdot h^{-1}$ and 2.78 $g \cdot L^{-1} \cdot h^{-1}$
302 compared to 4.00 $g \cdot L^{-1} \cdot h^{-1}$ and 1.92 $g \cdot L^{-1} \cdot h^{-1}$ in the fermentation without yeast extract.
303 Thus, lab and pilot scales fermentations were performed using the OFMSW hydrolysate
304 supplemented with yeast extract. Nonetheless, experimental work must be carried out in
305 the future to find an inexpensive replacement for the nitrogen source.




306

307 Figure 3: Effect of the addition of yeast extract (5 $g \cdot L^{-1}$) on the fermentation of OFMSW hydrolysate after
308 electrodialysis. Variation in the concentration of total sugars (○), glucose (□), disaccharides (Δ), xylose (+), lactic acid (◊)
309 and productivity (●) for the fermentations of OFMSW hydrolysate samples (a) without the addition of yeast extract
310 and (b) with yeast extract (5$g \cdot L^{-1}$) using *B. coagulans* A166.

311 **3.5 Lab and pilot scale fermentations**

312 Figure 4 shows the profiles for the lab (Figure 4a) and pilot (Figure 4b) scale
313 fermentations of the OFMSW hydrolysate, after electrodialysis and supplemented with
314 yeast extract (5 $g \cdot L^{-1}$), by *B. coagulans* A166. Initial sugar contents were above 80 $g \cdot L^{-1}$
315 from which glucose showed a concentration of approximately 50 $g \cdot L^{-1}$, lower than in all
316 the previous experiments. Oppositely, xylose had a concentration of about 25 $g \cdot L^{-1}$ a
317 value considerably higher than in the previous fermentations in which xylose varied
318 from 7.6 to 17.5 $g \cdot L^{-1}$. Due to the nature of the substrate, variations of this type between
319 batches are difficult to avoid.

As observed in Figure 4a' and 4b', after 6 h of fermentation, biomass nearly doubled from 2.61 to 4.71 $g \cdot L^{-1}$ and from 2.48 $g \cdot L^{-1}$ to 4.85 $g \cdot L^{-1}$ at the lab and pilot scale experiments respectively. It was also during this period that the productivities rapidly increased reaching $P_{max}$ values of 6.05 $g \cdot L^{-1} \cdot h^{-1}$ at the lab scale and 6.38 $g \cdot L^{-1} \cdot h^{-1}$ at the pilot scale. After 6 h, productivity values started to decrease. Values of P and $P_{exp}$ were 2.73 $g \cdot L^{-1} \cdot h^{-1}$ and 3.73 $g \cdot L^{-1} \cdot h^{-1}$ for the lab scale fermentation and, similarly, 2.68 $g \cdot L^{-1} \cdot h^{-1}$ and 3.63 $g \cdot L^{-1} \cdot h^{-1}$ for the pilot scale. Final LA concentrations reached 61.4 and 61.1 $g \cdot L^{-1}$, with yields of 0.97 and 0.94 $g \cdot g^{-1}$, in the lab and pilot scale respectively. In both cases, glucose was completely consumed after 17 h. The strain was able to consume xylose and glucose simultaneously, however, unlike in the case of glucose, consumption of xylose ceased without being completely depleted. Finally, enantiomeric purities for both fermentations were over 98.5% of L-LA.

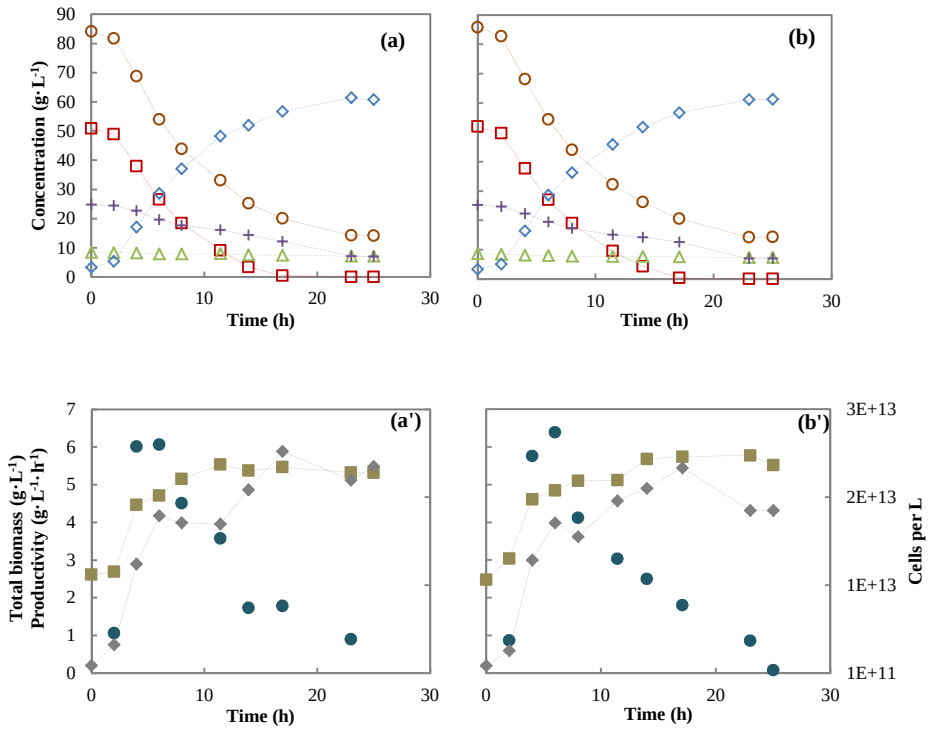



Figure 4: Fermentations profiles at the lab (a,a') and pilot (b,b') scales of the hydrolysate after electrodialysis using *B. coagulans* A166. Variation in the concentration total sugars (○), glucose (□), disaccharides (Δ), xylose (+), lactic acid (◊), cells per L (♦), total biomass (■) and productivity (●).

337 The implementation of the hydrolysate pre-treatment allowed for an important increase
338 in the optical purity of L-LA, from 93% reported in López-Gómez et al. (2019), to
339 98.7%, a value above the market requirements (Castro-Aguirre et al., 2016).
340 Additionally, a yield of 0.20 $g_{LA} \cdot g^{-1}_{dryOFMSW}$ was obtained after the fermentation step,
341 only slightly lower than in our previous results in which a yield of 0.23 $g_{LA} \cdot g^{-1}_{dryOFMSW}$
342 was achieved (López-Gómez et al., 2019).

343 A product with high purity is critical because polymer grade LA is, virtually, the
344 application with the upmost economic potential (Dusselier et al., 2013). In a recent
345 report, published by 'Grand View Research', PLA dominated the LA market during
346 2018 with a revenue share of over 27.8% (Grand View Research, 2018), a trend that is
347 forecasted to continue in the upcoming years. Furthermore, it is likely that due to the
348 nature of OFMSW, its application in other fields such as in the food or cosmetic
349 industries, in which the vast majority of non-polymer grade LA is used, can be difficult
350 due to the consumers' perception of the product and laws that could restrict its use.

351 **3.6 Lactic acid purification**

352 An effective purification is critical for some specialised applications which require
353 products with optimal specifications. In the case of PLA production for example,
354 besides a high optical purity, the total amount of impurities should not exceed 0.05 mol
355 % (Inkinen et al., 2011). Nevertheless, the developments of LA downstream and
356 purification methods are still behind the achievements obtained in the up-streams
357 processes (Alves De Oliveira et al., 2019). This problem is highlighted by the fact that
358 the downstream can account for 30-50% of the total cost of the process. Typically,
359 separation at the industrial scale is carried out in a 2-steps process in which $Ca(OH)_2$ is
360 firstly added to the fermentation broth resulting in a precipitate of calcium lactate.

Following that, $H_2SO_4$ is used to separate the LA. However, a downside of this method is the formation of large quantities of $CaSO_4$ (gypsum), a low-value solid waste which, without properly disposal or recycling, can have negative environmental effects (Alves De Oliveira et al., 2019; Komesu et al., 2017). Therefore, novel methods have been investigated to find a replacement for the purification of LA. Even though data in the literature regarding membrane electrodialysis for the separation of LA is scanty, it is an attractive alternative because the method does not produce harmful wastes, in can be easily scaled up and it allows for the recycling of chemicals (particularly the base for pH control) (Alves De Oliveira et al., 2019).

A series of filtration steps followed by membrane electrodialysis were used in the reported experiments for the separation of LA. Figure 5 shows the variation in the volume and concentration of ions and lactic acid after the fermentation and each downstream step. The numbers in brackets represent the stream of the process to which the samples were taken (see Figure 1). The total volume of the fermentation broth was 26.7 L with a LA concentration of 59.1 $g \cdot L^{-1}$. By the end of the purification the volume of the stream had been reduced to 1.13 L with 719 $g \cdot L^{-1}$ of LA which corresponds to a total LA recovery of 51.5 %.

It has been reported that values for the recovery of lactic acid using electrodialysis can exceed 90% when define and semi-defined mediums are used (Pleissner et al., 2017b; Wee et al., 2005). However, lower values are reported in the literature when complex substrates had been employed for the fermentation. Neu et al. (2016), reported a recovery of 38% when coffee mucilage was used for the fermentations whereas Pleissner et al. (2016), reported a value of only 23%. In these two cases, as for the case reported in this article, the microfiltration and nanofiltration steps accounted for most of

385 the losses during the purification. In this case, from the total LA losses, approximately
386 70% resulted from the microfiltration and nanofiltration steps. Similarly, 60% of LA
387 was lost during those two steps in the process reported by Neu et al. (2016). Recently, a
388 higher recovery of 62% has been reported when sweet sorghum juice was used for the
389 fermentations (Olszewska-Widdrat et al., 2019). In that case, the purification did not
390 include a microfiltration step, probably due to a low concentration of solids, which
391 would explain the higher recovery. Nonetheless, the ultrafiltration step was accountable
392 for about 20% of the LA losses.

393 The content of other ions was successfully reduced to only around 0.3 $g \cdot L^{-1}$, a value
394 lower than in the previous mentioned cases (Neu et al., 2016; Olszewska-Widdrat et al.,
395 2019; Pleissner et al., 2016). Likewise in those cases after distillation, sulphate ions
396 were the most predominant with a concentration of 0.25 $g \cdot L^{-1}$. Overall yield of the
397 process including the downstream was 0.10 $g_{LA} \cdot g^{-1}_{dryOFMSW}$. Even though, neither this
398 work nor the cited articles focused on the downstream of the process, undoubtedly
399 important improvements are necessary in this area to enhance the overall value of the
400 process.

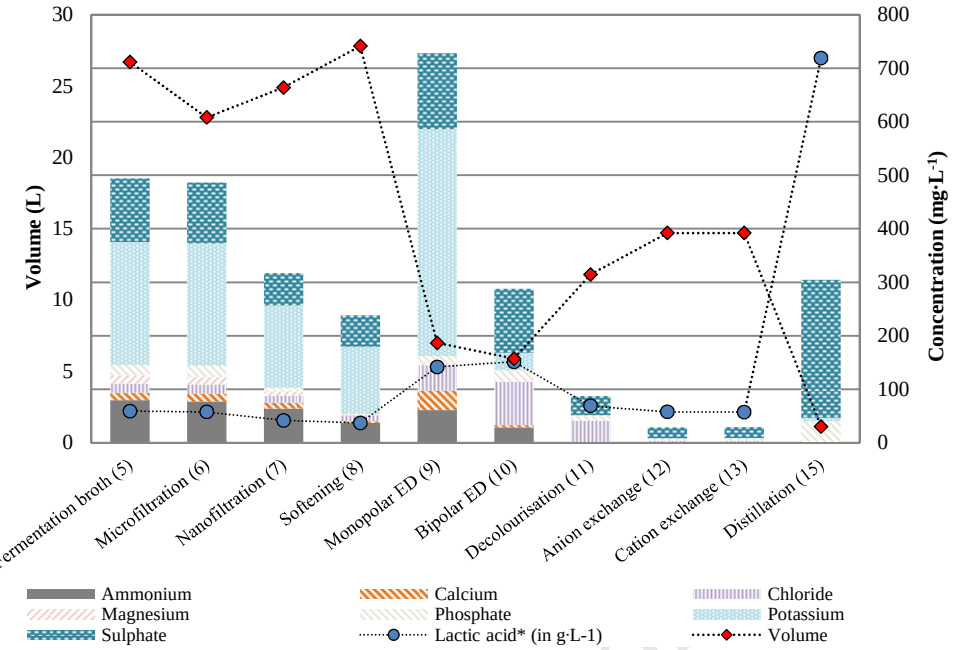



Figure 5: Variation in the volume and concentration of ions and LA after the downstream steps. LA concentrations are given in g·L$^{-1}$. The numbers in brackets indicate the streams from which they were taken in Figure 1.

## 4 Conclusion

This is the first report in which OFMSW has been utilised for the production of L-LA with high enantiomeric purity. The hydrolysates obtained from such a cheap and abundant biowaste proved to be a good substrate for the bioconversion of LA. However, high optical purities can only be accomplished with a pre-treatment step (to remove D-LA) after which, fermentations carried out by several *B. coagulans* isolates successfully achieved enantiomeric purities over 98% L-LA. Scale up of the process was carried out and at the pilot scale, a fermentation of the pre-treated hydrolysate, by *B. coagulans* A166, resulted in a final LA a concentration of 61.1 g·L$^{-1}$ and a yield 0.94 g·g$^{-1}$ were achieved. The downstream of the process resulted on a LA recovery of 51.5% and a purity of L-LA of 98.7 %. Additional experimental work should be carried out to optimise the hydrolysate pre-treatment stage, with perhaps investigations on alternative methods for LA removal. Moreover, further work on downstream steps optimisation is necessary to improve the performance of this critical stage of the process. Finally, future

work should investigate the economic feasibility of the process and evaluate if the increased costs, due to hydrolysate purification, are compensated by the production of LA with higher enantiomeric purity.

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Highlights

- High optically pure L-lactic acid was produced from organic municipal solid waste
- A pre-treatment of the waste is necessary to achieve high optical purity
- Pilot scale fermentations resulted in 61.1 $g \cdot L^{-1}$ of lactic acid and a yield 0.94 $g \cdot g^{-1}$
- Downstream recovery was 51.5 % with a L-lactic acid purity of 98.7 %

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: