Lactic acid bioprocess development: experiences using hydrolysates from the organic fraction of municipal solid wastes

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Keywords: *Bacillus coagulans*, electrodialysis, scale-up, polylactic acid, ethyl lactate
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1. Introduction

The organic fraction of municipal solid waste (OFMSW) is an abundant residue (estimated yearly production of about 140 Mt in the EU) produced in households, restaurants, yards and gardens (European Commission, 2018). Several fermentation processes have been investigated for the valorisation of the OFMSW, in addition to the extensively applied biogas production, e.g. for the production of chemicals such as biobutanol (Farmanbordar et al., 2018), biodiesel and ethanol (Nwobi et al., 2014), hydrogen (Lay et al., 1999), polyhydroxyalkanoates (Valentino et al., 2018) and lactic acid (LA) (Demichelis et al., 2017; Kim et al., 2016; Kwan et al., 2018; Probst et al., 2015b, 2015a). LA is one of the targeted product intermediates within the BBI-JU project 'Chemical Building Blocks from Versatile MSW Biorefinery – PERCAL'. The project investigates the conversion of OFMSW into intermediates which could in turn be used for the synthesis of value added chemicals such as polylactic acid (PLA), ethyl lactate (EL) and polyols.

In spite of its fermentative potential, OFMSW is a complex mixture of residues which can have significant variations due to geographical, seasonal and socioeconomic factors (Paritosh et al., 2018). Furthermore, due to their richness in carbohydrates, the wastes can naturally support the growth of indigenous bacteria. Such growth not only exhausts the sugars that could be used in a controlled fermentation but also results in the production of undesired metabolites which can act as inhibitors and could complicate the downstream. In particular, a high LA concentration and enantiomeric purity is necessary when the intermediate is intended to be used as the precursor for PLA. The work presented in this article showcases the development and adaptation of a bioprocess for the production of LA using OFMSW hydrolysates as substrates.

2. Materials and methods

There were three main challenges in the bioconversion of OFMSW hydrolysates into LA. The first challenge was to determine if the hydrolysates would be able to support the growth of LA producing bacteria. On a first step, the sugars and LA concentration of several OFMSW hydrolysates (from Valencia, Spain) produced throughout a year were measured. Additionally, the samples were used for the growth of several *Bacillus coagulans* strains (homofermentative L-LA producers) obtained from the collection of the Leibniz-Institute of Agricultural Engineering and Bioeconomy (Potsdam, Germany). The screening was performed at the microplate and lab scales. In general the fermentation conditions were 52 °C and a pH of 6 (controlled by the addition of 20 % $w \cdot w^{-1}$ NaOH). Conversion yields, final enantiomeric purities and productivities were used for strain selection.

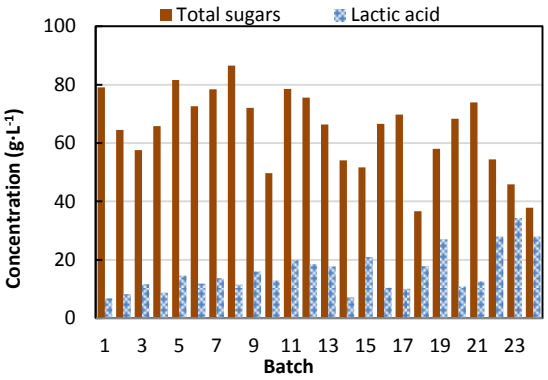
The second point was the evaluation of final enantiomeric purities that were achievable and the development of a process for the production of LA with high enantiomeric purity. Typically, OFMSW hydrolysates contain a racemic mixture of D- and L- LA. The initial LA mixture can reduce the final L-LA purity to levels in which it cannot be used for specialised applications which require high enantiomeric purities (over 98 %). Thus, a pre-treatment was developed based on microfiltration and monopolar electrodialysis for the removal of initial LA.

The third and final challenge was the scale up of the whole process. This involved studies using a 72 L BIOSTAT UD bioreactor (B-Braun Biotech, Germany) and the downstream and purification of LA. The process was scaled up for both cases, one in which the hydrolysates were pretreated (to produce LA with high enantiomeric purity) and one without pretreatment. The purification was based on 2 electrodialysis steps using 11 cation and 10 anion exchange membranes Type II (Fujifilm, The Netherlands). A detailed description of the downstream can be found in López-Gómez et al., (2020).

3. Results and Discussion

Twenty-three OFMSW hydrolysate batches were obtained during different times of the year and the analysis of sugars and LA is presented in Fig. 1. The first batches were prepared during the winter months and production continued until summer. As seen, considerable variations occurred amongst the samples, with total sugar concentration ranging from 86.5 to only 36.6 g L^{-1}. LA was present in every batch produced, with concentrations

ranging from 6.8 to 34.4 g L^{-1}. Lab scale fermentations were performed for the screening of several *B. coagulans* strains. Growth could be observed in all the OFMSW hydrolysates without the addition of any extra nutrients.**Error! Reference source not found.** shows the maximum growth rates (μ_{max}) with strains A20 and A166 showing the highest values.



Strain	A20	A166	A183	A300
μ (h^{-1})	0.056	0.054	0.028	0.031

Figure 1: Variation in the concentration of total sugars and lactic acid of 24 OFMSW hydrolysates and Maximum growth rates for the strains tested .

The initial LA content of the hydrolysates influences the final enantiomeric purities of the product. Therefore, a pretreatment was included in order to remove LA initially present in the hydrolysates. A detailed description of the pretreatment can be found in López-Gómez et al., (2020). Table 1 shows the strains tested, their internal code, from where they were obtained/isolated and summarizes the results of yield, productivities, LA titre, residual sugars fraction and final enantiomeric purity for the fermentations using hydrolysates after pretreatment. As seen high enantiomeric purities were achieved (over 97 %).

Table 1: Results for yield, maximum productivity, overall productivity, LA final concentration and optical purity for the screening of 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
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

*Calculated from the fraction of sugars consumed.

Finally, scaling up of the process was performed. Figure 2 shows the fermentation profile for one of the pilot scale (33.5 L working volume) experiments. Initial LA concentration was 6.5 g L^{-1} and sugars concentration was 51 g L^{-1} of glucose and 23.9 g L^{-1} of xylose. By the end LA had reached 60 g L^{-1} and the final enantiomeric purity was 95.1 % L- LA. The conversion yield of LA from total sugars was 0.65 g_{LA} g_{sugars}.

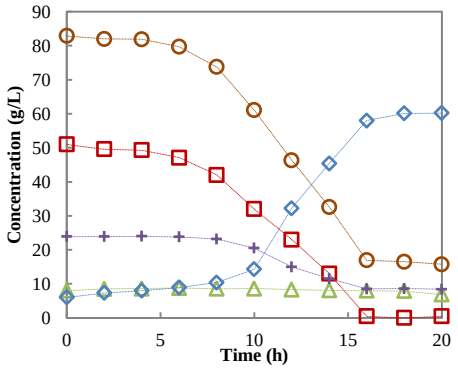


Figure 2: Fermentations in the technical scale (33.5 L working volume) of the hydrolysate after microfiltration with B. coagulans A166. Variation in the concentration of total sugars (○), glucose (□), disaccharides (△), xylose (+) and lactic acid (◊).

By the end of the downstream, a solution of 1.06 L containing 930 g L^{-1} LA was obtained. With a LA overall recovery of 45 %, the purification performance was in line with other reports in the literature using organic and agricultural residues as the substrates for the fermentation. Overall, a yield from OFMSW to LA of 0.11 g_{LA} $g^{-1}_{dryOFMSW}$ was achieved.

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