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ORIGINAL PAPER

Banana Peels as Substrate for Lactic Acid Production: Upstream Fermentation Bioprocess

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Abstract

Purpose Banana peels account for around 30–40% of the banana's weight and represent an industrial waste. This biomass can be used as an eco-friendly solution for biotechnological bioprocesses. Still, due to the fibrous nature of banana peels, pretreatment becomes essential to enhance the effectiveness of enzymes on the hydrolysis of complex carbohydrates. This study aims to determine the best pretreatment and enzymatic hydrolysis conditions to produce a fermentation substrate suitable for producing lactic acid from the peels.

Methods A diluted H_2SO_4 (0.5% and 0.25%) was used in an autoclave for 10 min at 121 °C. The pretreated material was subjected to several enzymatic hydrolysis tests in which two variables were evaluated for their influence: solid load (10, 15 and 20%), and H_2SO_4 concentration (0.5 and 0.25% *v/v*). Commercial enzymes (cellulolytic enzyme mixtures and pectinase) were used.

Results All enzymatic hydrolysis yields of cellulose referring to glucose released in the hydrolysates surpassed 60% in all the conditions tested after pretreatment. After evaluating the pretreatment efficiency, the ideal parameters were defined as 0.25% H_2SO_4, and cellulolytic enzyme mixtures with 15 and 20% solid load. Subsequently, a screening was carried out in MRS + broth with the most suitable pH and temperature conditions for lactic acid production by *Lacticaseibacillus rhamnosus.*

Conclusions The results showed the potential of BP medium for lactic acid production, with yields of 0.91 g/g sugar in sugar-containing media from enzymatic hydrolysis at 15% solid load and 0.82 g/g sugar in media from 20% solid load assays.

Statement of Novelty

This study addresses the challenge of banana peel waste, which constitutes 30–40% of the fruit's weight and is often discarded. By leveraging this biomass for biotechnological processes, it offers a sustainable alternative for lactic acid production. However, the fibrous nature of banana peels hinders enzymatic hydrolysis, making pretreatment crucial. This work optimizes pretreatment and enzymatic hydrolysis conditions, identifying a novel approach using diluted H_2SO_4 and cellulolytic enzyme mixtures. The findings demonstrate high hydrolysis yields and lactic acid production efficiencies (0.91 g/g and 0.82 g/g sugar), highlighting the feasibility of banana peel as a fermentation substrate. This study presents an innovative strategy for waste valorization in biotechnology.

Highlights

- Banana peels as an environmentally friendly biomass to produce lactic acid.
- Diluted acid and autoclaving pretreatment were used to enhance enzymatic hydrolysis.
- Enzymatic hydrolysis achieves over 60% glucose yield in all conditions tested.
- *Lacticaseibacillus rhamnosus* produced lactic acid, yielding 0.91 g/g and 0.82 g/g sugar.
- Efficacy of banana peel hydrolysates as a fermentation substrate.

Keywords Biowaste · Carbon source · Lactic acid · Hydrolysis

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Abbreviations

ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BP	Banana peels
BP medium	Banana peels medium
BP2022	Banana peels collected between August and December 2022
BP2023	Banana peels collected between April and August 2023
C	CellicCTec2
C + P	CellicCTec2 + Pectinase
C + V	CellicCTec2 + Viscozyme L
dwb	Dry weight basis
D-LA	D-lactic acid isomer
EH	Enzymatic hydrolysis
FF	Furfural
GOPOD	Glucose oxidase/peroxidase reagent
HMF	Hydroxymethylfurfural
HPLC	High-performance liquid chromatography
LA	Lactic acid
L-LA	L-lactic acid isomer
MFE	Banana peels medium prepared for fermentation
MRS (+)	MRS broth supplemented with 30 g/L of glucose and 30 g/L of fructose
NREL	National Renewable Energy Laboratory
P	Pectinase
PES	Polyethersulfone
PLA	Polylactic acid
SD	Standard deviation
V	Viscozyme L
VA	Vanillic acid

Introduction

Banana (*Musa* spp., Musaceae family) is a typical fruit from tropical and sub-tropical regions, that is consumed worldwide [1], with the main producers being India, China, Indonesia, Nigeria, Brazil and Ecuador. Over the years, banana production has grown steadily; in 1961 the world production was just 22 million tonnes, while in 2022 it reached 135 million tonnes (FAO, 2022). Beyond being the edible part of the plant, the pulp is of greater interest for the food industry, namely, to produce frozen banana, chips, jelly and cakes. As such, the peel is discarded and becomes an industrial waste. The weight of the banana peels (BP) accounts for around 30–40% of the fruit. In terms of annual production, these peels account for 40.5–54 million tonnes, representing a significant amount of biomass that could be incorporated into other biotechnological processes [3–5].

The economic factor is important in the transfer of a bioprocess to the industry, thus investigating and evaluating solutions that reduce costs is a way of approaching this transfer of technology. Industrial waste and by-products are potential substrates for conversion into added-value products, as these raw materials can be cheap and rich in carbohydrates, as well as not competing with food supply [6].

The composition of banana peel varies with factors such as variety and ripening stage [7], with most studies focusing on locally sourced bananas of the *Musa* genus. It is a fibrous biomass, with cellulose (7–38%), hemicellulose (6–38%), and pectin (9–32%) as major fractions, while lignin (3–30%) represents the most recalcitrant component [1–40]. High levels of extractives (30–64%), also including phenolics, flavonoids, and carotenoids, which provide antioxidant and antimicrobial properties [11–13]. Additionally, BP contains ash (1–18%) with minerals such as potassium, calcium, and magnesium, along with proteins (1–14%), lipids (7–16%), starch (3–11%), and soluble sugars [8]. Therefore, the significant carbohydrate content makes BP a versatile and potential carbon source for fermentation. Due to the nature of this material, pretreatment is essential to enhance the effectiveness of enzymes on complex carbohydrates in a hydrolysis process [9]. There are many biomass pretreatment classifications, but briefly, the pretreatment can be divided into four basic types: physical, chemical, physicochemical and biological. The choice will depend on the nature of the biomass, as well as the characteristics of the conversion process, in order to provide the major advantages [17]. Fermentation is the phase in which the matter is transformed by microorganisms, i.e. the carbon source is converted into the target compound. An example of a compound that can be produced through this bioprocess is lactic acid (LA) [18].

Lactic acid (2-hydroxypropanoic acid) ($C_3H_6O_3$) (LA) is a versatile molecule with numerous applications, spanning across food, cosmetics, textiles, pharmaceuticals, and chemicals. Despite its potential in various industries, a substantial 85% of the demand for lactic acid originates from the food industry. Lactic acid can be synthesized through two distinct processes: fermentation and chemical synthesis. The key differentiator lies in the resulting isomers (D- and L-lactic acid isomers). The chemical route yields a racemic mixture (DL-lactic acid), while fermentation allows for obtaining high yields and isomers in high purity (>98%), which will significantly influence the properties of downstream products, such as occurs in the production of biopolymers [19–21]. The growing environmental concerns associated with petroleum-derived plastics have driven a significant increase in the demand for bioplastics. Among the approaches to bioplastic production, LA fermentation has emerged as a promising and more sustainable pathway. LA is the precursor in

the synthesis of polylactic acid (PLA), a biodegradable and bio-based polymer that could aid the transition toward a circular economy, aligning with principles of waste valorization and environmental sustainability [22, 23].

Previous studies have reported lactic acid production from banana residues using various approaches. Belmakki et al. [24] investigated simultaneous saccharification and fermentation of unmatured BP and flesh, Mudifah and Wakayama [25] studied multiple parallel fermentation of BP and enzymes produced in situ by fungi, Azaizeh et al. [26] explored the valorization of banana peduncles through separate hydrolysis and co-fermentation, and Martínez-Trujillo et al. [10] evaluated the enzymatic saccharification of yellow ripe BP and sequential fermentation. In the context of the bioapplications of lactic acid, this study addresses, for the first time the use of discarded BP as a substrate to produce lactic acid through fermentation of BP-derived sugars using *Lacticaseibacillus rhamnosus*, evaluating the biomass chemical characterisation, the pretreatment behaviour, the enzymatic hydrolysis with different enzymatic combinations and the fermentation process itself. As well as, providing new insights into enhancing lactic acid production from BP under the process conditions.

Materials and Methods

Material and Reagents

The banana peels (BP) (Cavendish variety) were donated after being discarded by markets in the region of Bragança, Portugal. These peels were collected in two different periods, between August and December 2022 (BP2022) and between April and August 2023 (BP2023). The BP were collected at different periods because a large amount of material was needed for the whole process. Therefore, BP2022 was used in the phase of the pretreatment study and BP2023 was used in the phase of the fermentations. Each batch of bananas was chemically characterised before the different assays. Once the bananas were obtained, the peels were removed and dried at 40 °C for 5 days in an oven (Digitronic – TFT, J.P Selecta, Spain) until reaching a moisture of about 6%. After this process, the residue was ground in an electric grinder (28000 r/min, Cgoldenwall; US) to a particle size of ~70 mesh (0.212 mm), homogenised and stored protected from light and humidity until further steps.

All reagents and chemicals were purchased from scientific suppliers with analytical purity, and high-performance liquid chromatography (HPLC) grade when required.

Chemical Characterization of the Banana Peels

The chemical characterisation of BP was performed according to the National Renewable Energy Laboratory (NREL) (Colorado, USA) [27]. The content of extractives, structural carbohydrates (cellulose and hemicellulose), lignin and ash were determined in BP biomass following these methods. The crude protein and total starch contents were measured according to the Association of Official Analytical Chemists (AOAC). The former, relying on the Macro Kjeldahl method, using a conversion factor of 6.25 [28]; and the latter, using the total Starch (AA/AMG) Assay Kit (Megazyme).

For starch determination, 100 mg of the sample were first incubated with sodium acetate buffer and α-amylase at 100 °C for 15 min, to hydrolyse the starch into maltodextrin. Tubes were then cooled, the enzyme amyloglucosidase was added and they were incubated for a second time at 50 °C for 30 min, to hydrolyse the maltodextrins into D-glucose. After incubation, 2 mL of the solutions were collected, centrifuged and 0.1 mL of the supernatant were transferred to test tubes containing 3 mL of GOPOD (glucose oxidase/peroxidase) reagent. The tubes were subjected to a third and final incubation period of 50 °C for 20 min. In this last stage, a colorimetric reaction promoted the oxidation/peroxidation of D-glucose and consequent production of quinoneimine dye (indicator). Sample blanks (without added enzymes), reagent blanks and a glucose control with a standard solution (1.0 mg/mL) were run alongside the samples. The absorbances (510 nm) of the solutions were used to calculate the starch content [29].

Lactic Acid Production by Fermentation

Pretreatment

A diluted-acid pretreatment with sulphuric acid (H_2SO_4) was carried out in an autoclave for 10 min at 121 °C. The effect of the following variables on the enzymatic hydrolysis yield was evaluated: solid load (10, 15 and 20% (w/v) in dry weight basis (dwb)) and H_2SO_4 concentration (0.25 and 0.5% (v/v)) in the pretreatment. The biomass was weighed according to the solids load and incorporated into the acid solution at the above concentrations. The tests were carried out in Erlenmeyer flasks with a final volume of 50 mL. After autoclaving, the flasks were left at room temperature to cool down and proceed to the enzymatic hydrolysis (EH) stage. For this section, the BP2022 batch was used.

The experimental parameters were initially established based on literature from studies on the pretreatment of banana peels [7, 9]. Solid load was increased from 10% to 20% (w/v) in 5% increments, guided by both biomass handling characteristics and pretreatment performance. At 20%

Table 1 Parameters used in pretreatment tests

	1st test set	2nd test set	
Solid load (*w/v*dwb)	10%, 15 and 20%	15% and 20%	
H_2SO_4(*v/v*)	0%, 0.25% and 0.5%	0.25%	
Commercial enzymes	CellicCTec2;		CellicCTec2 CellicCTec2 + Viscozyme CellicCTec2 + Pectinase

Table 2 Coding of pretreatment tests

Solid load	Code	Description
10%	0.0Acid	Enzymatic hydrolysis control without H_2SO_4
	0.25Acid	Enzymatic hydrolysis with 0.25% H_2SO_4
	0.5Acid	Enzymatic hydrolysis with 0.5% H_2SO_4
15%	0.0Acid	Enzymatic hydrolysis control without H_2SO_4
	0.25Acid	Enzymatic hydrolysis with 0.25% H_2SO_4
	0.5Acid	Enzymatic hydrolysis with 0.5% H_2SO_4
20%	0.0Acid	Enzymatic hydrolysis control without H_2SO_4
	0.25Acid	Enzymatic hydrolysis with 0.25% H_2SO_4
	0.5Acid	Enzymatic hydrolysis with 0.5% H_2SO_4

solid load, the slurry exhibited high viscosity and reduced processability, coinciding with a decline in sugar yield. This condition was therefore defined as the upper operational limit for solids concentration of the study. Regarding acid concentration, trials were initiated with 0% and 0.5% (v/v) H_2SO_4, followed by a reduction to 0.25% to assess its influence. As no statistically significant differences were observed among these concentrations, 0.25% was selected for subsequent experiments, as it improved medium fluidity compared to 0% acid.

The sequence of tests was performed, at 0.25% (v/v) acid concentration and only at 15% and 20% (w/v) solid load, to test the effect of different combinations of commercial enzymes (described in point 2.3.2) in the sugar production yield under high solid load conditions.

In all tests, at the end of the autoclaving, aliquots were collected to measure the sugars for yield calculation. Table 1 summarises all the conditions used in the pretreatment trials including the experiments with different enzymes shown below in Sect. 2.3.2. Table 2 shows and describes the codes used in the graphs to identify each pretreatment test. The autoclave parameters were the same (121 °C and 10 min) and, as a control, a pretreatment run was carried out without acid in all tests.

The best pretreatment conditions for producing the fermentation medium were chosen based on the hydrolysis yields and the technical challenges of biomass management that may interfere with the yield and efficiency of the process.

Enzymatic Hydrolysis

After each pretreatment run, the pH of the pretreated slurry was adjusted with H_2SO_4 (1 M) to 5 for the enzymatic hydrolysis. The enzymatic hydrolysis (EH) tests were carried out in Erlenmeyer flasks (100 mL) with a final volume of 50 mL, in an orbital shaker (50 °C and 150 rpm) for 40 h. A commercial enzyme cellulase cocktail (enzyme blend, SAE0020, Sigma-Aldrich) (C) was used for the first two pretreatment sets of trials, at a dose of 34 mg protein/g pretreated material dwb. For the second study of the effect of supplemented enzyme cocktail at high solid loadings of 15 and 20% (w/v, dwb), in addition to C, Viscozyme L (KTN02158, Novozymes, Denmark) (V), at the same amount of C, and Pectinase (from *Aspergillus aculeatus*, P2611, Sigma-Aldrich) (P), in a dosage 10 times lower than C, were also used to study their influence on biomass enzymatic digestibility. Experimental parameters were defined based on literature [4, 30] and the established technical expertise of the laboratory where the studies were conducted. At this stage, BP2022 samples were used. Moreover, EH test was carried out in raw banana peels with 10% w/v of solid load (dwb) in water, without any pretreatment, as a control of EH in BP material.

The enzymatic hydrolysis yield of cellulose was calculated as the glucose content at the end of the enzymatic hydrolysis process minus the glucose content at the beginning (0 h) divided by the potential glucose of the cellulose contained in the pretreated BP (Eq. 1). The potential glucose content refers to the maximum theoretical amount of glucose obtainable from the cellulose fraction of the biomass, assuming complete hydrolysis. Therefore, for the first set of tests was considered to have a potential of 12% (w/v, dwb) of glucose, and 9.1% for the second set. The glucose content at 0 h corresponds to the free glucose present in the pretreated BP and the glucose from the enzyme solution.

$$EH_{yield}\,[\%] = \left(\frac{Glucose_{end} - Glucose_{0h}}{Potential\ Glucose_{Cellulose}} * \frac{[g]}{[g]} \right) * 100 \qquad (1)$$

To prepare the sugar-rich medium for lactic acid fermentation, EH trials were performed using 1 kg of pretreated BP material from each solid load (15 and 20% w/v dwb) and 0.25% v/v acid concentration as substrate, in a bioreactor

with a capacity of 15 L (Terrafors, Infors HT) for 42 h, using both C and V. By the end of the process, the hydrolysate went through two stages of centrifugation and filtration for maximum removal of any remaining solids or fines from the solution. For this second part, BP2023 material was employed. The medium was stored until it was used for the fermentation stage.

Microorganisms and Cell Propagation

Lacticaseibacillus rhamnosus DSM20711 was acquired as a freeze-dried stock from the Germany Collection of Microorganisms and Cell Cultures (DSMZ, Leibniz Institute, Germany). For preculture, cells were grown under anaerobic conditions in 0.5 L BIOSTAT® B plus, Sartorius fermenters (Sartorius AG, Goettingen, Germany) with magnetic stirring at pH 6.0 (pH control was done using 4 M KOH) at 35 °C for 18 h. MRS broth (Merck, Darmstadt, Germany) supplemented with 30 g/L of glucose and 30 g/L of fructose MRS (+), to resemble the sugar content of BP, was used as growth medium. MRS (+) was sterilized by filtering through a 0.22 µm PES filter unit (Thermo Fisher Scientific). Cells growing in exponential phase (OD600 nm≈2) were harvested by centrifugation (9000 rpm, 10 min), resuspended in five times lower volume of MRS (+) media containing 20% (v/v) glycerol, aliquoted in 5 mL vials and stored at −80 °C prior to inoculation.

Batch Lactic Fermentation

The two hydrolysates obtained from the chosen pretreatment trials described at the end of point 2.3.2. were used as the fermentation medium and supplemented with the same formula as the MRS. The experiments were conducted in bioreactors (BIOSTAT® B plus, Sartorius) in batch mode, under anaerobic conditions with N_2 injection (0.250 L min^{-1}), with a working volume of 200 mL and 300 rpm. A screening step was done in MRS (+), varying two factors at three levels, namely temperature (30 °C, 35 °C and 40 °C) and pH (5.0, 6.0, 7.0), to find the most suitable point to carry out the fermentation in the real medium (BP medium). The pH was maintained by adding potassium hydroxide (KOH). Samples were collected every 2 h to assess sugar consumption, cell growth and lactic acid production.

Analytical Methods

The measurement of sugars (glucose, xylose, galactose, arabinose, mannose, fructose and sucrose) in BP characterization solutions, hydrolysis and fermentation media was carried out by HPLC, using a Waters 2695 liquid chromatograph with a refractive index detector (Waters, Mildford, MA) and a column CarboSep CHO-782 Pb, (Transgenomic, Inc., Omaha, NE). Ultrapure water was used as eluent at a flow rate of 0.6 mL/min and a column temperature of 70 °C [31].

The LA produced in the fermentation step was also analysed by HPLC (Agilent, Waldbronn, Germany) equipped with a refractive index detector using a Aminex HPX-87 H (Bio-Rad Labs, Hercules, CA) at 50 °C. H_2SO_4 (5mM) column with a flow rate of 0.5 mL min^{-1}. The content of toxic degradation products of sugars (e.g. furfural, hydroxymethylfurfural, and formic acid) and acetyls was analysed by HPLC with a 1050 photodiode-array detector (Agilent, Waldbronn, Germany) and ICSep ICE−Corgel 87H3 column (Transgenomic, San Jose, CA, USA), operating at 65 °C with H_2SO_4 (5mM) as the mobile phase (0.6 mL/min) [32]. The optical LA purity was determined via HPLC using an Agilent 1100 series chromatograph HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent 1200 series Diode-Array with a chiral column Supelco Astec CLC-D (Merck, Darmstadt, Germany) column at 30 °C and UV detector at 254 nm. The mobile phase was 1 ml/min of 0.002 M CuSO4. A UV-Visible spectrophotomer (V-530, Jasco, Tokyo, Japan) was used to measure the solution absorbance (between 0.2 and 0.7) at 210 nm to quantify the acid soluble lignin content; sulphuric acid (4% v/v) was used as a blank. Cell growth was measured by optical density in a spectrophotometer (V-530, Jasco, Tokyo, Japan) at 600 nm. The relationship between absorbance (x) and cell dry weight (y) was determined by an appropriate calibration curve (Eq. 2), characterized by a zero intercept with $R^2 = 0.9978$.

$$\boldsymbol{y}\,[g/L] = 0.4369 * x\ [\boldsymbol{OD}\ 600] \tag{2}$$

Statistical Analysis

All data are expressed as mean ± standard deviation (SD). Biomass characterization analyses were performed in triplicate, whereas pretreatment and fermentation assays were conducted in duplicate as the prolonged duration of these procedures, combined with the limited experimental window, constrained the number of replicates feasible within these stages. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 28.0 (Armonk, NY: IBM Corp). A comparison between different samples was analysed through a student's T-test if only two levels were in study and a one-way analysis of variance (ANOVA) using a Tukey's as a post-hoc test, if more than two levels were analysed. If significant ($p < 0.05$), the values of the groups differed. Otherwise ($p > 0.05$), the values of the groups did not display significant differences and were considered "equal". For the first set of pre-treatments and to maximize

the response of the pretreatment, a linear model, applying the L-optimal design, was developed based on experimental data obtained from the pre-treatments. The factors studied were acid concentration (X1: 0, 0.25 and 0.5%) and solid load (X2: 10, 15 and 20%) with three levels each. The response variable was the sugars concentration (Y1: g/L) and yield (Y2: %). Data analysis was performed using Design Expert 13 (13.0.5.0) (Minneapolis, MN, USA).

Results and Discussion

Material Characterization

The characterisation of the key components of BP2022 and BP2023 samples was the first step, as from the results obtained it is possible to calculate the yields of the enzymatic hydrolysis and assess the potential of BP as a source of carbohydrates. Table 3 shows the values of the chemical characterisation of the banana peels. The values of total extractives, cellulose, total lignin, soluble lignin, acetyls and proteins were statistically different between the two BP samples determined by one-way ANOVA. While BP2022 had fewer extractives and a higher cellulose and lignin content, BP2023 had more extractives and less cellulose and lignin. In other words, BP2022 contains a slightly higher amount of sugars retained in the lignocellulosic structure. Extractives are defined as non-structural constituents of biomass that are soluble in neutral solvents. Water-soluble extractives typically include inorganic constituents, non-structural carbohydrates, and nitrogen-containing compounds. The inorganic fraction may originate from the plant matrix or from external sources such as adhering soil or residual fertilizer. Ethanol-soluble extractives generally consist of chlorophylls, waxes, and other minor lipophilic components [27]. Neither of the samples showed resistant starch, which is not digested in the human small intestine and is fermented in the large intestine by gut bacteria [33]. Comparing with the characterisation of banana peels from Cavendish bananas, Silva et al. [11] obtained higher values for extractives (62.9±0.4%), hemicellulose (6.0±1%) and cellulose (17.3±0.8%) and lower total lignin (9.9%), both soluble (1.01±0.02%) and insoluble (8.9±0.5%). The values for ash (13.22±0.01%) and extractives (62.97±2.04%) are in line with Pereira et al. [12], but those for lignin (30.77±2.62%), cellulose (29.28±0.39%) and hemicellulose (38.42±2.21%) are lower. Oberoi et al. [30] also obtained similar results for cellulose (12.17±0.21%), but almost twice as much for hemicellulose (10.19±0.12%). The ashes (11.86±0.37%) are also in agreement with Segura-Badilla et al. [14] as are the proteins (6.41+0.72%). In general, it can be stated that BP biomass presents significantly

Table 3 Banana peels characterisation

		Banana peels (2022) (% dwb)	Banana peels (2023) (% dwb)	*p*-value
Total extractives		44.8±0.2	52.951±0.007	0.002
Aqueous		51.93±0.08	57.9±0.6	0.005
Organic		4.9±0.1	7.2±0.1	0.002
Sugars in aqueous extractives				
	Glucose	10±1	10.6±0.3	0.853
	Xylose	0.200±0.005	0.228±0.003	0.340
	Galactose	0.0843±0.0003	0.088±0.003	0.345
	Arabinose	0	0	<0.0001
	Mannose	0.434±0.004	0	<0.0001
	Fructose	11±2	12.7±0.2	0.444
Cellulose (Glucans)		**11.42±0.09**	**8.3±0.1**	**0.002**
	Glucose	12.6±0.1	9.1±0.1	0.002
Hemicellulose		**5.9±0.3**	**5.3±0.2**	**0.179**
	Xylose	2.4±0.2	1.97±0.04	0.115
	Galactose	1.14±0.07	1.04±0.02	0.307
	Arabinose	2.20±0.07	2.0±0.1	0.172
	Mannose	0.89±0.02	0.93±0.02	0.338
Total lignin		**17.2±0.5**	**14.2±0.1**	**0.027**
	Soluble lignin	2.64±0.06	1.60±0.01	0.004
	Insoluble lignin	14.5±0.6	12.6±0.2	0.075
Acetyl groups		**0.37±0.02**	**0.152±0.003**	**0.010**
Ash		**14.0±0.1**	**13.433±0.006**	**0.057**
Protein		**6.90±0.07**	**7.99±0.09**	**0.006**
Starch		**2.7±0.1**	**2.73±0.02**	**0.678**

high extractives content, in comparison with other biomasses such as agricultural residues (wheat, corn stover), herbaceous species (*Miscanthus*) or wood, whose content does not usually exceed 15–20% dw [13]. About 50% of total extractives content consists monosaccharides, which can be considered an advantageous feature of this unique material, since it implies that a part of the sugars contained in it can be easily released without applying severe pretreatments. These results confirm that the total carbohydrate content measured in this work for BP biomass (38–40%) makes it a promising candidate for use as a source of sugars for further valorisation.

Pretreatment and Enzymatic Hydrolysis: Sugar Production

Evaluation of Pretreatment Conditions

The effect of pretreatment conditions (solid load and acid concentration) was evaluated through EH assays to determine the sugar production yield and to select the best conditions for the production of sugar-containing media for lactic fermentation.

Figure 1 shows the cellulose EH yields according to solid load and sulphuric acid concentration. The EH yield of the control used in this work, i.e., samples subjected to enzymatic hydrolysis without a previous pretreatment (53.5 ± 0.4%) was substantially lower than those reflected in Fig. 1, which indicates the suitability of a pretreatment stage. This value was statistically different ($p < 0.001$) from the yields of the samples pretreated in autoclave with H_2SO_4, in both acid concentrations. The pretreatment allowed all EH yields to be greater than 60% and most of the cases close to or exceeding 80%. The presence or absence of sulfuric acid did not cause significant changes in the EH yield within groups with 10% and 15% solid load. Despite this, when handling the samples and visually, it was possible to observe that the presence of acid influenced the viscosity and provided greater fluidity during hydrolysis, which facilitated the management process. At 10% solids, no significant differences ($p = 0.159$) were found between pretreatments at different acid concentration (0.0%: 80 ± 6; 0.25%: 83 ± 3%; 0.5%: 90 ± 3%). Similarly, the trials at 15% solid load did not result in major differences between the different acid concentrations (0.0%: 72 ± 6; 0.25%: 77 ± 4%; 0.5%: 81.4 ± 0.4%), statistically, there were no significant differences between the groups ($p = 0.215$). Nevertheless, for the 20% solid load, significant differences ($p = 0.036$) were found between the tests with different acid concentration, achieving much higher yields with 0.25% acid concentration (84 ± 1%) compared to 0.5% (63 ± 1%).

In the assays with 20% solid load, the media became thicker, similar to a "slurry", which made it more difficult to homogenise and correct the pH, and impaired fluidity in

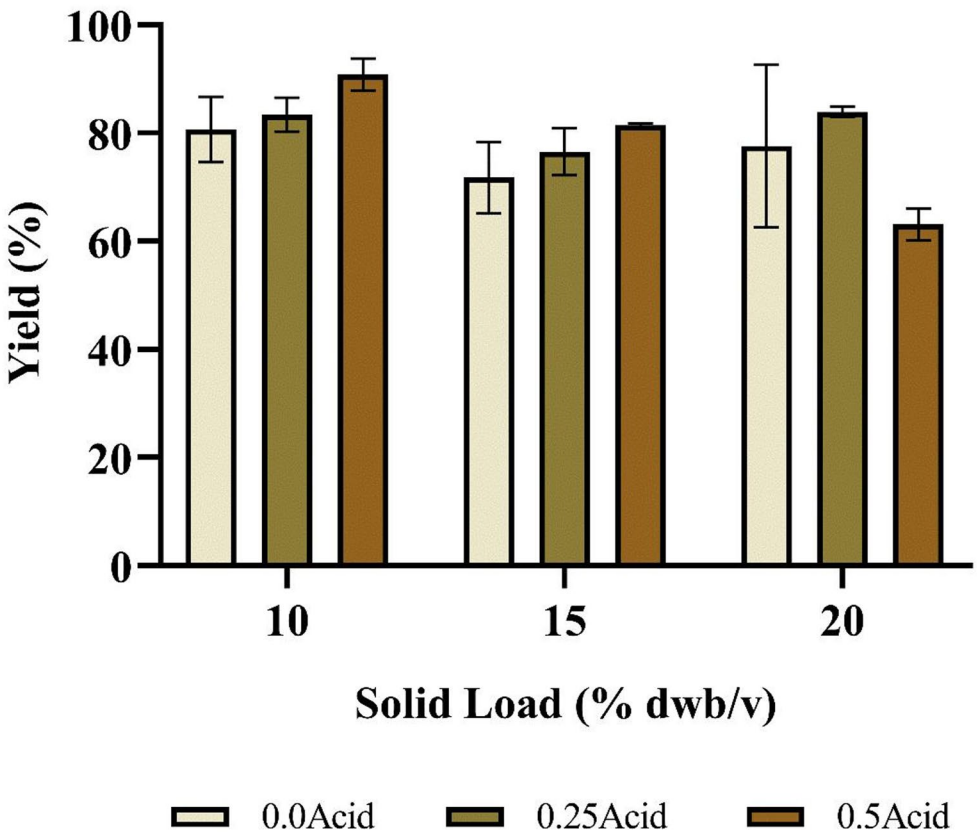

Fig. 1 Enzymatic hydrolysis yield of cellulose in pretreated BP enzymatic hydrolysis tests at different solid loads

the first hours of enzymatic hydrolysis. This, consequently, may have introduced mass transfer limitations and uneven enzyme substrate contact. These factors impacted the process efficiency and may explain variations in yields between the acid concentration. In the control treatment at 20% solid load this effect was particularly evident, where substantial variability was observed. Consequently, the large standard deviation obtained rendered the mean value statistically indistinguishable from those of other treatments. Therefore, any apparent differences in yield under these conditions should be interpreted as within the bounds of experimental error rather than as evidence of a true treatment effect. The data from 20% solid load might not be representative of what happens, as it deviates from the trend observed in the other solids loads.

Figure 2 summarises the concentration of total sugars obtained during hydrolysis at different solid load and acid concentration over 40 h. The sugar concentration measured in the experiments at the same solid load were within the same range of values. The highest rate of hydrolysis of the structural components occurred between 0 and 12 h. From the 12th hour onwards, the sugar content remained relatively constant and showed signs of the end of hydrolysis. The sugar concentrations among the solid load groups had a statistically significant difference ($p<0.001$). Within all solids loads, there was a significant difference between 0 and 12 h (10%: $p=0.022$; 15%: $p<0.001$; 20%: $p<0.001$). Only with 15% BP, the concentration of sugars was significantly different between 12 and 24 h ($p<0.001$) and statistically the same between 24 and 40 h. With 10% ($p=0.610$) and 20% BP ($p=0.510$), there were no significant differences between sugar concentrations from 12 h onwards. The presence or absence of H_2SO_4 did not cause significant differences between the samples within all different solid loads (10%: $p=0.553$; 15%: $p=0.895$; 20%: $p=0.890$). The limited increase in sugar release after 12 h could be attributed to product inhibition, as glucose and cellobiose accumulation may suppress cellulase and β-glucosidases activities through feedback inhibition, with cellulases being particularly sensitive by glucose. Thereby limiting the hydrolytic potential of cellulases and β-glucosidases. Similar behaviour has been reported for pretreated banana peels, where sugar concentrations plateaued after 12 h [30]. Additionally, pH shifts during fermentation, such as acidification, may further impaired enzyme activity and sugar release by altering the optimal pH for its action [10].

In their study, Mishra et al. [3] stated that acid pretreatment was more effective than alkaline pretreatment for delignification and exposed cellulose to enzymatic action on BP biomass. The authors postulated that the diluted acid is responsible for eliminating hemicellulose from the cell wall, which enhances the availability of cellulose for enzymatic hydrolysis and promotes better conversion of cellulose into monosaccharides. They studied the impact of H_2SO_4 pretreatment on temperature, incubation time, substrate and acid concentration on cellulose, hemicellulose and lignin. The accessibility of cellulose was primarily influenced by temperature and incubation time, while substrate and acid

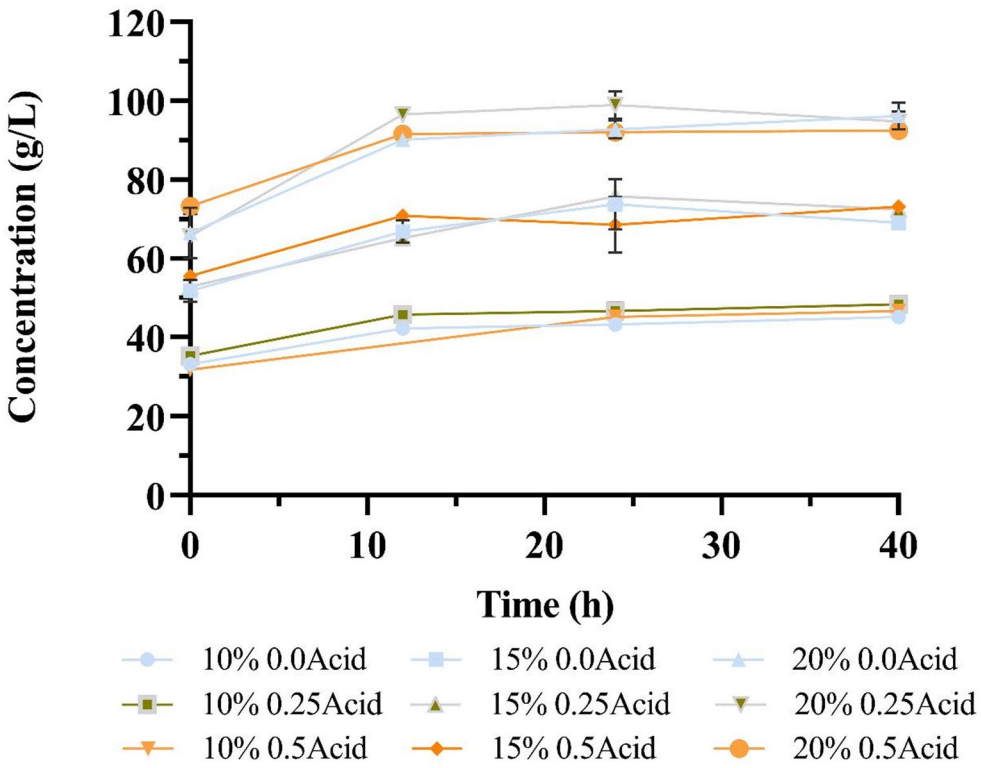

Fig. 2 Evolution of total sugar concentration in media from enzymatic hydrolysis of BP pretreated at different solid load and sulphuric acid concentration (0, 0,25 and 0.5 (v/v) and solid loads (15 and 20% w/w), over 40 h incubation time

concentration played significant roles in hemicellulose decomposition. Delignification, on the other hand, was predominantly affected by incubation time and substrate concentration. The results of the present work are partially in line with those found by Mishra et al. [3], as the autoclaving led to an increase in EH yield of cellulose. However, the addition of acid had a greater impact on the fluidity of the medium than on the EH yield. Substrate concentration also proved to be an important factor in determining the efficiency of the pretreatment process.

Palacios et al. [7] observed higher hydrolysis efficiency at high temperatures and in the presence of acid. In this case, the authors evaluated three concentrations of sulphuric acid (0, 0.5 and 1% v/v), two thermal treatments (autoclave 121 °C, 15 min and keep at 28 °C for 45 min) and three forms of biomass (pulverised, liquid, unmilled). The pulverised, acid-treated and autoclave-treated BP showed no hemicellulose content, which may may have been due to the size reduction of the biomass facilitating the hydrolysis of hemicellulose. The highest total sugar contents were found in BP liquefied using 0.5% v/v H_2SO_4 and autoclaving, and 1%v/v H_2SO_4 at 28 °C. In addition, the authors concluded that high temperatures and dilute acid can dissolve a large part of the hemicellulose, especially in pulverised samples, but they do not completely remove the lignin. The particle morphology also influences the hydrolysis efficiency of cellulose. In the current study, the sugar concentrations obtained over time were higher than those shown by Palacios et al. [7]. The use of high temperatures and dilute-acid pretreatment in the BP of this study proved to be adequate for the efficiency of the process. Although the particle size was not specifically studied, it may have had a positive impact on the hydrolysis yield.

Acid pretreatment can be highly effective in disrupting the biomass matrix by solubilising and depolymerising pectin and hemicellulose, which could lead to a reduction in viscosity [4, 9, 30]. However, the overall effect on sugar release may be offset by the formation of enzymatic inhibitors, suboptimal pH conditions for enzymatic activity or the production of intermediate oligosaccharides that inhibit other hydrolytic enzymes [12, 24]. Consequently, the improvement in fluidity resulting from the pretreatment may appear more pronounced than the actual incremental gains in final sugar concentration or hydrolysis efficiency.

To evaluate the presence of products formed in the hydrolysis, two samples were selected, one with acid and one without. The presence of contaminants was minimal, though it was greater in the acid-treated sample. The acid-treated sample contained 0.247 g/L of hydroxymethylfurfural (HMF) and 0.256 g/L of acetic acid, whereas the non-acid sample contained 0.030 g/L of HMF and 0.248 g/L of acetic acid. Both samples showed approximately 0.142 g/L of levulinic acid, less than 0.01 g/L of furfural (FF) and vanillic acid (VA), along with an unidentified peak (retention time = 7.88 min), which is probably a furfural derivative due to the similarity of the spectrum. In the acid-treated sample, this peak was almost double that in the non-acid sample.

Additionally, to complement the evaluation and considering the sugar concentration as the response variable, linear model was generated ($p < 0.0001$; Y = −0.44191 + 1.29151*Acid concentration + 4.71967 * Solid Load), identifying the solid load as the only significant factor ($p < 0.0001$). Under this model, the optimal pretreatment conditions were an acid concentration of 0.00–0.077% and a solid load of 20%, resulting in a theoretical maximum yield of 94.0508 g/L of lactic acid.

Testing of Different Enzyme Cocktails in EH

EH tests were carried out with supplementary enzyme cocktails (Viscozyme L and Pectinase) aimed at improving the hydrolysis of all components contained in BP pretreated biomass and maximize sugar production. For these trials, H_2SO_4 at 0.25% v/v was used because although the EH results were similar with and without acid, its addition favoured the fluidity of the biomass throughout the process.

Figure 3a and b plot the sugar concentration obtained over 24 h with the different enzyme mixtures and solids concentrations 15% and 20%, respectively. The results obtained for 20% solid load condition, comparing each acid concentration, were more heterogeneous than those obtained with 15% solid load, which again could be explained by the high solid loads and low mobility of the medium. The results from assays at 15 and 20% solid loads were significantly different ($p < 0.001$), with 20% solid load showing higher sugar concentration results, which is to be expected due to the greater sample mass. Within each solid load group, considering the 3 tests (C, C + V, C + P) to evaluate the influence of time, the sugar concentration increased from 0 h to 12 h and remained practically constant at 24 h, with no significant differences in any of the groups (15%: $p = 0.990$; 20%: $p = 0.958$). Despite this, a trend was observed in which hydrolysis with viscozyme L showed better results than with the other enzyme combinations, both in terms of concentration and biomass fluidity during the whole process. Practically, at both solid loads, after the first sample collection, the medium with viscozyme L was always more liquid than the others. Statistically, cellulase plus viscozyme L showed a significant difference ($p = 0.037$) compared to the use of cellulase alone. Thus, viscozyme L together with cellulase were chosen to produce the fermentation BP medium.

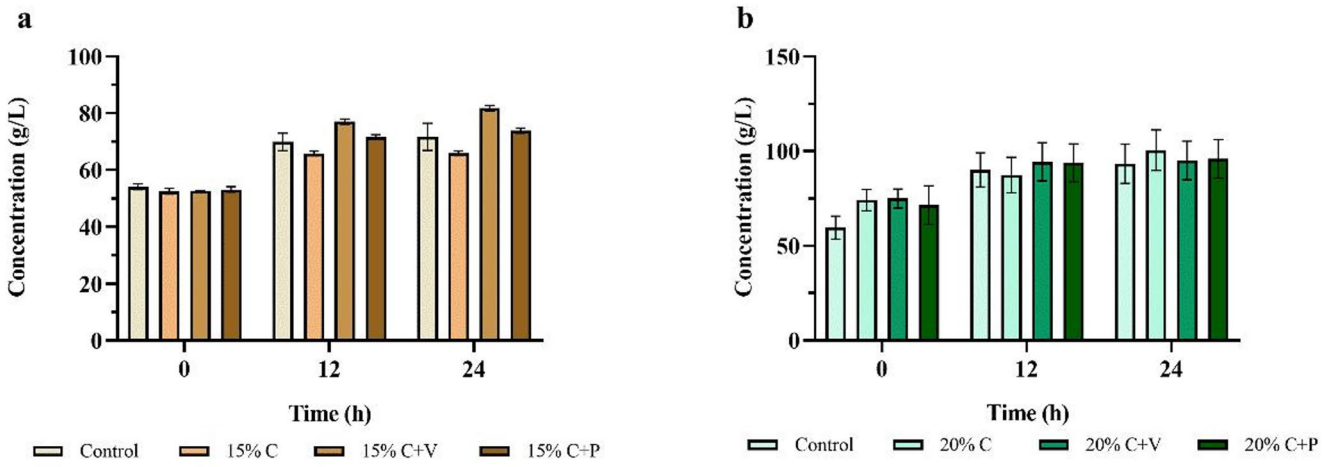

Fig. 3 Total sugar concentration at 0, 12, and 24 h of incubation time in media from enzymatic hydrolysis of BP pretreated at 0.25 sulphuric acid and (**a**) 15% solids and (**b**) 20% solids, with selected enzyme mixtures

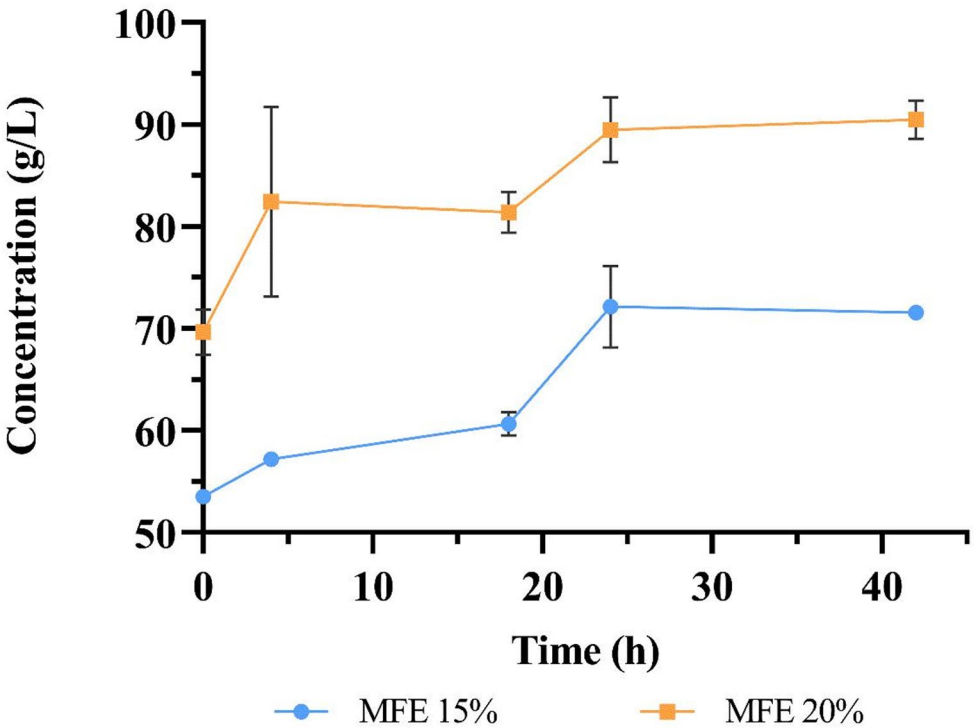

Fig. 4 Evolution of total sugar concentration in fermentation media from enzymatic hydrolysis of BP at 15% and 20% solids

Sugar Media Production for Lactic Acid Fermentation Assays

Once the pretreatment conditions and enzymes had been selected based on the results obtained in the trials described above, the fermentation medium was prepared at the highest solids loads. The parameters defined were the use of C+V enzymes and 0.25% of acid.

Figure 4 depicts the concentration of sugars in the medium prepared for fermentation (MFE), obtained immediately after autoclaving and during hydrolysis over 42 h with 15% and 20% solid loads. The concentration of sugars in MFE at both solids loads (15%: 71.6±0.1 g/L and 20%: 90±2 g/L) was lower than in the evaluation of pretreatment conditions stage. The final concentration of sugars in the evaluation tests was 72±1 g/L with 15% solid load and 95±3 g/L with 20% solid, using 0.25% v/v acid concentration and C. In the subsequent trial using C+V, the values were 82±1 g/L and 95±10 g/L, respectively. These differences could be explained by the increase in pretreatment scale volume, 1000 g of dry sample per batch, difficulties in homogenizing the entire substrate, as well as the fluidity of the substrate, which could have affected the enzymatic action. However, visually, after about 4 h of treatment, the

biomass became less viscous, as a liquid solution. Comparing both solids loads, the values were statistically different ($p < 0.001$) regarding the sugars content. Despite the larger volume used to produce the MFE, the hydrolysis time trend remained the same, after 24 h of hydrolysis most of the structural components had already been hydrolysed, the sugars concentration remained stable and, statistically, no significant differences were found in either case between 24 and 42 h. For 15% solid load, the sugar concentration increased between the autoclave and the start of pretreatment (0 h), remained statistically constant until 18 h and increased again until 24 h. For 20% solid load, the values were similar between autoclave and 0 h, increased until 4 h, remaining constant until 18 h and increasing again until 24 h.

The calculation of the EH yields for the trials to generate MFE provided values of 70% for 15% solid load and 64% for the experiment at 20% consistency. When comparing the yields between them, the groups exhibited significant differences ($p = 0.026$). The 15% BP showed a slightly higher yield compared to the 20% BP, which can be attributed to difficulties in poor mixing and mass and heat transfer constraints in the 20% trial, which diminished the efficiency of the enzymes in the initial stages of the hydrolysis.

Consistent sugar yields across varying solid load suggest that increased solids concentration does not drastically hinder enzymatic hydrolysis efficiency. Higher solid load could be economically advantageous due to resource consumption. However, this could be offset by operational challenges, increased viscosity and mass/heat transfer limitations. Optimizing solid load would require balancing sugar yield with process scalability. As well as, further investigation, including pilot-scale studies and rheological analysis, to determine the most technically and economically feasible conditions for scaling up the process.

Lactic Acid Fermentation

Selection of Optimal pH and Temperature Conditions

Before performing the lactic acid production tests with the real medium, the optimal temperature and pH conditions of *L. rhamnosus* on the MRS (+) medium were determined. The fermentation time was set at 28 h, since after 24 h, almost all the sugars had been consumed and LA production slowed to a standstill. At 28 h, there was no significant sugar content left (< 1.000 g/L).

Table 4 Fermentation parameters in temperature screening assays in MRS (+); maximum lactic acid concentration, yield and volumetric productivity

T (ºC)	Max. concentration (g/L)	Yield (g/g)	Vol. productivity (g/L h)
30	69 ± 3	0.91 ± 0.04	2.5 ± 0.1
35	75 ± 3	0.98 ± 0.04	2.7 ± 0.1
40	69 ± 3	0.92 ± 0.04	2.5 ± 0.1

To determine the effect of temperature on the production of lactic acid, the maximum lactic acid concentration, fermentation yield and volumetric productivity of the fermentations were calculated at 30, 35 and 40 °C, at a constant pH of 6 (Table 4). The results at 30 and 40 °C were rather similar in terms of maximum concentration, yield and volumetric productivity, while the results at 35 °C were higher in lactic acid concentration, yield and productivity.

Figure 5a shows lactic acid productivity over the course of fermentation. The maximum productivity values were found between 4 and 8 h (greatest slope), reaching 1.3 ± 0.6, 2.9 ± 0.6 and 3.7 ± 0.6 g/L h for 30, 35 and 40 °C, respectively. This indicates the influence of temperature on the initial speed of LA production. As the temperature increases, the rate of lactic acid production also rose, reaching the highest rate at 40 °C. When considering the productivity at the end of 28 h, 30 °C and 40 °C samples were equal (2.463 and 2.461 g/L h, respectively), which suggests that at 40 °C there was a higher initial production rate (8 h), that decreased over time, as well as a lower concentration of lactic acid and yield at the end of the process. This could be explained by the fact that fermentation ended earlier, after 24 h. The increase in lactic acid from 57.0 to 57.9 g/L between 24 and 28 h represents a very low variation. Fermentations at 35 °C outperformed those at 30 °C and 40 °C in lactic acid yield and productivity by *L. rhamnosus*, which is consistent with the optimal growth range generally reported for lactic acid bacteria (30–37 °C) [22, 34, 35]. At 30 °C, suboptimal enzyme activity and slower metabolic rates likely reduced productivity, while at 40 °C, partial enzyme destabilization and stress responses may have diverted resources toward cell maintenance or byproduct formation [4, 22, 36, 37]. Thus, 35 °C appeared to provide the most favourable balance between enzymatic efficiency and cellular stability, minimizing inhibitory effects of product accumulation for lactic acid production. Since the best results in terms of fermentation yield and productivity were achieved at 35 °C, this temperature was selected to evaluate the effect of pH on fermentation.

Regarding the pH effect on the production of lactic acid, Table 5 shows the results of the maximum LA concentration, yield and volumetric productivity of the fermentation tests at pH 5.0, 6.0 and 7.0, at constant temperature of 35 °C. The results at pH 5 and 6 were similar. On the other hand, at pH 7, the results for maximum lactic acid concentration, yield and productivity were lower, including when comparing the results obtained when screening at different temperatures (at pH 6). For instance, at 40 °C, 69.2 g/L of lactic acid was obtained, with a yield of 0.920 g/g and a productivity of

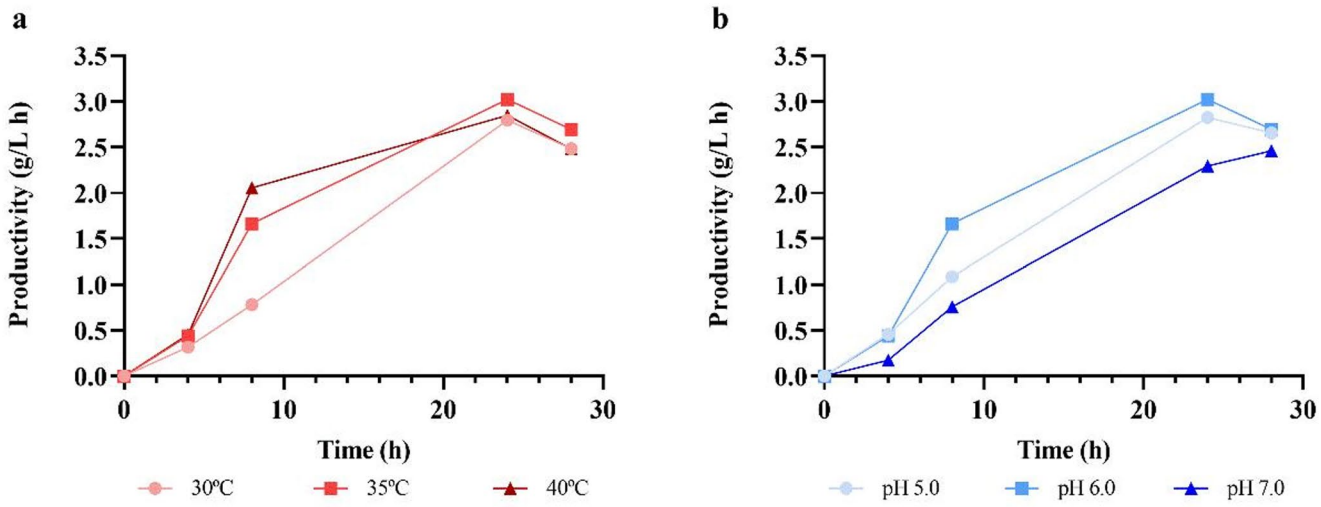

Fig. 5 Evolution of productivity values during (**a**) pH screening fermentation assays in MRS (+) (pH 5.0, 6.0, 7.0), maintaining a constant temperature of 35 °C and (**b**) temperature screening fermentation assays in MRS (+) (30 °C, 35 °C and 40 °C), maintaining a constant pH of 6.0

Table 5 Fermentation parameters in pH screening assays in MRS+: maximum lactic acid concentration, yield and volumetric productivity

pH	Max. concentration (g/L)	Yield (g/g)	Vol. productivity (g/L h)
5.0	74±3	0,97±0.04	2.6±0.1
6.0	75±3	0.98±0.04	2.7±0.1
7.0	68±3	0.90±0.04	2.4±0.1

2.461 g/L h, which are higher values than those obtained at pH 7. Therefore, it was possible to observe that *L. rhamnosus* prefers slightly acidic conditions, pH 5 or 6.

The maximum productivity over time (Fig. 5b) was also found between 4 and 8 h, reaching 1.7±0.6, 2.9±0.6, and 1.3±0.6 g/L h for pH 5.0, 6.0 and 7.0, respectively. The influence of pH on the rate of lactic acid production was also observed, with pH 6 being the most suitable among the pH studied due to its higher productivity, and pH 7 having the most negative impact on productivity. Considering the productivity at the end of the process, although lower fermentation yield and lactic acid concentration were obtained at pH 7, for all pH values the productivities were very similar (2.635, 2.671 and 2.442 g/L h for pH 5.0, 6.0 and 7.0, respectively). Abedi & Hashemi [36] summarised the productivity and yield of different agro-industrial biomasses (e.g.: apple pomace, cellulosic biosludge, white rice bran hydrolysate, cassava wastewater, solid carob waste, brewer's spent grain, yam tuber starch and others) for the production of LA by *L. rhamnosus*. The yields ranged from 0.38 to 0.99 g/g, with only a few values higher than 0.90 g/g. Regarding productivity, the range was between 0.58 and 8.77 g/L h, with most values below 3.0 g/L h, which is where the productivity obtained in this study was found. Ngouénam et al. [15] compared the fermentation of three tropical fruit peels: banana, pineapple and papaya. The BP was the sample with the highest LA concentration and productivity of 1.65 g/h L, while productivity was similar between the 3 fruit peels. In this study, the lactic acid bacteria were isolated from the fruits themselves. Hassan et al. [16] also evaluated the influence of BP medium concentration (4 to 20% w/v dwb) on LA production by *Enterococcus durans* BP130 and obtained yields ranging from 0.75 to 0.9 g/g, except with 20% solid load, which had a negative impact, yielding only 0.62 g/g. The productivity obtained was 1.2 g/L h.

The chirality of the LA produced, L-LA or D-LA, was identified through MRS tests. Aliquots were also collected in the pH tests: pH 5, 97.4% L-LA and 2.6% D-LA; pH 6, 94.7% L-LA and 5.3% D-LA; and pH 7 95.1% L-LA and 4.9% D-LA. The D-LA and L-LA synthesis depends on lactic acid-producing strains and is mediated by D-lactate dehydrogenase [38]. According to the results, 35 °C was chosen for the BP medium fermentations for lactic acid production. Although good results were obtained for both pH 5 and 6, pH 6 was selected since the results were slightly higher.

Lactic Acid Production from BP EH Media (MFE)

After defining the most suitable conditions (35 °C, pH 6), fermentation tests were carried out in BP medium for 15% and 20% solid load and the fermentation kinetics were evaluated to understand the potential of banana peels as a substrate for lactic acid production.

Figure 6 illustrates the fermentation parameters, i.e., the consumption of sugars (glucose and fructose), lactic acid and biomass produced in real medium over time. In MFE, fermentation was carried out for periods of 48 h, but

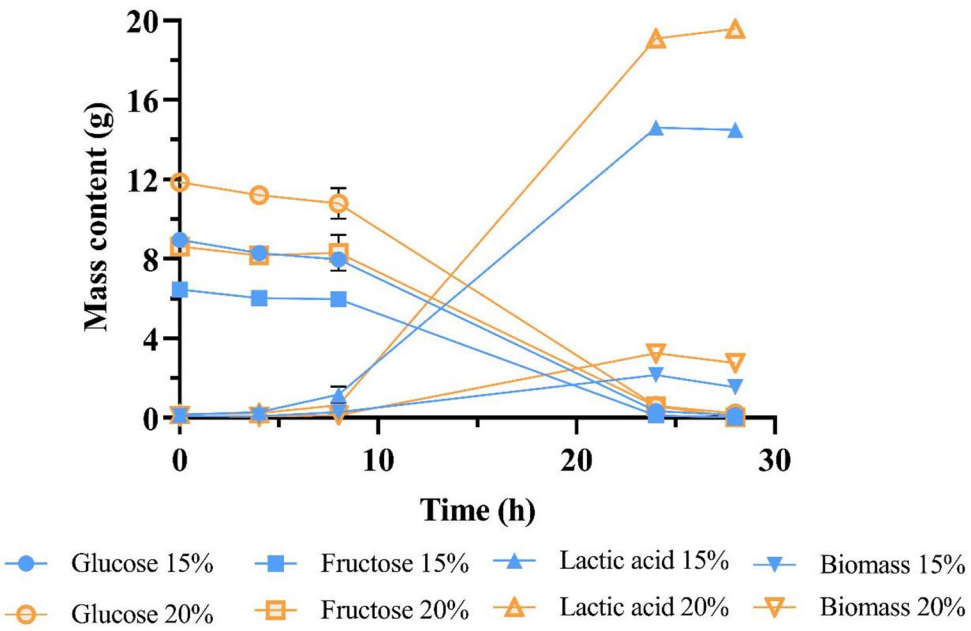

Fig. 6 Evolution of glucose, fructose, LA and biomass production over 28 h fermentation time in the EH media produced with pretreated BP at 15% and 20% solids (w/w) and 0.25% acid concentration

Table 6 Fermentation results using media from enzymatic hydrolysis of pretreated BP at 15 and 20% solids and 0.25% acid concentration: maximum lactic acid concentration and yield and volumetric productivity

% solid load	Max. concentration (g/L)	Yield (g/g)	Vol. productivity (g/L h)
15	82.3 ± 0.7	0.99 ± 0.01	2.94 ± 0.03
20	106.4 ± 0.9	0.975 ± 0.002	3.80 ± 0.03

after approximately 28 h, there were no longer significant quantities of sugars available, and the amount of lactic acid remained constant. Fermentation in real medium was expected to take longer than in synthetic medium due to the presence of inhibitor compounds and/or other unknown substances, as fermentation only starts once these inhibitor compounds, such as HMF and FFs have been consumed. However, no large quantities of inhibitor compounds were observed in the medium (data not shown). The process lasted around 2–4 h longer both to start the more pronounced consumption of sugars and to finish than in MRS (+).

Glucose and fructose were consumed simultaneously. Batches with 15% solid load started producing lactic acid earlier. However, after 24 h, both solid loads had already finished the process. 20% solid load produced a greater amount of LA, which is to be expected due to the greater number of initial sugars in the medium. The biomass content peaked at around the same time as the maximum LA concentration. After this period, there was no significant LA production, but aliquots continued to be removed from the fermentation broth, which may explain the decrease in biomass values.

Table 6 shows the results obtained for the maximum lactic acid concentration, yield and volumetric productivity of the fermentation tests with 15% and 20% solid loads. The results were rather similar in terms of yield, while the results with 20% solid load were higher in maximum concentration and productivity. The higher productivity explains why the maximum LA production and the end of fermentation were also reached at 28 h, even with a higher source of sugars with 20% solid load. In addition, it demonstrates the microorganism's ability to synthesise LA with higher sugar contents, i.e. more concentrated medium.

The maximum productivity values over the course of fermentation **(**Fig. 7**)** for both solid loads were found between 8 and 24 h, later than in the MRS (+) medium (4 h and 8 h), reaching 4.6 ± 0.1 and 5.92 ± 0.07 g/L h for 15% and 20% solid load, respectively. The 20% BP took longer to start fermentation, which suggests a lower initial production rate, but once started, productivity increased over the hours and consumed most of the sugars by 28 h.

Regarding fermentation yields, the values were similar to those achieved in synthetic medium under the optimum conditions, demonstrating the potential of the medium made from banana peels for lactic acid production. Among the fermentation tests in real medium, the assays with 15% solid load had a slightly higher yield, 0.99 g/g, than with 20% solid load, 0.975 g/g. Marques et al. [39] studied the production of lactic acid from recycled paper sludge and obtained productivity between 0.7 and 0.91 g/g in the different trials using *L. rhamnosus.* The authors also observed the influence of pH on LA production, because when pH was not controlled, the production decreased. Moreover, when pH reached 3, fermentation stopped, which could

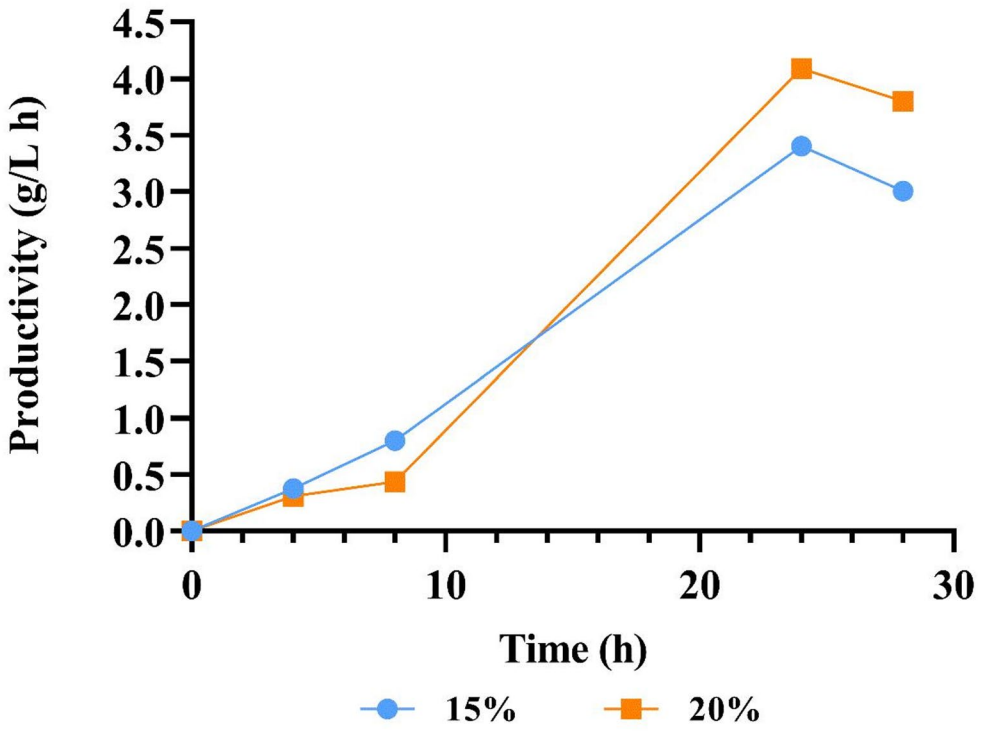

Fig. 7 Evolution of productivity values over 28 h fermentation time at 35 °C and pH 6.0, in EH media produced with pretreated BP at 15% and 20% solids (w/w) and 0.25% acid concentration

be explained by the pKa (3.86) of LA. For fermentation to proceed properly, the pH of the medium has to be higher than the pKa of LA, as the dissociation of LA causes inhibition of cell growth. In one of the preliminary BP fermentation trials in the current study, when the pH reached around 4.0, LA production ceased, and the process stalled. When the pH was again raised to above 5.0, production resumed, agreeing with what was explained in Marques et al. [39] study. Radosavljević et al. [37] also obtained a yield of up to 93.03% of LA by fermenting brewer's spent grain and malt rootlets in batches, as well as supplementing with other by-products related to the brewing industry. Senedese et al. [40] evaluated the production of LA by *L. rhamnosus* ATCC 10,863 (same collection) by batch and fed batch, using sugar cane molasses. In batch fermentation, the maximum amount of LA produced occurred at 14 h and the process was finalised between 18 and 20 h, as LA was no longer being produced, and minimal amounts of sugars remained. The glucose yield was 92.4% and the fructose yield 98.5%. In the fed-batch, maximum production occurred at 46 h and the yield of glucose was 93.8% and fructose 98.3%. The higher productivity compared to previous studies could be related to the greater availability of sugars in the BP for fermentation.

Productivity at 20% was similar to 15%, which is a significant productivity value. By the end of the process, it was possible to obtain a maximum concentration of 106.4 g LA/L (18.76 g LA) with 20% BP and 82.81 g/L (14.59 g LA) with 15% BP. Therefore, 20% solid load was chosen due to the better production conditions and the greater amount of LA that can be achieved in the end of fermentation.

Conclusion

BP is a by-product of the banana processing industry with potential for valorisation. The use of agro-industrial by-products is a way of contributing to minimising the generation of wastes in this type of industry and so, comply with circular economy. Overall, in this work BP proved to be a suitable source of carbon for the production of lactic acid through a bioconversion process, comprising diluted-acid pretreatment, enzymatic hydrolysis of pretreated material and fermentation of released sugars. The diluted acid-pretreatment is proven to be satisfactory for banana peels, improving the hydrolysis yield considerably in comparison with untreated BP. The different pretreatment conditions tested generated materials with cellulose conversion yields above 60%, with most of the samples yielding between 70 and 90%. The hydrolysate obtained was adequate for lactic acid fermentation, with a low presence of degradation products, i.e. toxins. The comparison of fermentation parameters in tests carried out in MRS (+) and media from EH shows that *L.rhamnosus* has a good performance of in real media, with similar fermentation yield values and higher volumetric productivity in media from EH experiments, performed at 15 and 20% solid loads. Moreover, the possibility of carrying out successfully high-gravity EH experiments

allowed reaching elevated lactic acid concentration values of 106 gL^{-1} after 28 h fermentation. The main challenges were the homogenization of samples with higher solid loads and the fines that remain in the medium, even after some centrifugation and filtration steps, which were overcome to finally obtain both good hydrolysis and fermentation yields. Despite the satisfactory outcomes of the present work, further studies are required to evaluate the conversion process in terms of energy consumption, water and the use of enzymes, to finally determine its real potential to convert BP biomass into a high-value product such as lactic acid.

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Data Availability The authors confirm that the data supporting the findings of this study are available within the article or its supplementary materials.

Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

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