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Upgrading pasta wastes through lactic acid fermentations

José Pablo López-Gómez[a,*], Peter Unger[a], Roland Schneider[a], Marie-Aline Pierrard[b], Joachim Venus[a]

[a] Leibniz Institute for Agricultural Engineering and Bioeconomy, Max-Eyth-Allee 100, Potsdam, Germany
[b] IDELUX, Drève de l'Arc-en-Ciel, 98, 6700 Arlon, Belgium

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ABSTRACT

During its production process, every kilogram of pasta manufactured generates about 23 g of pasta wastes (PW). Considering the global pasta production, there are about 376 kilotonnes of PW produced every year. In this work, PW were characterised and used as the substrate in lactic acid (LA) fermentations. Enzymatic hydrolysis of 200 g/L of PW allowed for the liberation of sugars with a yield 0.81 g_s/g_{dryPW}. After the screening of several *B. coagulans*, the strain A559 was selected for experiments at the lab and pilot scales. Two fermentation modes were tested during lab scale experiments namely, simultaneous saccharification and fermentation and sequential hydrolysis and fermentation with the latter showing higher yields. The process was scaled up to 50 L where a LA concentration of 47.67 g/L and yield of 0.67 g_{LA}/g_{drydPW} were achieved.

1. Introduction

Waste prevention is of particular interest to the food industry, not only due to the economic benefits that it could represent, but also due to the social pressure associated to food waste. Nonetheless, some type of wastes are simply unavoidable and thus, the Circular Economy Package from the European Union (EU) establishes that for such residues reutilisation and recycling must be implemented (European Commission, 2015). Consequently, numerous studies have been carried out for the development of bioprocesses for the conversion of organic residues, which previously were simply disposed, in order to increase their value. Such trend, has focused the interest of companies and industry towards the valorisation of waste streams with bioconversion potential that were previously overlooked.

The European Commission classifies food wastes according to the stage in the supply chain in which they originate (Primary production, processing, distribution and retail and lastly, consumption) (Sanchez Lopez et al., 2020). Wastes generated during the production processing stage are homogeneous in composition, produced in large volumes and at high concentrations (Albizzati et al., 2021). These properties make them attractive fermentation substrates and provide the opportunity to valorise them through the generation of biochemicals.

Pasta is part of people's diet all around the world, with a total global production of 16 Mt in 2019 (International Pasta Organisation, 2020). A recent report by Principato et al., (2019), examined the food loss and waste for the case of Barilla, one of the leading producers of pasta worldwide. Their results showed that 1979 g of food losses and wastes are produced per each kilogram of pasta manufactured. The authors quantified the wastes and classified them according to the stage in the production chain where they occur into wastes in the cultivation, milling, production, retail and consumption. Most of the wastes (68.89%) are produced during the cultivation, including the straw left in the field and grain losses. Following that, milling and consumption stages account for 17.22 % and 12.61 %, respectively. Finally, pasta wastes obtained during the production process (PW)

* Corresponding author.
E-mail address:
plopezgomez@atb-potsdam.de (J.P. López-Gómez).
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are approximately 1.2 % and wastes from the retail stage account for only 0.1 %.

Every kilogram of pasta manufactured generates about 23.55 g of PW (Principato et al., 2019). Thus, considering the global annual pasta production, there are about 376.8 kt of PW produced every year. The majority of these wastes are scrap residues as the result of equipment cleaning or modifications for changing the pasta shapes (Principato et al., 2019). The application of such type of waste in fermentation processes is very interesting and bares some advantages over the other most abundant wastes of the pasta production chain. For example, unlike wheat straw, which requires a pre-treatment to break its lignocellulosic structure, PW only requires the reduction in particle size, water and enzyme addition for hydrolysis. Another type of pasta waste is the one originated during the consumption stage but this could only be used as part of the organic fraction of municipal solid waste (OFMSW) mixture (López-Gómez et al., 2020b). The utilisation of OFMSW carries its own difficulties e.g. the presence of inert materials, a heterogeneous composition and the spontaneous growth of other microorganisms. In contrast, PW have a known and homogeneous composition which simplifies their use in fermentations.

Lactic acid (LA) is an organic acid molecule with multiple uses in industry and that has had a resurgence in interest, due to its application as a precursor for polylactic acid. LA can be produced chemically, by the reaction of acetaldehyde with hydrogen cyanide which produces lactonitrile that can be then hydrolysed to LA. However, fermentative production of LA is preferred since it can be stirred to produce optically pure D- or L-LA. Most commonly, LA is produced from sugars obtained from corn, sugarcane or cassava (Grand View Research, 2019), nevertheless, alternative substrates have been investigated for its production. In particular, residues and wastes have undergone increasing interest due to their lower costs and waste management benefits (López-Gómez et al., 2020b). In this article, the valorisation of PW through fermentation has been explored using the production of lactic acid (LA) as a case study. The article firstly covers the characterisation of the PW and their enzymatic hydrolysis. Following that, various strains were evaluated for the production of LA. After microorganism selection, fermentation studies were carried out using two methods: separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). Finally, the process was tested at the pilot scale (50 L) and results obtained were used for a basic estimation of the potential global production of LA from PW.

2. Materials and methods

2.1. Substrate, enzymes and microorganisms

PW were obtained from a company in Belgium. The material was in the form of lasagne sheets of various dimensions. Before hydrolysis experiments, some water was added together with the PW into a mixer and shredded. Following that, water was added to the substrate to obtain a medium with the desired pasta concentration. Finally, the substrate was autoclaved at 121 °C for 15 min.

Bacillus coagulans isolates (internal identification numbers: A20, A28a, A59, A166, A203 and A559), belonging to the strain library of the Bioengineering Department of the Leibniz Institute for Agricultural Engineering and Bioeconomy (Potsdam, Germany) were used for the LA fermentation. All the strains used in this study were homofermentative and thermophilic L-LA producers. The preparation of seed cultures was performed in 250 mL shake flasks containing de Man, Rogosa and Sharpe medium (MRS) (Merck, Germany) supplemented with 0.7 g EVERZIT Dol 0.5–2.5 mm (Evers, Germany) for pH stabilization. The pre-culture conditions were 52 °C for 12–16 h at an agitation of 150 rpm.

2.2. Pasta hydrolysis studies

Several enzymes dosages were used to evaluate the effect of enzyme concentration in the hydrolysis. The enzyme cocktail used during the hydrolysis experiments was Stargen™002 (Genencor International, USA), a commercial mixture of enzymes containing α-amylase and glucoamylase. The enzyme activity as defined by the producer is 570 GAU/g (Hargono et al., 2018). Flasks with a working volume of 0.1 L and containing 200 g/L of PW, or approximately 80 g/L of dry PW (dPW) and enzyme concentrations of 0.64, 1.28, 1.92, 2.56 and 3.20 ($\mu L/g_{dPW}$) were prepared in triplicate. Following that, the flasks were incubated at 50 °C and samples were taken at 24 and 48 h for the analysis of released sugars. Additionally, an experiment in 2 lab sale bioreactors (Sartorius AG, Germany) with 1 L working volume containing 200 g/L of PW, was carried out to compare the enzyme concentrations of 1.28 and 2.56 $\mu L/g_{dPW}$ and used as the basis for the pilot scale experiments described in Section 2.4.4. Hydrolysis yields were calculated as the mass of sugars obtained divided by the initial mass of dry PW ($Y_{s/dPW}$).

2.3. Screening of Bacillus coagulans strains for the fermentation of pasta hydrolysates

Six *B. coagulans* strains were used to evaluate their performance during the fermentations of pasta hydrolysates. *B. coagulans* are facultative anaerobes thus, the fermentations were carried out under aerobic conditions without the supply of oxygen. The preparation of the hydrolysates for the screening was performed in 5 L bioreactors with a working volume of 4 L and a pasta concentration of 200 g/L. Hydrolysis process conditions were 50 °C, 200 rpm, enzyme dosage was 2.56 $\mu L/g_{dPW}$ without pH control. Fermentation experiments were carried out in Eloferm bioreactor units (Biotronix GmbH, Hennigsdorf, Germany). The working volume was 0.5 L, agitation was set to 150 rpm and temperature to 52 °C. The pH was maintained at 6 by the addition of NaOH 20% (w/v). Bioreactors were inoculated with 25 mL of a preculture prepared as described in Section 2.1. Samples were taken throughout the fermentations for the quantification of sugars and LA. Fermentations for each strain were carried out with and without the addition of yeast extract (5 g/L).

2.4. Hydrolysis and fermentation set-up

Two methods were used for the evaluation of the hydrolysis and fermentation of the PW. In the first one, hydrolysis and fermentation were carried out separately and sequentially (SHF). The second process was an SSF. Experiments were carried out in duplicate. Yields were calculated in the experiments for the production of LA from dry PW $Y_{LA/dPW}$ and for the production of LA from sugars obtained after hydrolysis.

2.4.1. *Sequential hydrolysis and fermentation*

Hydrolyses were carried out using a 2 L bioreactor, with a 1 L working volume. Two concentrations of pasta were tested 200 and 300 g/L. Hydrolyses were carried out at 50 C°, without pH control and a stirrer speed of 300 rpm in a 2 L BIOSTAT bioreactor (Sartorius AG, Germany). After hydrolysis (24 h), the fermentation was carried out in the same bioreactor with a supplementation 5 g/L of yeast extract. Temperature for the process was 52 °C, agitation was lowered to 200 rpm and pH was maintained at 6 by the addition of NaOH 20% (w/v). The inoculation volume of the strain A559 was 50 mL, i.e. 5% (v/v). Samples were taken during the whole process for the quantification of sugars and LA. The process was stopped once LA production ceased.

2.4.2. *Simultaneous saccharification and fermentation*

Similarly to the process described in Section 2.4.1, the SSF was performed in a 2 L bioreactor with a 1 L working volume. The conditions of the process were 52 °C, pH 6 controlled by adding NaOH 20% (v/v), agitation was set to 200 rpm and the substrate concentrations were 200 and 300 g/L (supplemented with 5 g/L of yeast extract). The enzyme dosage was 2.56 $\mu L/g_{dPW}$. Exactly ten minutes after the addition of enzymes, 0.05 L of a seed culture of A559 were added to the bioreactor. Samples were taken regularly during the whole process for the quantification of sugars and LA. The process was stopped once LA production ceased.

2.4.3. *Fermentations without sterilization*

Once the best conditions for the hydrolysis and fermentations were stablished an experiment was carried out in which the PW medium was not autoclaved. Instead, before hydrolysis, 1 L of PW medium (200 g/L of PW) were placed in in a 1 L bioreactor at 80 °C for 30 min. Following that, the system was cooled down to 50 °C and the hydrolysis was performed with Stargen™002 (2.56 $\mu L/g_{dPW}$). After 24 h of hydrolysis, the temperature was adjusted to 52 °C, the medium was supplemented with yeast extract (5 g/L) and the hydrolysate was inoculated with *B. coagulans* A559. The experiment was carried out in duplicate.

2.4.4. *Pilot scale fermentations*

Pilot scale experiments were carried out in duplicate, in a 72 L BIOSTAT UD bioreactor (B-Braun Biotech, Germany) with a working volume of 50 L. PW were added at a concentration of 200 g/L and an inactivation step at 80 °C for 30 min was performed. Following that, the temperature of the bioreactor was adjusted to 50 °C, agitation was set to 200 rpm and enzymes (1.28 $\mu L/g_{dPW}$) were added. After 24 h of hydrolysis, the bioreactor's temperature and pH were adjusted to 52 °C and 6, respectively, and it was supplemented with 5 g/L of yeast extract. The inoculum (A559) for the fermentation was prepared as described in Section 2.1 (MRS medium) in a 2 L BIOSTAT bioreactor (Sartorius AG, Germany). During the process, NaOH (20% v/v) was used for maintaining the pH at 6. Samples were taken regularly, inactivated at 95 °C for 20 min and kept at – 20 °C until further analysis.

2.5. *Analytical assays*

Dry matter content was measured by weighing 50 g of PW and dried at 105 °C until constant weight. The total amount of organic matter was calculated after 10 g of PW were combusted at 550 °C for 5 h in a furnace. The weight of the ashes that remained corresponded to the inorganic matter present in the PW sample. That inorganic matter weight was subtracted from the dry matter weight to define the organic matter content. Total nitrogen was determined by the Kjeldahl method (Kjeldahl Sampler System K-370/371,BÜCHI, Essen, Germany). The Ewers' Polarimetric Method (ISO 10520, 1997) was used for the quantification of starch in the PW. Organic acids concentrations (including LA, acetic acid) as well as sugars concentrations were measured using high performance liquid chromatography (HPLC), an Eurokat H (300 mm × 8 mm × 10 µm; Knauer, Berlin, Germany) and a refractive index detector RI-71 (SHODEX, Tokyo, Japan). The mobile phase was 5 mM H_2SO_4 (flow rate=0.8 mL/min), sample volume was 10 µL. LA L-/D-isomer ratio was measured by HPLC (Chiralpak®MA(+) column (50 mm × 4.6 mm × 3 µm; DAICEL, Tokyo, Japan) with a 2 mM CuSO4 mobile phase and an UV detector.

3. Results and discussion

3.1. *Pasta waste composition and hydrolysis studies*

The total amount of solids in the PW was 40.37% (w/w), with a corresponding total organic matter content of 98.82%. Starch was the main component in the solids with a value of 61.58% dry basis (db). Additionally, crude protein and sucrose were found to be present in smaller quantities of 6.29% and 0.41% (w/w db), respectively. Thus, 1 kg of fresh PW contains approximately 248.6 g of starch (24.86%).

In its dry form, pasta is mostly constituted by carbohydrates (approximately 70–75%) from which starch is the main component, with smaller proportions of sugars and dietary fibres (Gull et al., 2018). Starch is a polymer of glucose units joined by glycosidic bonds. In industry, energy intensive processes, at high temperatures (60–65 °C), are typically used for the hydrolysis and liquefaction of starch for example in the ethanol production (Xu et al., 2016). However, it has been already argued that using amylases in processes with milder conditions can offer good results with less energy expenditure (Xu et al., 2016).

During optimization experiments one of the most important tasks is to increase the amount of solids to hydrolyse i.e. the solids loading. A higher solids loading translates into more sugars and therefore, a higher concentration of the final product which in turns makes the downstream and purification more economically efficient (Ioelovich and Morag, 2012). Further savings can be achieved if the concentration of enzymes used is reduced, since enzyme costs is one of the major bottlenecks in the development of the processes (Klein-Marcuschamer et al., 2012; Marzo et al., 2020).

On a first step, enzyme dosage optimization experiments were carried out. As observed in Table 1, most of the sugars were obtained during the first 24 h of the process, with an increase in concentration of sugars of less than 10% from 24 h to 48 h for almost all the enzyme dosages tested (besides 0.64 $\mu L/g_{dPW}$). Sugars concentration increased as the enzyme dosages increased, reaching a maximum value (110 g/L) in the flasks with 3.20 $\mu L/g_{dPW}$. However, sugar concentration by itself is not a good indicator of hydrolysis efficiency and thus, yields of sugars from dry PW ($Y_{s/dPW}$) were calculated. As seen, the $Y_{s/dPW}$ value increases until reaching its maximum at 2.56 $\mu L/g_{dPW}$ ED.

Table 1 – Effect of the enzyme dosage on the yield of sugars per gram of dry pasta waste ($Y_{s/dPW}$) during the hydrolysis of 200 g of fresh pasta waste in shake flasks experiments. Values show the average of three samples at 24 h and 48 h of hydrolysis.

Enzyme dosage (μL/g_{dPW})	$Y_{s/dPW}$ (g_{sugars}/g_{dPW})			
	24 h	SD	48 h	SD
0.64	0.75	± 0.05	0.89	± 0.03
1.28	0.82	± 0.02	0.90	± 0.01
1.92	0.86	± 0.02	0.93	± 0.02
2.56	0.87	± 0.04	0.96	± 0.05
3.20	0.87	± 0.06	0.95	± 0.05

SD: standard deviation

In addition to the breakage of polymeric carbohydrates, enzymes also act as liquefying agents. During the process of hydrolysis, viscosity of the medium is reduced which allows for mixing to occur more easily, improving heat and mass transfer within the system. Evidently, a higher concentration of pasta in the medium represents more carbohydrates available for hydrolysis. However, a higher solids loading can also have a negative effect in the mixing, diminishing the mass transfer of the enzymes and resulting in low hydrolysis yields (Ioelovich and Morag, 2012).

3.2. *Strain selection*

Hydrolysates for the screening were prepared in three bioreactors batches. Hydrolyses in bioreactor experiments provided more stable and similar results, in replicated experiments, than those carried out in flasks did. The hydrolysis of 200 g/L of pasta, shown in Fig. 1, occurred rapidly with around 90% of the total sugars liberated during the first 10 h. After the 10 h mark, the concentration of sugar continued increasing but at a lower rate until stabilising (variation of less than 5%) after 24 h. Such fast action of α-amylase and glucoamylase enzymes has been reported before, for example during the hydrolysis of mixed food wastes and corn flour, where most of the sugars were obtained during the first 8 h (Demichelis et al., 2017; Sun et al., 2014; Xu et al., 2016). Most likely, the absence of other materials in the pasta waste and the availability of starch creates good conditions for a fast hydrolysis. Furthermore, the initial pH of the PW medium prepared in this research was always approximately between 4.2 and 4.3 and did not vary throughout hydrolyses. Such value lies within the ideal pH range (3.3–4.5) reported by the supplier of the enzyme for its optimum performance (Strąk et al., 2017), which favours the stability of the process.

After 48 h, the hydrolysis yielded approximately 0.67 g_s/g_{dPW} with a conversion yield from starch into glucose of 1.08 g/g. The hydrolysis of starch consists of the breakage of the glycosidic bonds and the addition of an oxygen molecule to the released monomeric sugars, forming a glucose molecule. Stoichiometrically, the theoretical yield of glucose from starch is 1.11 g/g, thus, the experimental value obtained in the hydrolysis was 98 % of the maximum theoretical value.

Experiments in small bioreactors were used for the screening of several *B. coagulans* strains. Despite the high content of organic matter in the PW, its reduced nitrogen content does not provide the best conditions for the fermentation. Every strain tested during the screening showed a better performance (in terms of productivities) when the

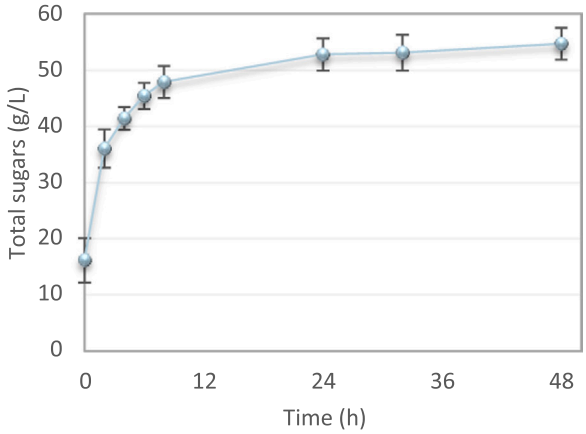

Fig. 1 – Hydrolysis profile of pasta waste (200 g/L) showing average total sugars concentration of three separate experiments with their standard deviation.

hydrolysates were supplemented with yeast extract (5 g/L), e.g. Figs. 2a and 2b show the fermentation profile for the strain A559 (profiles for the other strains can be found in the supplementary file of this article). As shown, total fermentation times were generally shortened by more than 24 h with the supplementation of yeast extract with a respective increase in productivities (Fig. 2c). Other studies using alternative nutrient sources have demonstrated that it is possible to replace the expensive yeast extract by cheaper alternatives (Alexandri et al., 2020; Schroedter et al., 2020). The evaluation of other nitrogen sources should be considered in the future to make the valorisation of PW more economically attractive. On the other hand, lactic acid yields from sugars $Y_{LA/s}$, were unaffected by the addition of yeast extract with values ranging from 0.8 g_{LA}/g_s to 0.9 g_{LA}/g_s. Yields of LA from dry PW ($Y_{LA/dPW}$) were around 0.5 and 0.61 g_{LA}/g_{dPW} for the strains tested.

3.3. *Fermentation mode comparison*

Even though the yields for the screened strains were similar, the strain A559 showed a slightly higher productivity and thus was selected for the evaluation of both SHF and SSF. The profiles for both processes are shown in Fig. 3. After assessing the experiments with 200 g/L of PW, a further experiment to evaluate the performance of the fermentation with a higher concentration of PW (300 g/L) in the medium was also included. This allowed obtaining a higher concentration of sugars, which is desirable in order to increase LA at the end of the fermentation and thus reduce downstream and purification costs.

As in the smaller scale experiments, during the SHF, the first 10 h of the hydrolysis showed a sharp increase in the concentration of sugars, for both 200 and 300 g/L of PW, reaching 59 and 75 g/L in 7.5 h, respectively. However, the $Y_{s/dPW}$ for 300 g/L of PW was 0.62 g_s/g_{dPW} whereas the corresponding value for the hydrolysis with 200 g/L of PW was 0.73 g_s/g_{dPW}. This result highlights that, although more sugars were liberated in the hydrolysis of 300 g/L, in fact, the process is less efficient and the conversion yield is better at 200 g/L. This was consistent through the hydrolysis and at 24 h, when the hydrolysis was stopped, a total 65.31 and 84.6 g/L of glucose were present in the samples. At that point, the yields of sugars from dry PW were high at 0.79 and 0.70 g_s/g_{dPW} for 200 and 300 g/L of PW, respectively.

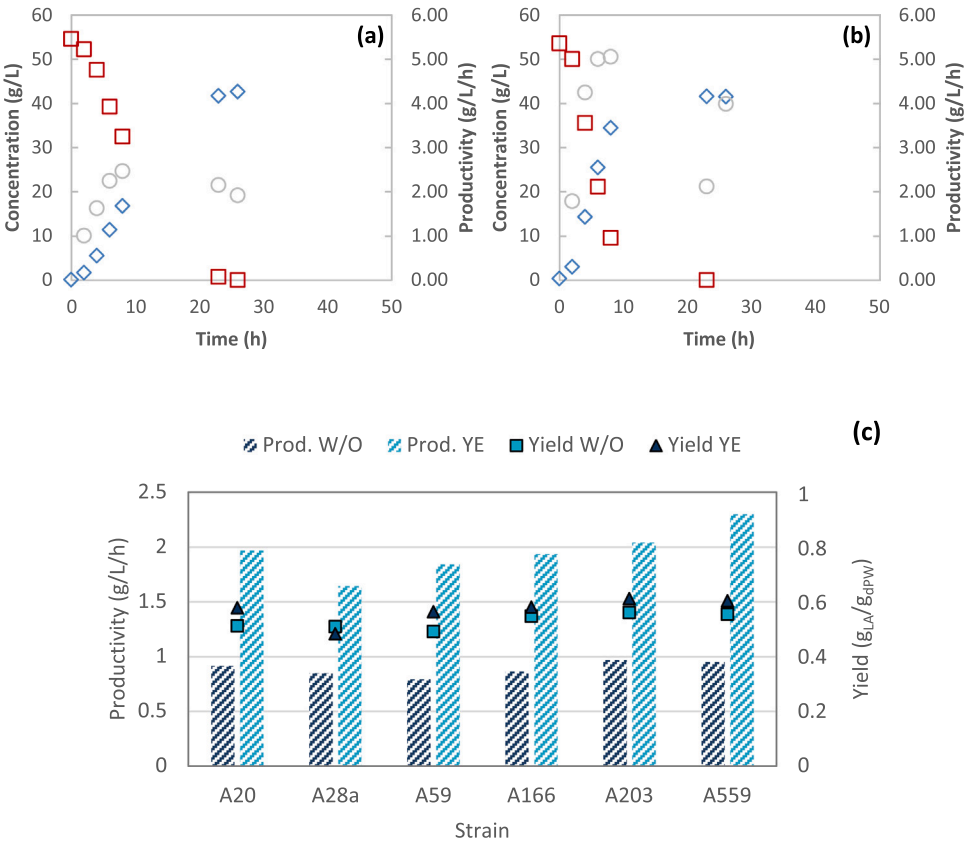

Fig. 2 – Effect of the addition of yeast extract in the fermentation of pasta waste hydrolysates. (a) Fermentation by strain A559 without the addition of yeast extract. (b) Fermentation by strain A559 with the addition of 5 g/L yeast extract. Concentrations of lactic acid (···◊···), glucose (···□···) and productivities (···○···). (c) Yields in grams of lactic acid per gram of dry pasta waste and productivities of various *B. coagulans* tested in fermentations without (W/O) and with yeast extract (YE).

In the SHF experiments, the addition of *B. coagulans* A559 caused a sharp reduction in the concentration of glucose, with total consumption after approximately 28 h of inoculation (52 h total process) for both PW concentrations. By the end of the process, LA reached 47.37 and 57.1 g/L for the experiments with 200 and 300 g/L of PW, respectively. The corresponding $Y_{LA/dPW}$ were 0.58 and 0.49 g_{LA}/g_{dW}. The simultaneous saccharification and fermentation (SSF) showed a much shorter process time (Fig. 3) of only 26 h. Nevertheless, LA only reached 37.4 and 47.5 g/L for the experiments with 200 and 300 g/L of PW, respectively (corresponding $Y_{LA/dPW}$ 0.41 and 0.39 g_{LA}/g_{dPW}). Most likely, the lower efficiency of the SSF process is related to the pH of the process. As described in Section 3.2, the optimum pH for the action of Stargen™002 lies between 3.3 and 4.5, however, the SSF process was carried out at pH 6, the optimal pH for growth of *B. coagulans*. Future work could be performed with the intention of exploring the best condition of pH and investigate if a balance can be found between the optimal pH of the hydrolysis and the fermentation.

3.4. *Pilot scale fermentations with non-sterilised pasta waste*

Normally, fermentation processes using pure cultures require the sterilization of the medium. Such step is most critical in fermentations that involve mesophilic strains, with typical optimum growing temperatures ranging from 30° to 40°C. Nonetheless, the use of sterilization implicates very high temperatures and pressures, specialised equipment, as well as steam and cooling water, in addition, to extra time (Xiao et al., 2012). All these factors add to the production costs and are especially counterproductive in processes in which there is already little room to achieve feasibility. This is the case for most of the systems based in the biotransformation of waste materials for the generation of chemicals, which directly compete with their counterparts from the petrochemical industry or processes that are based on simple sugars as substrates. In order to address this problem, the utilisation of thermophilic organisms or even extremophiles, has been explored as an alternative to carry out fermentations without the need for costly sterilisation processes (Xiao et al., 2012; Zeldes et al., 2015).

Taking into consideration that the PW comes straight from the production plant and that the optimum temperatures for hydrolysis (50 °C) and fermentation (52 °C) used in this research, could limit the growth of contaminants, the fermentation of PW without sterilization was studied. Heat inactivation methods have been reported for dealing with vegetative contaminants that might be present in the materials (Zeldes et al., 2015). Thus instead of autoclaving, experiments were carried out in which the PW medium was inactivated at 80 °C for 30 min before the hydrolysis. At the lab scale, fermentations of PW showed a $Y_{s/dPW}$ of 0.81 g_s/g_{dPW} while the $Y_{LA/dPW}$ was 0.60 g_{LA}/g_{dPW}. This values are practically the same as the ones achieved for the same process but with sterilization ($Y_{s/dPW}$= 0.79 g_s/g_{dPW}, $Y_{LA/dPW}$=0.58 g_{LA}/g_{dPW}, see Section 3.3), which indicated that the process could be carried out without the need of autoclaving. However, test at the lab scale using PW without autoclaving or without the inactivation step showed contamination of the medium and thus, pilot scale experiments were performed only with the inactivation step. In the future,

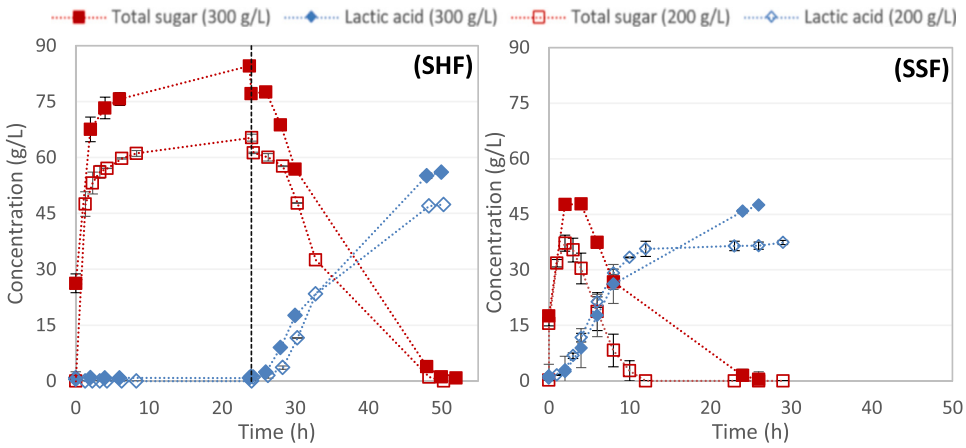

Fig. 3 – Profile for (SHF) sequential hydrolysis and fermentation and, (SSF) simultaneous saccharification and fermentation of pasta wastes. The graphs show the average concentrations of lactic acid and glucose for experiments using 200 (◇,□) and 300 g/L (◆,■) of pasta waste, inoculation time for (SHF) is marked at 24 h (———). Error bars show the values for the duplicate experiments.

experimental work will be performed to investigate if the temperature difference between the hydrolysis (50 °C) and fermentation (52 °C) is necessary, or if on the contrary, a single temperature can be used for the whole process without affecting yields and productivities.

Furthermore, results at the 1 L bioreactor lab scale showed that an enzyme concentration of 1.28 μL/g_{dPW} yielded practically the same concentration of sugars in the hydrolysis of 200 g/L of PW (see supplementary material). Thus, 1.28 μL/g_{dPW} of enzyme was used for the hydrolysis in the pilot scale experiments. The fermentation profiles for the two pilot scale experiments for 200 g/L of PW are shown in Fig. 4. As seen, the production of sugars followed a very similar behaviour as in the laboratory scale experiments (see Fig. 3). Hydrolysis of starch occurred rapidly and most of the conversion into glucose happened during the first 4 h. After 24 h of hydrolysis, the concentration of sugars was over 60 g/L for both experiments, with an average $Y_{s/dPW}$= 0.81 g_s/g_{dPW}, the same as in the lab scale fermentations.

There was a noticeable increase in the lag phase time of the fermentations of almost 10 h, which was not the case for the lab scale experiments that exhibited almost no lag phase. Such an increase in the lag phase can have a substantial impact in the economic feasibility of the process. Previous experiences in our lab have shown that remaining solid particles after the hydrolysis can extended the lag phase. In the future, work will be carried out to resolve potential issues that may be causing the delay in exponential growth at the pilot scale. Nevertheless, by the end of the process, the average LA concentration was 47.65 g/L, a value consistent with the results obtained in the lab scale. The optical purity of L-LA was 99% for the two fermentations (a value consistent with the optical purities observed throughout the fermentations reported in this article).

Remarkably, the $Y_{LA/dPW}$ showed a significant improvement (> 10%) over the result obtained in lab scale with a value of 0.67 g_{LA}/g_{dPW}. The LA yield from sugars ($Y_{LA/s}$) was 0.81 g_{LA}/g_s.

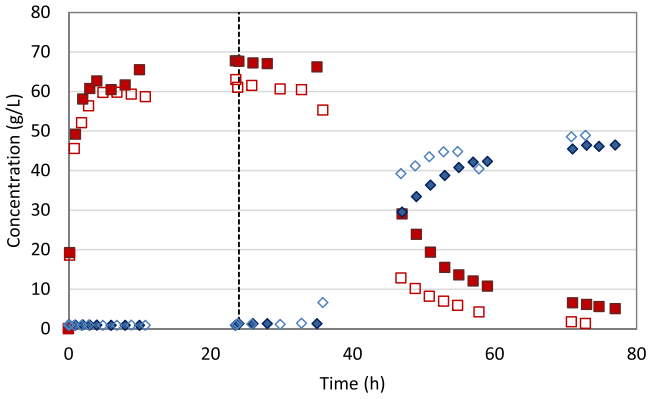

Fig. 4 – Fermentation profile for pilot scale experiments using 200 g/L of pasta waste. The plot shows the results of duplicate experiments and the variation in total sugars concentration (□, ■) and lactic acid (◇,◆). The inoculation time is marked at 24 h (———).

4. Potential global production of LA from pasta waste

To the best of the authors' knowledge, this is the only report available in the literature that utilizes PW. As shown in Table 2, other reports have used pasta wastes generated during the consumption stage e.g. in the mixture of leftovers from canteens and restaurants or the organic fraction of municipal solid wastes (OFMSW). However, a direct evaluation of the efficiency of LA fermentations using PW in such of mixtures is impossible. One of the main issues with those kinds of mixtures is that variations in the composition of the waste are common, which somehow complicates the replication of the experiments. Furthermore, and especially for the case of OFMSW, the content of lignocellulosic materials can be high. In those instances, harsher pretreatments are necessary to maintain yields. Thus, a continuous analysis of the composition of the substrates is required in order to define the need of changes in the process. On the other hand, the utilisation of PW (or other similar homogeneous residues) has the advantage of reducing the risk of lower fermentation performances due to variations in the composition of the substrate (López-Gómez et al., 2020b), a situation that is similar amongst residues directly obtained from food processing plants.

In comparison to LA fermentations of other starchy food wastes (Table 2), the yield obtained using PW in this research was remarkably high. This is expected considering the high

Table 2 – Lactic acid production from various wastes.

Substrate	Microorganism	Sugars (g/L)	T (°C)	pH	Working volume (L)	LA (g/L)	$Y_{LA/drywaste}$ (g/g)	Ref.
Pasta waste	*Bacillus coagulans* A559	64.6	52	6	50	47.6	0.67	This study
Canteen wastes	*L. plantarum* TD46	45	30	5.5–6	0.15	28.8	0.39	(Wang et al., 2005)
	Streptococcus A620	100[a]	35	6	40 kg	60.5	0.25	(Pleissner et al., 2017)
	Streptococcus A620	67.3	35	6	1	39.2	0.33	(Demichelis et al., 2017)
	Indigenous microorganisms	79.3	35	6	0.25	50	0.21	(Peinemann et al., 2019)
	L. rhamnosus	100[a] (db)	35	5	0.25	45.5	0.45	(Wang et al., 2009)
Catering and bakery wastes	*L. casei* Shirota	100.2	37	6	2.5	94	0.27	(Kwan et al., 2016)
Bakery wastes	*T. aotearoense*	92	55	5.5	2	78.4	0.18	(Yang et al., 2015)
Potato residue	*G. stearothermophilus*	–	60	7	0.5	59	–	(Smerilli et al., 2015)
Rice noodle residue	*L. plantarum* S21	134	37	6	7	102	0.76	(Unban et al., 2019)
OFMSW	*B. coagulans* A166	82	52	6	33.5	60	0.22	(López-Gómez et al., 2020c)

[a] g/L of food waste

starch content in the PW and its homogeneity (i.e. without being mixed with other wastes).

Considering that every year 376.8 kt of PW are produced (see Section 1), the yield of $Y_{LA/dPW}$= 0.67 g_{LA}/g_{dPW} obtained in the pilot scale experiments and the dry weight of the substrate, a total 100.9 kt of LA could be produced solely from PW. Usually, losses ranging between 40% and 60% during the downstream and purification of LA are expected (López-Gómez et al., 2020a). Thus, after the purification around 40–60 kt of LA could be produced from PW. The global production of LA was 1220 kt in 2016 and it is forecasted to reach 1960 kt by 2025 (López-Gómez et al., 2018), PW could be used to cover part of this increasing LA demand. Furthermore, LA was used as the model product in this research, however, considering the excellent properties of the PW for the fermentations, biochemical products which could hold higher value should also be considered. Ultimately, the goal must be to establish the most optimal upgrading routes for such type of wastes.

5. Conclusion

PW was used for the production of LA. The homogeneity of the material and its high starch content provide great conditions for both the hydrolysis and fermentation. Hydrolysis experiments using commercial enzymes showed a high conversion yield of $Y_{s/dPW}$ = 0.81 g_s/g_{dryPW}. Lab scale fermentations showed that a SHF was more efficient than a SSF. At the pilot scale, SHF of PW successfully maintained the hydrolysis yield while the fermentation of LA achieved a $Y_{LA/s}$ = 0.81 g_{LA}/g_s. Overall, a high LA yield from dry PW was obtained, $Y_{LA/dPW}$= 0.67 g_{LA}/g_{dPW}.

Declaration of Competing Interest

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fbp.2022.07.010.

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