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# Assessing the organic fraction of municipal solid wastes for the production of lactic acid

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## HIGHLIGHTS

- Hydrolysates were prepared using OFMSW from different sources.
- OFMSW hydrolysates showed total sugars concentration above 70 $g{\cdot}L^{-1}$.
- *B. coagulans* could grow in all OFMSW hydrolysates without any extra nutrients.
- Final LA concentrations of 60 $g{\cdot}L^{-1}$ with a yield of 0.71 $g{\cdot}g^{-1}$ were achieved.
- It was estimated that 0.23 g of LA could be produced from one g of dry OFMSW.

## ARTICLE INFO

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## ABSTRACT

With an estimated yearly production of about 140 Mt in the EU, conventionally, the organic fraction of municipal solid wastes (OFMSW) has been disposed in landfills with negative environmental effects. Nonetheless, the chemical composition of this residue make it a substrate with great bioconversion potential. In this study, OFMSW from Spanish municipal treatment plants, were evaluated for the production of LA. Samples were identified according to the sorting mechanisms employed for their collection in: (A) separately collected, (B) non-separately collected and (C) separately collected + paper/cardboard. Enzymatic hydrolysis was used to produce hydrolysates A, B and C accordingly. Hydrolysate A showed the highest total sugars and glucose content with values of 70 and 55 $g{\cdot}L^{-1}$, respectively. Following the characterisation, a screening showed that growth of *B. coagulans* was possible in all three hydrolysates. Furthermore, lab scale fermentations showed that LA final concentrations could reach around 60 $g{\cdot}L^{-1}$, with yields from total sugars of above 0.60 $g{\cdot}g^{-1}$. A technical scale fermentation of the hydrolysate A resulted in a final LA concentration of 60.7 $g{\cdot}L^{-1}$, a yield of 0.71 $g{\cdot}g^{-1}$ with a productivity of 2.68 $g{\cdot}L^{-1}{\cdot}h^{-1}$. Overall, it was estimated that 0.23 g of LA could be produced from one g of dry OFMSW.

## 1. Introduction

During the last decades, increasing environmental concerns, together with the acknowledgment of the unsustainability of a fossil fuel-based energy system, have fuelled the search for renewable and more environmentally friendly alternatives for the generation of energy and products. Such rethinking has transformed the way in which the efficiency of a process is evaluated, not only it needs to be profitable, but also sustainable while reducing its negative impact on the environment. The 'Bioeconomy' concept surges as a result of this pursuit and it prioritises the utilization of renewable biological resources for the sustainable production of food, energy and industrial goods [1]. In addition to the development of novel processes and products based on biomass, the valorisation of wastes is a critical aspect for the growth of a bioeconomy. In other words, residues from a process should not be considered as such until their full potential has been exploited.

The organic fraction of municipal solid wastes (OFMSW) is an abundant type of biowaste (with a yearly production of about 140 Mt in the EU) that comes from households, restaurants, small businesses, yards and garden wastes, etc. [1]. Traditionally, OFMSW has been landfilled with negative effects on the environment [2]. Therefore, new legislations have imposed targets and restrictions on its disposal

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OFMSW. For example, the European Union action plan for the Circular Economy (Directives 2018/850,851), entered into force in July 2018 and revised the legislative framework on waste, setting targets for recycling 65% of MSW, limiting its disposal in landfilling to a maximum of 10% and making obligatory the separate collection of biowaste by 2023 [1]. In turn, investigations into new methods for the processing of the OFMSW have been carried out and its value started to be recognised.

The composition of OFMSW depends upon different factors such as season, climate, geographic location, etc., but in average, it is mainly constituted of approximately 30–69 % carbohydrates (starch, cellulose and hemicelluloses), 5–10 % proteins and 10–40 % lipids [3,4] which makes it a substrate with high potential application in biotechnological processes [5]. Anaerobic digestion is acknowledged as one of the main alternatives for the valorisation of the OFMSW and it has been fully scaled up during the recent decades [5–7]. However, anaerobic digestion only generates low-value products, biogas and compost. Therefore, more recent studies have focused on the production of other higher value added chemicals such as biobutanol [8], biodiesel and ethanol [9], hydrogen [10], polyhydroxyalkanoates [11] and lactic acid (LA) [5,12–15].

LA is an organic acid widely utilized in the food, pharmaceutical, textile, medical, leather and chemical industries and, lately, the production of polylactic acid (PLA) has also increased the interest in its production [16]. Industrially, LA is manufactured by the conversion of simple sugars through fermentation. However, more recently, the utilization of other cheap substrates has been the objective of research targeting cuts in production costs and the addition of value to residues from other processes [16]. The application of the OFMSW to produce LA perfectly fits the bioeconomy concept; an interesting alternative that could result in the reduction of costs in LA production while dealing with the processing of the OFMSW in a more effective manner.

Only a couple of reports have focused on the production of LA by fermentations of OFMSW. Probst et al. [17] showed how naturally occurring microorganisms present in the OFMSW could be used for the fermentation of the waste into LA. However, these investigations did not use a pre-treatment stage for the hydrolysis of the OFMSW limiting carbohydrate utilization by the microorganisms. Moreover, a mixed culture of indigenous microorganisms, which not solely produce LA, most likely results in low conversion yields and productivities [5]. A pre-treatment can enhance the liberation of sugars by enzymatic hydrolysis as demonstrated by Nwobi et al., [9]. In their study, results showed that the pre-treatment of OFMSW and hydrolysis enhanced the production of ethanol and LA. Furthermore, the mild pre-treatment conditions used avoided the formation of inhibitory compounds, such as hydroxymethylfurfural (HMF) and phenolic compounds, which is typical of harsh thermochemical treatments.

Various publications dealing with the utilization of food waste (FW) for the production of LA can be found in the literature [5,12,18–20]. In a recent study, LA production from FW was attempted using a mesophilic *Streptococcus* [5]. They concluded that a separate hydrolysis (instead of simultaneous saccharification and fermentation) enhanced LA productivities. Even though studies using FW can serve as a basis for research using the OFMSW, there are significant differences between their compositions. Residues obtained from kitchens, are usually cooked food residues rich in starch, proteins and free sugars [5]. The cooking process is, in essence, a pre-treatment which facilitates the saccharification and fermentation processes. Moreover, as specified in [5,18,20], the FW used in their studies were leftovers from canteens and bakeries which mainly contained noodles, potatoes, vegetables, fruits, meat, sauce, rice, cake, bread and pastries. On the contrary, OFMSW is a more complex, variable and heterogeneous residue which, in addition to leftovers, also contains for example, peels and seeds from vegetables and fruits, garden wastes, spent coffee, uncooked food residues, etc. Furthermore, though in a lower proportion, it can also contain other inert materials such as food packaging, paper and non-biodegradable materials (plastics, glasses, metals, textiles, etc.) because of an inefficient waste sorting. The removal of these inert materials is crucial to increase the efficiency of subsequent biological treatments and, typically, a mechanical pre-treatment stage is included in MSW treatment plants to separate those inert fractions [21]. Therefore, an appropriate evaluation of OFMSW as feedstock for LA production is impossible without using real samples from industrial MSW treatment plants.

This study focuses on the valorisation of three representative OFMSW streams from municipal plants. The hydrolysates produced from the three streams were used in fermentations with *Bacillus coagulans* to produce LA. *B. coagulans* strains are homofermentative L-LA bacteria, with low nutritional requirements and that can grow on a variety of carbon sources which makes them of high interest for industrial applications [22]. Furthermore, the strains are thermophilic which facilitates the processes since it can work under non-sterile conditions reducing contamination problems [23]. To the best of our knowledge, it is the first time that a study is devoted to the evaluation of OFMSW, from MSW industrial treatment plants, for the production of LA.

## 2. Materials and methods

### 2.1. OFMSW samples and hydrolysate production

The validation of LA fermentation was carried out with three different OFMSW feedstocks from Spanish MSW treatment plants (Table 1). Besides the location, the main difference between the samples was collection system used in the different municipalities. (A) Separately collected biowaste refers to biowaste which has gone through a pre-sorting at its source of origin i.e. houses, restaurants, gardens, etc. (B) Non-separately collected biowaste refers to the biowaste fraction remaining after the mechanical pre-treatments carried out to the mixed urban waste fractions, in mechanical-biological treatment plants, in which recyclables are recovered from this stream. Finally, (C) refers to samples from (B) that have been mixed with paper/cardboard fractions in a rotary drum for several days in the MSW plants. Fig. 1 shows the images from the 3 types of OFMSW used for the experiments reported.

Samples ranging from 50 to 100 kg, with moisture contents between 70–80 %, were obtained from the industrial plants after passing different mechanical sorting pre-treatments. The samples were collected during different periods of the year and stored at −20 °C until they were used for the hydrolysis. Inert materials in the samples such as glass, plastics, stones, textiles, etc. were manually removed. After that, the samples were homogenized using a technical hammer mill and sterilized by autoclaving at 121 °C for 1 h before hydrolysis.

Enzymatic hydrolyses were carried out in 5 L or 50 L jacketed reactors for 72 h, with a stirring rate of 150 rpm. The solids-to-liquid ratio

**Table 1**
OFMSW feedstock used for the validation of LA production and hydrolysate produced.

| OFMSW substrate description | Hydrolysate | Municipality of MSW treatment plant |
|---|---|---|
| Separately collected municipal biowaste | A | Valencia metropolitan area |
| Non-separately collected municipal biowaste | B | |
| Non-separately collected municipal biowaste + contaminated paper and cardboard | C | Barcelona metropolitan area |

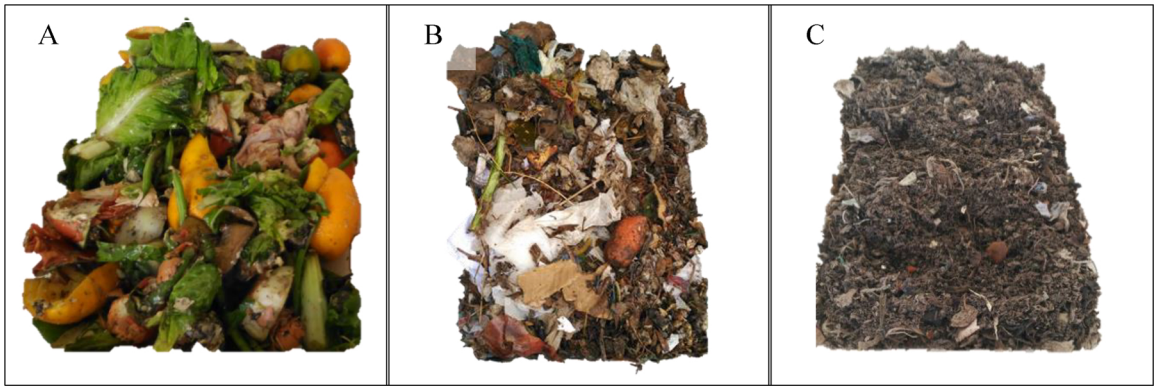


**Fig. 1.** OFMSW samples (A) separately collected, (B) non-separately collected and (C) non-separately collected + mixed cardboard and paper.

was 20% ($w{\cdot}w^{-1}$) and the temperature of the process 50 °C. The pH was maintained at 5 by the addition of NaOH (20% $w{\cdot}w^{-1}$), although minimal pH fluctuations occurred during the hydrolysis. The enzyme cocktail used which was tailor made for OFMSW substrates and supplied by Novozymes, was mainly based on cellulases and amylases. Samples were withdrawn during the hydrolysis to measure the variation in the sugar content and the formation of growth inhibitors.

Hydrolysate samples were coarse filtered using a filter bag (Ø = 150 µm) before the fermentations. A microfiltration was carried out, after the coarse filtration, for some specified cases. The microfiltration was performed using a cross-flow micro-filtration system (UFITEC, Germany), pore size = 0.2 µm, at 1.5 bar and 15 °C, equipped with 4 TAMI membranes (TAMI Industries, France).

### 2.2. Microorganism and screening

A selection of *B. coagulans* strains, obtained from the department of Bioengineering at the Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB, Germany) was used for the screening of the substrates and subsequent fermentations. Inocula for the fermentations were prepared in de Man, Rogosa and Sharpe (MRS) medium as described by [24] with an incubation time of 12 h.

A screening was carried out to evaluate if the hydrolysates obtained from the different OFMSW samples could support the growth of 11 *B. coagulans* isolates. Before the screening, hydrolysate samples were centrifuged at 4800 rpm for 15 min. After centrifugation, the supernatant was removed, its pH was adjusted to 7.0 with NaOH (20% $w{\cdot}w^{-1}$) and used for the fermentations at 52 °C. Screening was carried out using Bioscreen C (Growth Curves USA) equipment which provides optical density (OD) measurements in a microplate. A volume of 300 µL of hydrolysate and 10 µL of the inoculum was used per well. Triplicates were carried out for each one of the strains tested. Measurements were performed at 3 min intervals and wavelengths ranging from 420 to 580 nm.

After the screening, the strains A20 and A166 identified by the Leibniz Institute's German Collection of Microorganisms and Cell Cultures (DSMZ) as DSM 2314 and DSM ID-14300 respectively, were used in bioreactor experiments.

### 2.3. Fermentations

#### 2.3.1. Lab scale fermentations

Hydrolysates A and B, obtained from separately collected OFMSW and non-separately collected OFMSW respectively, were selected for further fermentations in small scale bioreactors. Two sets of fermentations were performed. In the first fermentation, the hydrolysates were used after being coarse filtrated. The second set was carried out with a further removal of solids in which, after coarse filtration, hydrolysate A was microfiltrated (as described in section 2.1). Each fermentation was carried out in duplicate, at 52 °C and 400 rpm, using an Eloferm multifermentation system (Biotronix GmbH, Germany). The working volume for the fermentations was 300 mL and the pH was controlled at 6.0 with NaOH 20% ($w{\cdot}w^{-1}$). The bioreactors were inoculated with *B. coagulans* A166 at a concentration of 6% ($v{\cdot}v^{-1}$). During the fermentation, samples were taken at different times, inactivated by heating at 95 °C for 20 min and stored at −20 °C before the quantification of sugars and LA. Similarly, fermentations of the coarse filtrated and microfiltrated hydrolysate A, were carried out with *B. coagulans* A20. Productivities were calculated at every sampling point during the fermentation with the highest value corresponding to the maximum productivity ($P_{max}$). Additionally, the global productivity of the fermentation ($P_g$) was used to define the productivity of the fermentation from the time of inoculation until the variation of LA concentration ceased, i.e. the beginning of the stationary phase. The LA yield (Y) was calculated from the sugars consumed whereas the (Y') denotes the LA yield obtained from the total concentration of sugars at the beginning of the fermentation.

#### 2.3.2. Technical scale fermentation

A technical scale fermentation was performed in a 72 L BIOSTAT UD bioreactor (B-Braun Biotech, Germany). A total of 30 L of hydrolysate A were used as the substrate for the fermentation. The process was carried out at 52 °C and the pH was controlled at 6.0 by the addition of NaOH (20% $w{\cdot}w^{-1}$). A double Rushton turbine was used for agitation at 300 rpm. The reactor was inoculated with *B. coagulans* A166 at 3% ($v{\cdot}v^{-1}$). The inoculum was prepared in a 3 L reactor, containing 1 L of hydrolysate A. Samples were taken regularly for the quantification of sugars, LA, living cells and total cells.

### 2.4. Analytical assays

Analytical essays for the quantification of sugars and LA concentration and LA enantiomeric purity were carried out as detailed in [25]. High Pressure Liquid Chromatography (HPLC) (Coregel 87H3 7.8 mm × 300 mm column, Chrom Tech, USA) was used for the quantification of sugars and potential inhibitors, such as furfural and 5-HMF, for the evaluation of the hydrolysis.

The quantification of sugars and LA was performed using HPLC (Dionex, USA) and a Eurokat H column (300 mm × 8 mm × 10 µm, Knauer, Germany). The mobile phase was an aqueous solution of 5 mM $H_2SO_4$ with a flow rate of 0.8 $mL{\cdot}m^{-1}$. Injection volume for each sample was 10 µL and the detection was carried out with a refractive index detector (RI-71, Shodex, Japan). The enantiomeric quantification of LA was also carried out via HPLC (Dionex, USA) with a Phenomenex Chirex 3126 column (150 × 4.6 mm ID, Phenomenex, USA) at 30 °C coupled to an ultraviolet detector. The mobile phase was 1 mM $Cu_2SO_4$ flowing at 1 $mL{\cdot}m^{-1}$.

A THOMA cell chamber (Glaswarenfabrik Karl Hecht GmbH & Co

**Table 2**
Average concentration of hydrolysate from different OFMSW sources. Hydrolysate A shows the average of 13 batches from separately collected OFMSW, hydrolysate B shows the average of 10 batches from non-separately collected OFMSW and hydrolysate C shows the average from 3 samples from separately collected OFMSW mixed with cardboard and paper.

| Hydrolysate | Glucose ($g{\cdot}L^{-1}$) | Xylose ($g{\cdot}L^{-1}$) | Disaccharides ($g{\cdot}L^{-1}$) | Arabinose ($g{\cdot}L^{-1}$) | Lactic acid ($g{\cdot}L^{-1}$) | Acetic acid ($g{\cdot}L^{-1}$) |
|---|---|---|---|---|---|---|
| A | 55.41 ± 2.01 | 10.13 ± 1.2 | 8.30 ± 0.62 | 1.19 ± 0.50 | 5.69 ± 0.88 | 3.20 ± 0.23 |
| B | 47.16 ± 2.88 | 8.76 ± 0.84 | 6.46 ± 0.69 | 1.13 ± 0.50 | 13.76 ± 1.05 | 2.83 ± 0.23 |
| C | 36.84 ± 3.02 | 6.78 ± 0.44 | 4.00 ± 0.45 | 0.30 ± 0.00 | 17.9 ± 1.54 | 4.04 ± 0.03 |

KG, Germany) was used for the determination of total number of cells while the number of living cells was determined by the number of colony forming units as described in [25].

## 3. Results and discussion

### 3.1. Hydrolysate characterization

The reason to select three representative OFMSW streams was to evaluate the effect that different municipal waste collection systems could have in the production of OFMSW hydrolysates. A separate collection of biowaste reduces the content of inert materials in the hydrolysate, which in turns increases the content of organic matter available for its conversion into sugars [21]. Furthermore, the mixture of streams to produce hydrolysate C increased the heterogeneity of the substrate for which a homogenizing process was required. The impact that an appropriate sorting of OFMSW has can be seen in Table 2.

Considerable variations were observed between the hydrolysates obtained from different sources of OFMSW but in all cases, glucose was the main sugar available for fermentation. Before the hydrolysis, the amount of extractable sugars was around 20–30, 10–15 and 5–8 $g{\cdot}L^{-1}$ for hydrolysates A, B and C, respectively. Hydrolysate A showed the highest glucose concentration (55.41 ± 2.01 $g{\cdot}L^{-1}$), followed by hydrolysate B (47.16 ± 2.88 $g{\cdot}L^{-1}$) and C (36.84 ± 3.02 $g{\cdot}L^{-1}$). These values are considerably higher than the reported in [15] where, without hydrolysis, the organic municipal solid wastes had a concentration of reducing sugars of only 23.7 ± 1.1 $g{\cdot}L^{-1}$. As anticipated, glucose concentrations were lower than the values reported in [5], in which the hydrolysates were prepared solely from kitchen wastes, achieving 67.3 $g{\cdot}L^{-1}$ of glucose. In contrast to other lignocellulosic substrates, such as hardwood and softwood, which have a higher hemicellulose content, the xylose content in the OFMSW hydrolysates was much lower than glucose content [26]. Even lower was the concentration of disaccharides reaching 5.87 ± 0.62 $g{\cdot}L^{-1}$ for hydrolysate A and around 2 $g{\cdot}L^{-1}$ for hydrolysates B and C. No specific inhibitory compounds such as furfural and 5-HMF were detected in the samples. This fact was expected since these inhibitors are usually generated when harsh thermochemical pre-treatments are carried out, but not when enzymatic processes are used [9,27].

Lactic and acetic acids were present in the 3 types of hydrolysate produced. These organic acids were already detected from the beginning of the hydrolysis which indicates that they were produced by naturally occurring organisms present in the OFMSW. The highest concentration of these acids was detected for the hydrolysate C samples. Biowaste + paper fractions were mixed for several days in a rotatory drum which probably promoted the activity of microorganisms present in the waste and could explain the lower concentration of sugars and the highest concentration of LA. LA was present in the hydrolysates with concentrations of 5.69 ± 0.88, 13.76 ± 1.05 and 17.90 ± 1.54 $g{\cdot}L^{-1}$ for A, B and C, respectively and analysis of the enantiomeric LA purity revealed a racemic mixture of D- and L-. This is in congruence with [15,28], that showed a natural production of an isomeric racemic mixture of LA in kitchen FW and OFMSW. Even though racemic LA can be used, for example in the production of organic solvents e.g. ethyl lactate [15], a high optical purity is paramount for other applications. In particular, for the production of PLA in which the ratio D- to L- LA has an important effect on the final product properties such as its thermal stability, crystallinity and biodegradation, and commercially, a mixture with higher amount of L-LA is used [29–31]. Since an effective separation of the enantiomers is not feasible yet, a high optical purity after the fermentation can be crucial. In the case of the FW studies aforementioned, LA formation by natural occurring bacteria was likely not a problem since there were short periods between the collection and the fermentation [32], a condition which would be much more difficult to meet in the case of the OFMSW from industrial plants.

### 3.2. Screening

The results for the concentrations of sugars and nutrients obtained in the characterisation of the substrate (Section 3.1) suggested that OFMSW hydrolysate could be a good substrate for microbial fermentations. Nevertheless, the heterogeneous nature of the OFMSW could result in hydrolysates that, although having available carbohydrates for fermentation, lack the required nutrients to support growth. Furthermore, even though the formation of some inhibitory compounds (such as furfural) was measured during the hydrolysis, such a complex substrate could contain other elements which could hinder growth. Therefore, a preliminary evaluation of the hydrolysates was carried out at the microscale level to determine if the substrate could indeed support the growth of *B. coagulans* without the addition of any extra nutrients. Fig. 2 shows the variation in OD, from initial to maximum OD achieved, for the strains tested (internal code at ATB).

Values for the maximum growth rates ($\mu_{max}$) were calculated for the strains and are reported in Table 3. The strains A20 and A166, both homofermentative L—LA producers, were the strains with the highest $\mu_{max}$, and thus, they were selected for further experiments. Previous experience within this research group has shown the good performance of these strains for the consumption of various sugars in complex substrates [33,34].

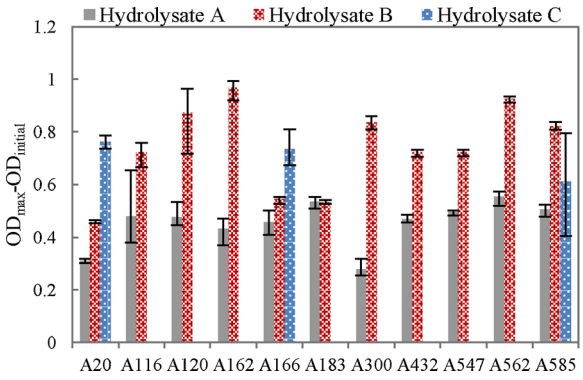


**Fig. 2.** Difference between initial and maximum optical density achieved in a microplate reader by different *B. coagulans* isolates. Substrates for the fermentations were hydrolysates produced from different OFMSW sources. Results show the average of triplicates with error bars showing the maximum and minimum value for each set of samples. Only strains A20, A166 and A585 were tested for the case of hydrolysate C.

**Table 3**
Maximum growth rates for the strains tested.

| Strain | A20 | A116 | A120 | A162 | A166 | A183 | A300 | A432 | A547 | A562 | A585 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| μ ($h^{-1}$) | 0.056 | 0.043 | 0.036 | 0.033 | 0.054 | 0.028 | 0.031 | 0.006 | 0.039 | 0.018 | 0.038 |

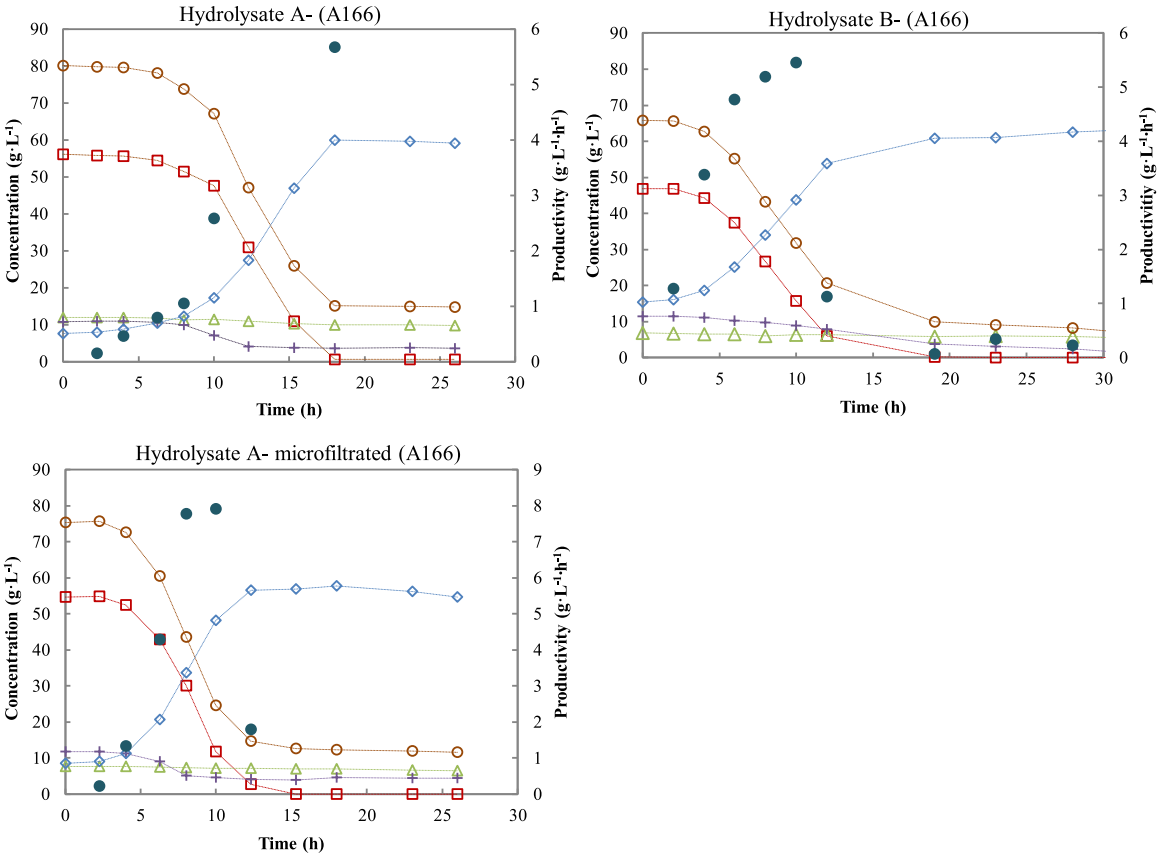


**Fig. 3.** Variation in the concentration of total sugars (○), glucose (□), disaccharides (△), xylose (+), lactic acid (◊) and productivity (•) for the fermentations of separately collected biowaste (Hydrolysate A) and non-separately collected biowaste (Hydrolysate B) using *B. coagulans* A166. Graphs show the results obtained for the substrates after coarse filtration and after microfiltration. Results show the averages of experiments done in duplicate.

### *3.3. Fermentations*

#### *3.3.1. Lab scale fermentations*

Based on their higher sugar content, hydrolysates A and B were used in small scale bioreactors experiments. Fermentations were carried out using *B. coagulans* A166 with the hydrolysates after being coarse filtered.

The profile for the fermentations is shown in Fig. 3. As before, hydrolysate A had a higher concentration of total sugars and glucose (80.1 and 56.1 $g{\cdot}L^{-1}$) than hydrolysate B (66.0 and 46.8 $g{\cdot}L^{-1}$). After the lag phase, glucose concentration sharply decreased, and it was totally consumed after 18 h for hydrolysate A and 19 h for hydrolysate B. After glucose, xylose was the most consumed sugar with a decrease in concentration from 10.8 to 3.7 $g{\cdot}L^{-1}$ and from 11.5 to 3.1 $g{\cdot}L^{-1}$ for hydrolysate A and B respectively. Concentrations for disaccharides and arabinose remained almost constant throughout the process. Both fermentations had a high Y = 0.91 $g{\cdot}g^{-1}$ and values for Y' were 0.63 $g{\cdot}g^{-1}$ and 0.54 $g{\cdot}g^{-1}$ for hydrolysate A and B, respectively. Similar values for $P_{max}$ of 5.67 ± 0.32 and 5.45 ± 0.11 $g{\cdot}L^{-1}{\cdot}h^{-1}$ for hydrolysate A and B were achieved. $P_g$ values were 2.95 ± 0.00 $g{\cdot}L^{-1}{\cdot}h^{-1}$ for A and 2.89 ± 0.24 $g{\cdot}L^{-1}{\cdot}h^{-1}$ for B.

Initial concentrations of LA for hydrolysate A and B were 7.6 and 15.0 $g{\cdot}L^{-1}$ respectively. By the end of the fermentation the concentration of LA was 60.0 $g{\cdot}L^{-1}$ for hydrolysates A with a L-LA percentage of approximately 93%, this value is exactly at the theoretical threshold determined by Castro-Aguirre et al. for PLA to crystallise [35]. Similarly, hydrolysate B reached a LA concentration of 63.0 $g{\cdot}L^{-1}$ however, due to its higher initial LA content, an optical purity of only 83% L-LA was obtained.

The higher sugars content, together with the lower concentration of initial LA made hydrolysate A a more attractive substrate and, thus, it was used for subsequent experimental work. A further removal of solids, by microfiltration, was implemented to evaluate if it could improve fermentation performance. After microfiltration, hydrolysate A showed a total sugars and glucose concentration of 75.2 and 54.6 $g{\cdot}L^{-1}$ respectively. Initial LA concentration was similar to that from the coarse filtrated sample with approximately 8 $g{\cdot}L^{-1}$ while a final LA concentration of 57.7 $g{\cdot}L^{-1}$ was achieved with an optical purity of 93% L-LA. In terms of conversion yields, an increment was observed with values for Y and Y' of 0.97 and 0.63 $g{\cdot}g^{-1}$. Values for $P_{max}$ and $P_g$ also showed an increase with 7.89 and 2.98 $g{\cdot}L^{-1}{\cdot}h^{-1}$.

A set of fermentations was carried out using the strain A20 to compare its performance against the A166, results of the experiments are shown in Fig. 4.

Similarly to the fermentations with the A166 strain, it is apparent that the microfiltration step causes a slight reduction in the total sugars concentration and shortens the process time. However, LA yield from consumed sugars was considerably lower for the fermentation using the

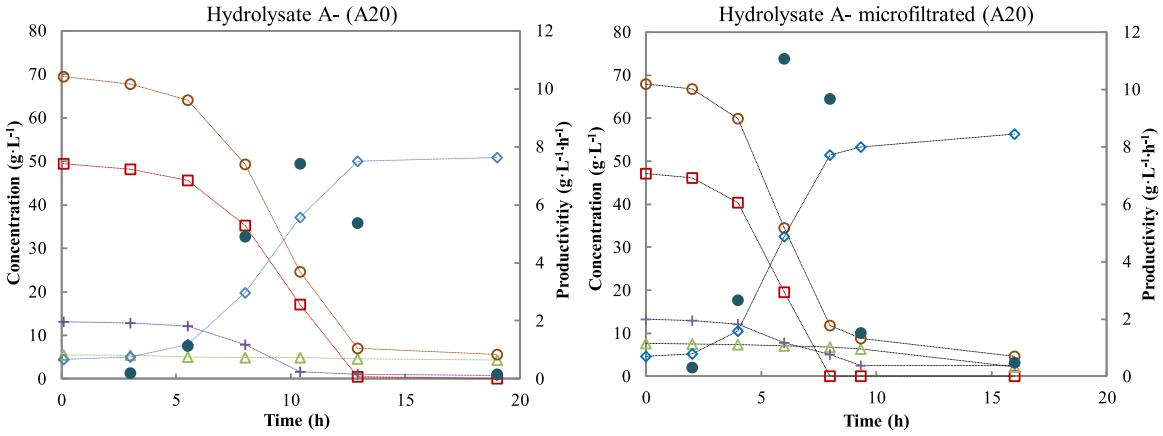


**Fig. 4.** Variation in the concentration of total sugars (○), glucose (□), disaccharides(△), xylose (+),lactic acid (◊) and productivity (●) for the fermentations of separately collected biowaste (Hydrolysate A) after coarse filtration (150 μm) and microfiltration (0.2 μm) using *B. coagulans* A20. Results show the averages of experiments done in duplicate.

**Table 4**
Evaluation of the fermentations using hydrolysates A and B.

| Hydrolysate | Strain | Treatment | Y ($g \cdot g^{-1}$) | Y' ($g \cdot g^{-1}$) | $P_{max}$ ($g \cdot L^{-1} \cdot h^{-1}$) | $P_g$ ($g \cdot L^{-1} \cdot h^{-1}$) | Initial LA ($g \cdot L^{-1}$) | Final LA ($g \cdot L^{-1}$) | L-LA (%) |
|---|---|---|---|---|---|---|---|---|---|
| A | A166 | Coarse filtrated | 0.91 | 0.63 | 5.67 | 2.95 | 7.6 | 60.0 | 93 |
| | | Microfiltrated | 0.97 | 0.63 | 7.89 | 2.98 | 8.0 | 57.7 | 93 |
| | | Technical scale /microfiltrated | 0.94 | 0.71 | 7.50 | 2.84 | 7.3 | 60.7 | 93 |
| | A20 | Coarse filtrated | 0.80 | 0.65 | 7.40 | 3.58 | 4.0 | 51.0 | 93 |
| | | Microfiltrated | 0.90 | 0.69 | 11.07 | 3.38 | 4.6 | 56.3 | 93 |
| B | A166 | Coarse filtrated | 0.91 | 0.54 | 5.45 | 2.89 | 15.0 | 63.0 | 83 |

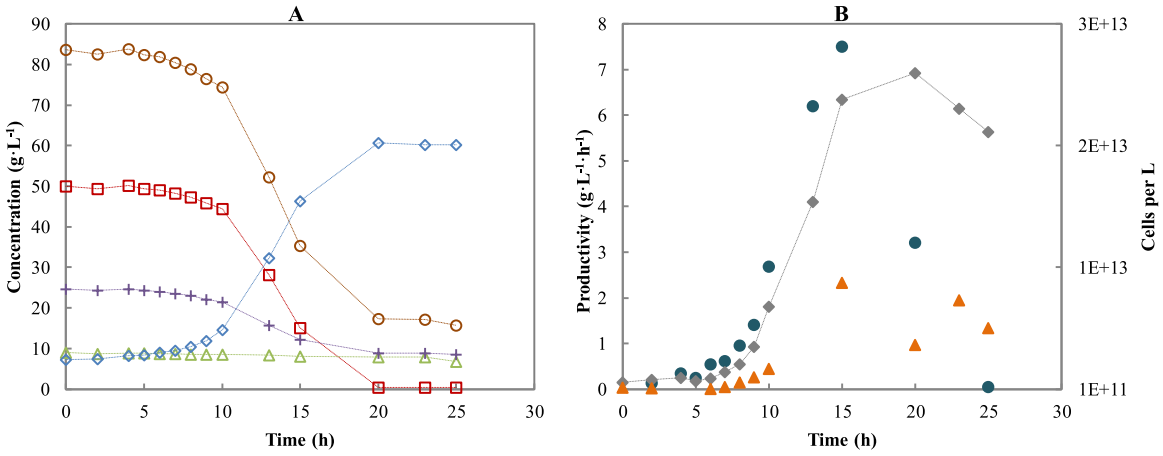


**Fig. 5.** Fermentations in the technical scale (30 L working volume) of the hydrolysate A after microfiltration with *B. coagulans* A166. (A) Variation in the concentration of total sugars (○), glucose (□), disaccharides (△), xylose (+) and lactic acid (◊). (B) Measurements of total cells (♦), living cells (▲) and productivity (●).

coarse filtrated hydrolysates with a value of 0.80 $g \cdot g^{-1}$ with an increase after microfiltration to 0.90 $g \cdot g^{-1}$ but still lower than all the yields obtained using the A166 strain. Values for Y' were 0.65 and 0.69 $g \cdot g^{-1}$ for the fermentations with the coarse filtrated and microfiltrated hydrolysate respectively. Interestingly, the strain showed higher values than A166 of productivities with 3.58 $g \cdot L^{-1} \cdot h^{-1}$ for the coarse filtrated sample and 3.38 $g \cdot L^{-1} \cdot h^{-1}$ for the microfiltrated one.

Table 4 is a summary of the results for yields, productivities, titres and optical purities for the fermentations. Hydrolysate A showed, in general, better results than hydrolysate B with slightly higher productivity values. Moreover, the values of optical purity were considerably lower for the case of hydrolysate B. Additionally, the microfiltration enhanced the performance of the strains with higher values for Y, Y' and $P_{max}$.

### 3.3.2. Technical scale fermentation

The profile for the technical scale fermentation of hydrolysate A is shown in Fig. 5. The batch of hydrolysate A used in this fermentation showed the heterogeneity of the OFMSW. Although the concentration of total sugars was 83.70 $g \cdot L^{-1}$, similar to the previous fermentations, the fractions of xylose was 0.29, considerably higher than in the previous cases in which the fraction of xylose was between 0.12 and 0.18. Like in the lab scales fermentations, glucose was completely consumed while the concentration of xylose went from 24.60 to 8.80 $g \cdot L^{-1}$ by the end of the process. However, as in the previous fermentations, there was no significant variations in the concentration of disaccharides and, by the end of the fermentation, a total of 15.8 $g \cdot L^{-1}$ or residual sugars were left unfermented. It has been previously reported that some *B. coagulans* strains struggle with the consumption of disaccharides with

**Table 5**
Production of LA using OFMSW and kitchen food wastes.

| Substrate | Microorganism | Process | P ($g \cdot L^{-1} \cdot h^{-1}$) | Final LA ($g \cdot L^{-1}$) | Ref. |
|---|---|---|---|---|---|
| OFMSW | *B. coagulans* | Enzymatic hydrolysis & fermentation | 2.84 | 60.7 | This study |
| | Mixed culture | Direct fermentation | 1.00 | 29.9 | [14] |
| | Mixed culture | Fermentation with temperature gradient | 0.79 | 29.7 | [15] |
| Kitchen food waste | *B. coagulans* NBRC12583 | Enzymatic hydrolysis & fermentation | 0.72 | 86.0 | [38] |
| | *Lactobacillus* and *Streptococcus* sp. | Simultaneous saccharification and fermentation | 2.16 | 60.5 | [32] |
| | *Lactobacillus casei* Shirota | Fungal hydrolysis & fermentation | 2.61 | 94.0 | [18] |
| | *Streptococcus* sp. | Enzymatic hydrolysis & fermentation | 3.38 | 66.5 | [5] |

$\mu_{max}$ values 50% lower than those obtained from glucose [36] which could explain why the concentration of disaccharides did not show a significant change throughout the process. Unlike in some cases in which the consumption of carbohydrates occurs sequentially [37], the bacteria was able to metabolise glucose and the other sugars simultaneously. Thus, an alternative reason for the incomplete consumption of sugars could be that, although the hydrolysate contains enough nutrients to support the growth during the first hours, as the fermentation progresses, they become depleted, halting the fermentation at a certain point.

As seen in the figure, the initial LA concentration was 7.30 $g \cdot L^{-1}$ reaching 60.70 $g \cdot L^{-1}$ by the end of the fermentation. This value is higher than the ones reported in the literature when real samples of OFMSW were used (see Table 5). As shown in Fig. 5B the productivity of the strain started increasing after 5 h and reached a maximum value of 7.50 $g \cdot L^{-1} \cdot h^{-1}$ at 15 h which is congruent with the value obtained in the lab scale fermentation of 7.89 $g \cdot L^{-1} \cdot h^{-1}$. After 20 h, the global productivity was 2.84 $g \cdot L^{-1} \cdot h^{-1}$, a result comparable to the values obtained using FW. A productivity of 4.47 $g \cdot L^{-1} \cdot h^{-1}$ was achieved in this study during the exponential phase. The same productivity had a value of 3.38 $g \cdot L^{-1} \cdot h^{-1}$ in the study by Demichelis et al., [5] in which FW was hydrolysed and then fermented. Finally, the yields where similar to the previous experiments using hydrolysate A with values for $Y = 0.94\ g \cdot g^{-1}$ and $Y' = 0.71\ g \cdot g^{-1}$.

### 3.4. Mass balance

The hydrolysates were prepared at 20% solids loading, thus, every kg of hydrolysate contained 200 g of dry OFMSW. After hydrolysis, an average concentration of total sugars of 70 $g \cdot L^{-1}$ was achieved. Average density for the hydrolysate was 1.07 $kg \cdot L^{-1}$, hence, every kg of hydrolysate contained 65.4 g of sugars. During the technical scale experiment the LA yield from total sugars achieved was 0.71 $g \cdot g^{-1}$. Therefore, approximately 46 g of LA could be produced per kg of hydrolysate i.e. 200 g of dry OFMSW, which corresponds to a yield of 0.23 $g_{LA} \cdot g^{-1}_{dryOFMSW}$. The study by [15] is one of the few available in the literature in which LA was produced from real OFMSW samples. Nonetheless, they used the indigenous mixed microbial population of the waste to produce LA, hence, productivities and yields reported were low. Fewer more studies using food waste from cafeterias and restaurants can be found in the literature that can be used as reference. In processes with separate hydrolysis and fermentation the yields ($g_{LA} \cdot g^{-1}_{dryFW}$) were 0.27 [18] and 0.33 [5]. Thus, considering that samples of OFMSW obtained from MSW treatment plants were used, the yield obtained in this study is promising.

## 4. Conclusion

The OFMSW obtained from different MSW treatment plants was used to produce LA. The bioresidues were classified according to the waste collection system into separately collected OFMSW, non-separately collected OFMSW and separately collected OFMSW + paper/cardboard. The separately collected OFMSW yielded the highest concentration of sugars and lowest concentration of LA after hydrolysis which highlights the importance that the collection system has in the process. Remarkably, microbial growth of *B. coagulans* could be observed in all the OFMSW hydrolysates without the addition of any extra nutrients. A higher enantiomeric purity for L-LA (93%) was obtained in fermentations with hydrolysate A compared to fermentations using hydrolysate B that only achieved 83%. Furthermore, fermentation results showed that LA yields and productivities comparable to those from FW studies are achievable. In conclusion, the production of LA is an interesting alternative, to more common methods, such as anaerobic digestion, which could further add value to OFMSW. Future experimental work will be carried out to optimise the fermentation process and increase final lactic acid concentrations. Additionally, studies involving the pre-treatment of the hydrolysate (to reduce its initial LA content), will be carried out to assess the feasibility of producing LA with high optical purity from OFMSW.

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## References

[1] European Commission, A Sustainable Bioeconomy for Europe: Strengthening the Connection Between Economy, Society and the Environment Updated Bioeconomy Strategy, (2018), https://doi.org/10.2777/792130.
[2] L. Alibardi, R. Cossu, Composition variability of the organic fraction of municipal solid waste and effects on hydrogen and methane production potentials, Waste Manag. 36 (2015) 147–155, https://doi.org/10.1016/j.wasman.2014.11.019.
[3] D. Pleissner, C.S. Lin, Valorisation of food waste in biotechnological processes, Sustain. Chem. Process 1 (2013) 21, https://doi.org/10.1186/2043-7129-1-21.
[4] E. Uçkun Kiran, A.P. Trzcinski, W.J. Ng, Y. Liu, Bioconversion of food waste to energy: a review, Fuel 134 (2014) 389–399, https://doi.org/10.1016/j.fuel.2014.05.074.
[5] F. Demichelis, D. Pleissner, S. Fiore, S. Mariano, I.M. Navarro Gutiérrez, R. Schneider, J. Venus, Investigation of food waste valorization through sequential lactic acid fermentative production and anaerobic digestion of fermentation residues, Bioresour. Technol. 241 (2017) 508–516, https://doi.org/10.1016/j.biortech.2017.05.174.
[6] J. Mata-Alvarez, S. Macé, P. Llabrés, Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives, Bioresour. Technol. (2000), https://doi.org/10.1016/S0960-8524(00)00023-7.
[7] Y. Li, S.Y. Park, J. Zhu, Solid-state anaerobic digestion for methane production from organic waste, Renew. Sustain. Energy Rev. 15 (2011) 821–826, https://doi.org/10.1016/j.rser.2010.07.042.
[8] S. Farmanbordar, H. Amiri, K. Karimi, Simultaneous organosolv pretreatment and detoxification of municipal solid waste for efficient biobutanol production, Bioresour. Technol. (2018), https://doi.org/10.1016/j.biortech.2018.09.017.
[9] A. Nwobi, I. Cybulska, W. Tesfai, Y. Shatilla, J. Rodríguez, M.H. Thomsen, Simultaneous saccharification and fermentation of solid household waste following mild pretreatment using a mix of hydrolytic enzymes in combination with Saccharomyces cerevisiae, Appl. Microbiol. Biotechnol. 99 (2014) 929–938, https://doi.org/10.1007/s00253-014-5977-z.
[10] J.-J. Lay, Y.-J. Lee, T. Noike, Feasibility of biological hydrogen production from organic fraction of municipal solid waste, Water Res. 33 (1999) 2579–2586,

https://doi.org/10.1016/S0043-1354(98)00483-7.
[11] F. Valentino, M. Gottardo, F. Micolucci, P. Pavan, D. Bolzonella, S. Rossetti, M. Majone, Organic fraction of municipal solid waste recovery by conversion into added-value polyhydroxyalkanoates and biogas, ACS Sustain. Chem. Eng. 6 (2018) 16375–16385, https://doi.org/10.1021/acssuschemeng.8b03454.
[12] T.H. Kwan, Y. Hu, C.S.K. Lin, Techno-economic analysis of a food waste valorisation process for lactic acid, lactide and poly(lactic acid) production, J. Clean. Prod. 181 (2018) 72–87, https://doi.org/10.1016/j.jclepro.2018.01.179.
[13] M.-S. Kim, J.-G. Na, M.-K. Lee, H. Ryu, Y.-K. Chang, J.M. Triolo, Y.-M. Yun, D.-H. Kim, More value from food waste: lactic acid and biogas recovery, Water Res. 96 (2016) 208–216, https://doi.org/10.1016/j.watres.2016.03.064.
[14] M. Probst, J. Walde, T. Pümpel, A.O. Wagner, I. Schneider, H. Insam, Lactic acid fermentation within a cascading approach for biowaste treatment, Appl. Microbiol. Biotechnol. 99 (2015) 3029–3040, https://doi.org/10.1007/s00253-015-6414-7.
[15] M. Probst, J. Walde, T. Pümpel, A.O. Wagner, H. Insam, A closed loop for municipal organic solid waste by lactic acid fermentation, Bioresour. Technol. 175 (2015) 142–151, https://doi.org/10.1016/j.biortech.2014.10.034.
[16] M.A. Abdel-Rahman, Y. Tashiro, K. Sonomoto, Recent advances in lactic acid production by microbial fermentation processes, Biotechnol. Adv. 31 (2013) 877–902, https://doi.org/10.1016/j.biotechadv.2013.04.002.
[17] M. Probst, A. Fritschi, A. Wagner, H. Insam, Biowaste: a *Lactobacillus* habitat and lactic acid fermentation substrate, Bioresour. Technol. 143 (2013) 647–652, https://doi.org/10.1016/j.biortech.2013.06.022.
[18] T.H. Kwan, Y. Hu, C.S.K. Lin, Valorisation of food waste via fungal hydrolysis and lactic acid fermentation with *Lactobacillus casei* Shirota, Bioresour. Technol. 217 (2016) 129–136, https://doi.org/10.1016/j.biortech.2016.01.134.
[19] G. Choi, J. Kim, C. Lee, Effect of low pH start-up on continuous mixed-culture lactic acid fermentation of dairy effluent, Appl. Microbiol. Biotechnol. 100 (2016) 10179–10191, https://doi.org/10.1007/s00253-016-7871-3.
[20] J. Tang, X. Wang, Y. Hu, Y. Zhang, Y. Li, Lactic acid fermentation from food waste with indigenous microbiota: effects of pH, temperature and high OLR, Waste Manag. (2016), https://doi.org/10.1016/j.wasman.2016.03.034.
[21] S. Ponsá, T. Gea, A. Sánchez, The effect of storage and mechanical pretreatment on the biological stability of municipal solid wastes, Waste Manag. 30 (2010) 441–445, https://doi.org/10.1016/J.WASMAN.2009.10.020.
[22] F. Su, P. Xu, Genomic analysis of thermophilic *Bacillus coagulans* strains: efficient producers for platform bio-chemicals, Sci. Rep. 4 (2015) 3926, https://doi.org/10.1038/srep03926.
[23] K. Ma, G. Hu, L. Pan, Z. Wang, Y. Zhou, Y. Wang, Z. Ruan, M. He, Highly efficient production of optically pure L-lactic acid from corn stover hydrolysate by thermophilic Bacillus coagulans, Bioresour. Technol. 219 (2016) 114–122, https://doi.org/10.1016/j.biortech.2016.07.100.
[24] J.P. López-Gómez, M. Alexandri, R. Schneider, J. Venus, A review on the current developments in continuous lactic acid fermentations and case studies utilising inexpensive raw materials, Process Biochem. (2018), https://doi.org/10.1016/j.procbio.2018.12.012.
[25] M. Alexandri, A.-K. Neu, R. Schneider, J.P. López-Gómez, J. Venus, Evaluation of various *Bacillus coagulans* isolates for the production of high purity L-lactic acid using defatted rice bran hydrolysates, Int. J. Food Sci. Technol. (2018), https://doi.org/10.1111/ijfs.14086.
[26] H. Sixta, Handbook of Pulp Vol 1 Strauss GmbH, Mörlenbach, 2006.
[27] L.J. Jönsson, B. Alriksson, N.-O. Nilvebrant, Bioconversion of lignocellulose: inhibitors and detoxification, Biotechnol. Biofuels 6 (2013) 16, https://doi.org/10.1186/1754-6834-6-16.
[28] K. Sakai, Y. Murata, H. Yamazumi, Y. Tau, M. Mori, M. Moriguchi, Y. Shirai, Selective proliferation of lactic acid Bacteria and accumulation of lactic acid during open fermentation of kitchen refuse with intermittent pH adjustment, Food Sci. Technol. Res. 6 (2000) 140–145, https://doi.org/10.3136/fstr.6.140.
[29] K.J. Jem, J.F. van der Pol, S. de Vos, Microbial Lactic Acid, Its Polymer Poly(lactic acid), and Their Industrial Applications, (2010), pp. 323–346, https://doi.org/10.1007/978-3-642-03287-5_13.
[30] S. Klotz, N. Kaufmann, A. Kuenz, U. Prüße, Biotechnological production of enantiomerically pure d-lactic acid, Appl. Microbiol. Biotechnol. 100 (2016) 9423–9437, https://doi.org/10.1007/s00253-016-7843-7.
[31] T. Tabi, I.E. Sajo, F. Szabo, A.S. Luyt, J.G. Kovacs, Crystalline structure of annealed polylactic acid and its relation to processing, Express Polym. Lett. 4 (2010) 659–668, https://doi.org/10.3144/expresspolymlett.2010.80.
[32] D. Pleissner, F. Demichelis, S. Mariano, S. Fiore, I.M. Navarro Gutiérrez, R. Schneider, J. Venus, Direct production of lactic acid based on simultaneous saccharification and fermentation of mixed restaurant food waste, J. Clean. Prod. 143 (2017) 615–623, https://doi.org/10.1016/j.jclepro.2016.12.065.
[33] R. Glaser, J. Venus, Co-fermentation of the main sugar types from a beechwood organosolv hydrolysate by several strains of *Bacillus coagulans* results in effective lactic acid production, Biotechnol. Rep. Amst. 18 (2018) 22–27, https://doi.org/10.1016/j.btre.2018.e00245.
[34] R. Alves de Oliveira, R. Schneider, C.E. Vaz Rossell, R. Maciel Filho, J. Venus, Polymer grade l-lactic acid production from sugarcane bagasse hemicellulosic hydrolysate using *Bacillus coagulans*, Bioresour. Technol. Rep. 6 (2019) 26–31, https://doi.org/10.1016/j.biteb.2019.02.003.
[35] E. Castro-Aguirre, F. Iñiguez-Franco, H. Samsudin, X. Fang, R. Auras, Poly(lactic acid)—mass production, processing, industrial applications, and end of life, Adv. Drug Deliv. Rev. 107 (2016) 333–366, https://doi.org/10.1016/j.addr.2016.03.010.
[36] M. Aulitto, S. Fusco, S. Bartolucci, C.J. Franzén, P. Contursi, *Bacillus coagulans* MA-13: a promising thermophilic and cellulolytic strain for the production of lactic acid from lignocellulosic hydrolysate, Biotechnol. Biofuels 10 (2017) 210, https://doi.org/10.1186/s13068-017-0896-8.
[37] R. Brückner, F. Titgemeyer, Carbon catabolite repression in bacteria: choice of the carbon source and autoregulatory limitation of sugar utilization, FEMS Microbiol. Lett. 209 (2002) 141–148, https://doi.org/10.1111/j.1574-6968.2002.tb11123.x.
[38] K. Sakai, Y. Ezaki, Open L-lactic acid fermentation of food refuse using thermophilic *Bacillus coagulans* and fluorescence in situ hybridization analysis of microflora, J. Biosci. Bioeng. 101 (2006) 457–463, https://doi.org/10.1263/JBB.101.457.