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Bioresource Technology Reports

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Bioconversion of potato-processing wastes into an industrially-important chemical lactic acid

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ARTICLE INFO

Keywords: Potato processing waste Lactic acid Lactobacillus pentosus Calcium lactate Stirred tank reactor

ABSTRACT

Lactic acid (LA) is an important biomolecule applied in food, pharmaceutical and chemical areas, mainly to produce biodegradable polymers, such as poly–lactic acid (PLA). In this work, an efficient fermentative process for LA production was developed using potato processing waste (PPW) hydrolysate with *Lactobacillus pentosus*. After optimization and kinetics studies, LA production reached 150 g/L with a productivity of 1.6 g/L.h in Erlenmeyer flasks. LA production was also conducted in STR where 110 g/L were reached with a productivity of 2.4 g/L.h. LA recovery consisted of a clarification step, with powdered activated carbon, with further precipitation at low temperature and acidification of calcium lactate for conversion to LA. The process was effective for contaminants' removal and clarification, and LA concentration to 416 g/L. Good perspectives for LA production, recovery and clarification were observed. Future studies will be carried out for LA purification and polymerization for PLA synthesis.

1. Introduction

LA is the popular name given to hydroxypropanoic acid ($C_3H_6O_3$). The molecule has an asymmetric carbon and it exists in the form of two optically active isomers: L(+)lactic acid or S-lactic acid and D(-)lactic acid or R-lactic acid. LA has a large variety of applications primarily in the food industry, but it is also applied in pharmaceutical and chemical industry, with a strong focus on the production of biodegradable polymers such as PLA (Ameen and Caruso, 2017; Gao et al., 2011). PLA has several industrial applications, varying from packaging-material to its uses in biomedical field. Its versatility is due to its characteristics and biocompatibility approved by the United States Food and Drug Administration (FDA) (Casalini et al., 2019).

LA production may be carried out by chemical-synthesis or through microbial-synthesis in fermentation process. When chemically synthesized, LA consists of a racemic mixture (50/50) of D- and L- forms, whereas LA obtained by fermentation is optically active (L or D), depending on the employment of the producer strain of bacteria, resulting in significantly different properties when polymerized

(Alexandri et al., 2019; Komesu et al., 2017; Singhvi et al., 2019). Pure isomers are more valuable for specific industrial applications than the racemic form (Abdel-Rahman et al., 2011). For this purpose, some microbial strains have been reported as LA producers including Lactic Acid Bacteria (LAB) that produce LA as a major metabolic product (Cubas-Cano et al., 2018; Reddy et al., 2008).

Glucose, sucrose and lactose as the main carbon sources are reported for LA production. However, the use of alternative low-cost renewable carbon sources, such as agricultural residues cassava bagasse and sugarcane bagasse (Pandey et al., 2001), and food-industry byproducts including molasses, whey and starch wastes etc. can reduce production costs of LA (Ahmad et al., 2020; Nampoothiri et al., 2010).

Potato (*Solanum tuberosum* L.) is a tuber of high nutritional relevance grown globally as part of staple diet. World potato production achieved 368 million tonnes in 2018. Following the main producers China (24.5%) and India (13.2%) Europe represents 12.7% of the production quantity with about 105 million tonnes (*Eurostat (European Commission*), 2020). In 2019, Brazilian production reached almost 4 million tonnes and about 40% of its consumption refers to industrially-

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processed potatoes, such as pre-fried and chips (Inácio et al., 2019). Potato is usually consumed fresh, but its industrial products such as starch and potato chips are of great importance (Carvalho et al., 2014; Gélinas and Barrette, 2007). During potato chips and other products manufacturing, peeled and sliced potatoes are washed with water to remove the starch from their surface, thus generating the potato processing waste PPW. Due to its high starch content, PPW may be used as a good and low-cost substrate for LA production by microbial fermentation (Ghaffar et al., 2014).

Downstream processing for recovery and purification of LA can also add the production costs, using different methods reported such as broth filtration, adsorption using active carbon or ionic resins, evaporation, hydrolysis, esterification, extraction, electrodialysis, distillation, ultrafiltration and chromatographic methods (Bayazit et al., 2011; Castillo Martinez et al., 2013; Oliveira et al., 2018; Singhvi et al., 2019).

The aim of current study involved the development of LA production in submerged fermentation using potato processing wastes by a selected bacterium *Lactobacillus pentosus*, and optimization of an effective downstream recovery process of LA from the fermented broth.

2. Material and methods

2.1. Microorganism

Lactobacillus pentosus NRRL B-227 was cultivated in Man-Rogosa-Sharpe (MRS) broth at 35 $^{\circ}\text{C}$ for 24 h, with periodic culture maintenance and storage at -20 $^{\circ}\text{C}$ with glycerol addition.

2.2. Substrate

Potato processing waste (PPW) was obtained from Wanflo Industry (Campo Largo, Brazil) with a composition of: 72,4% of total carbohydrates (66.8% of starch), 14.7% protein, 7.7% ash, 0.9% lipids. PPW was acid hydrolyzed using hydrochloric acid at 1.5% (w/w) concentration for 15 min at 121 °C, following a process of Woiciechowski et al. (2002) with a solid concentration of 25%. The pH of hydrolyzed potato processing waste (HPPW) was adjusted to 7.0 and HPPW was used as the substrate in fermentation medium for LA production.

2.3. Optimization of lactic acid production using HPPW as substrate

2.3.1. Inoculum preparation

The selected bacterium L. pentosus NRRL B-227 inoculum was cultivated in a HPPW medium with 20 g/L total reducing sugar (TRS) and yeast extract (YE) (25 g/L) to adapt the strain to PPW hydrolysate with 10% (ν/ν) of inoculum. Erlenmeyer flasks (250 mL), which were closed with cotton plugs, with 75 mL of HPPW medium were incubated at 30 °C and 120 rpm for 24 h under aerobic conditions.

2.3.2. Optimization of lactic acid production

The optimization of LA production was carried out in three steps with the use of experimental designs. In the first step, nitrogen source's influence on LA production was tested using organic and inorganic sources including YE (HiMedia) 10 g/L; YE (Biorigin YE-MF) 10 g/L; and YE (Biorigin-CMF) 10 g/L; peptone 10 g/L; urea 2.4 g/L; (NH₄)₂SO₄ 5.2 g/L; and (NH₄)₂HPO₄ 5.2 g/L. Each nitrogen source was individually added in separate triplicate sets of flasks with medium composed of HPPW with 50 g/L of TRS from HPPW and 40 g/L of CaCO₃.

In the second step, a Plackett & Burman (PB) experimental design was employed to test the influence of different components on biosythesis of LA using K_2HPO_4 2 g/L; $C_2H_3O_2Na$ 5 g/L; Na_2SO_4 2 g/L; $FeSO_4$ 0.05 g/L; $MnSO_4$ 0.05 g/L; Tween 80 1 g/L; and Tween CaCO₃ 40 g/L. The components were added to fermentative medium composed of HPPW with 50 g/L of TRS and 10 g/L YE (data not shown).

In the third and final step of optimization study included experiments on the effect of different concentrations (g/L) of TRS from HPPW

(50, 70, 100, 130, 150), YE (1.6, 5, 10, 15, 18) and CaCO₃ (46, 60, 80, 100, 114) on LA production using central composite rotatable design (CCDR) totalizing 17 assays (Table 1).

Fermentation essays were carried out in 250 mL Erlenmeyer flasks, which were closed with cotton plugs, with 75 mL medium, 10% (ν / ν) of inoculum, incubated at 30 °C, 120 rpm for 72 h. LA concentration was analyzed by HPLC. Results were analyzed using Software Statistica 5.0 (StatSoft, Tulsa, USA).

2.3.3. Kinetics of LA production using HPPW in flasks and Stirred Tank Reactor (STR)

LA production using HPPW was carried out on small scale in Erlenmeyer flasks and also in larger volumes using a 7-L capacity STR (Laboratory bioreactor model MDL, B.E. Marubishi, Thailand). In both systems, fermentative medium was composed by 10 g/L of YE, 80 g/L of CaCO3, and 160 g/L TRS and 135 g/L TRS from HPPW for Erlenmeyer flasks and STR essays, respectively. The inoculum rate was 10% (ν / ν) prepared in HPPW medium with 20 g/L TRS and 25 g/L of YE. Process of fermentation was carried in Erlenmeyer flasks at 30 °C and 120 rpm for 96 h, whereas in a 7-L STR, 4 L working volume fermentation was carried out at 30 °C and 150 rpm for 96 h. All cultivations were done with at least three repetitions. Samples were withdrawn periodically for LA and TRS analysis.

2.4. Recovery and purification of lactic acid

Recovery and purification of LA from fermented broth was optimized according to specific steps, these have been presented in Fig. 1.

Fermented broth was heated to 50 °C for dissolution of calcium lactate and then centrifuged at $1800 \times g$ for 20 min (Centribio, model 80-2B) to remove suspended bacterial cells and solids. The supernatant was then submitted to clarification that was conducted with PAC (C118-CB), obtained from Carbomafra S/A (Brazil), under different conditions. A full factorial experimental design 2(4–0) was employed to study the influence of temperature (30, 40 and 50 °C), agitation (0, 50, 100 rpm), time of contact (5, 15, 25 min) and PAC concentration (5, 10, 15% (w/v)). Essays were conducted in 125 mL Erlenmeyer flasks with a volume of 50 mL. Incubation occurred in water-bath with agitation (Ethiktechnology, model 501-D). PAC was removed by centrifugation at 1800 ×g for 20 min in a temperature range between 30 and 50 °C.

After PAC removal, the clarified broth was precipitated to obtain solid calcium lactate. The precipitation was carried out in glass beaker at 4 $^{\circ}$ C under static conditions for 24 h. The solid salt was recovered by filtration and the filtrate containing part of the calcium lactate was then returned for recovery process. Results were analyzed using Software Statistica 5.0 (StatSoft, Tulsa, USA). Recovered calcium lactate was

Table 1CCDR experimental design for the optimization of LA production using HPPW.

Run	HPPW with g/L TRS	YE g/L	CaCO ₃ g/L	LA g/L	Productivity g/L.h
1	70	5	60	69.7	0.968
2	70	5	100	67.6	0.939
3	70	15	60	63.4	0.880
4	70	15	100	59.7	0.829
5	130	5	60	96.4	1.339
6	130	5	100	110.7	1.537
7	130	15	60	116.1	1.613
8	130	15	100	122.9	1.707
9	50	10	80	46.1	0.640
10	150	10	80	135.0	1.875
11	100	2	80	73.6	1.022
12	100	18	80	94.2	1.308
13	100	10	46	85.8	1.191
14	100	10	114	94.4	1.311
15 (C)	100	10	80	97.8	1.358
16 (C)	100	10	80	110.4	1.533
17 (C)	100	10	80	106.5	1.479

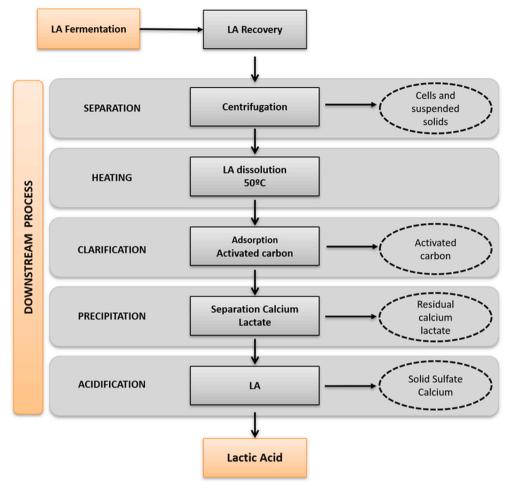


Fig. 1. Steps of LA production and recovery.

converted to LA using sulfuric acid at different concentrations (1, 2, 3, 4 and 5 mol/L). The acidification was performed in glass beaker under agitation. The yield of LA conversion was determined.

2.5. Analytical methods

Fermented broth was acidified with sulfuric acid to convert calcium lactate to LA and centrifuged at 1800 $\times g$ (times gravity) for 20 min

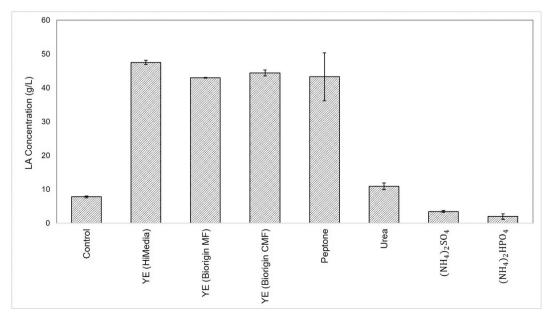


Fig. 2. LA production under different nitrogen sources.

(Centribio, model 80-2B). The supernatant was filtered through a 0.22 μm cellulose acetate membrane. LA and TRS content were analyzed by HPLC (Shimadzu LC 10AD, detector RID 10A), using an Aminex HPX-87H column, mobile phase of 5 mM H₂SO₄, 0.6 mL/min, at 60 °C. Standards for chromatography analyses were L-lactic acid 99% (Sigma Aldrich), D-glucose (Sigma Aldrich) and D-maltose (Sigma Aldrich).

Isomers D and L concentration was determined by D-Lactate Colorimetric Assay Kit (Sigma-Aldrich, USA). In this assay, D-Lactate is specifically oxidized by D-Lactate hydrogenase and generates a proportional colorimetric product measured at 450 nm.

3. Results and discussion

3.1. Production of lactic acid using HPPW

3.1.1. Optimization of lactic acid production

changes and may be inhibited due to pH reduction during high concentrations of LA production (Es et al., 2018; Singh et al., 2006). Also, pH values have significant interferences concerning the downstream process, in which, LA is generally found in its dissociated form when pH value is above its pKa value. For a better purification, LA needs to be found in its free form (López-Gómez et al., 2019; Wang et al., 2015). A higher LA production was obtained with TRS and YE concentrations higher than 140 g/L and 10 g/L, respectively. The model adjustment of experimental design is 0.98 (p < 0.05). Increasing LA

influence LA production at the studied range, neither the interaction

between other variables. Even so, the presence of CaCO3 in the culture

medium is important due to medium neutralization during LA fermen-

tation, thus forming calcium lactate. In fact, LAB is sensitive to pH

concentrations could be reached with higher TRS concentrations and central levels of YE (from 10 to 18 g/L). The mathematical model is described by Eq. (1), where z: LA concentration; x: TRS concentration; y:

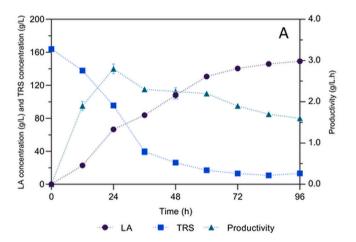
$$z = -49.996 + 0.813x - 0.004x^2 + 3.117y - 0.264y^2 + 0.0384xy + 0.0056wx - 0.0114wy + 1.156w - 0.0093w^2$$
 (1)

The influence of different nitrogen sources on LA production by L. pentosus NRRL B-227 is shown in Fig. 2. YEs from different origins (HiMedia, Biorigin MF, and Biorigin CMF) and peptone led to a similar yield of LA, 47 g/L, 42 g/L, 44 g/L and 43 g/L, respectively. The YE-CMF Biorigin, which is composed of inactivated yeast, was chosen for the development of LA production process, due to its lower cost (~2-4 US \$/kg) compared to commercial nitrogen sources such as commercial yeast extract (\sim 100–200 US\$/kg) and peptone (\sim 140–160 US\$/kg) sources. Inorganic nitrogen sources and urea did not promote LA production due to the lack of nutrients such as amino acids, proteins and vitamins. Nancib et al. (2005) have studied LA production using date juice as substrate and different nitrogen sources (YE, ammonium sulfate, tryptic soy, urea, peptone and casein hydrolysate). YE showed the greatest enhancing effect on LA production; however, authors concluded that part of the YE could be replaced by ammonium sulfate and B vitamin. Wang et al. (2020) also noticed the nitrogen importance and influence, not only directly to cell growth, but when they increased nitrogen concentrations (5 g/L to 15 g/L), was observed a gradually LA yields augmentation in L. pentosus ATCC8041 fermentation.

The influence of YE, K₂HPO₄, C₂H₃O₂Na, Na₂SO₄, FeSO₄, MnSO₄, Tween 80 and CaCO₃ on LA production was studied with the support of a Plackett & Burman experimental design (data not shown). The highest LA production was achieved (45.4 g/L) with YE, C₂H₃O₂Na, polysorbate 80 and CaCO3. Lower LA yields (4.4 g/L) were obtained without a nitrogen source (control). It means that a certain C/N is needed for bacterial growth and LA accumulation. YE and CaCO3 were significant for LA production at the studied levels (p < 0.05) and R²=0.734. Therefore, these factors were selected to HPPW medium in the next steps of optimization.

The third step of LA production optimization employed a CCDR experimental design, totalizing 17 essays (Table 1). The highest LA production (135 g/L) and productivity (1.875 g/L.h) were reached using a medium composed of HPPW with 150 g/L TRS, 10 g/L of YE and 80 g/ L of CaCO₃ (run 10), corresponding to the higher level for sugar concentration and central levels for YE and CaCO3. The lowest LA production and productivity were 46.4 g/L and 0.644 g/L.h, respectively, using HPPW with 50 g/L TRS, 10 g/L of YE and 80 g/L of CaCO₃ (run 9).

It was possible to observe that higher C/N ratios favored LA production according to Pareto chart (data not shown), which showed the significant interaction between the two variables (p-value<0.05). TRS and YE concentration influenced LA production positively as well as the interaction between the two variables. CaCO3 concentration did not YE concentration; w: CaCO₃ concentration.



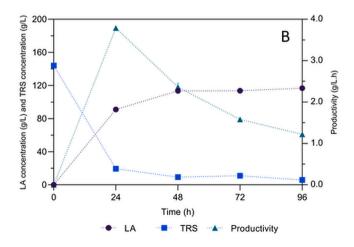


Fig. 3. A) Kinetics of LA production using HPPW in: A) Erlenmeyer flasks; and B) in STR.

The results were validated experimentally. According to the Eq. (1), LA production achieved 140.8 g/L using HPPW with 164 g/L TRS, 10 g/L of YE and 80 g/L of CaCO $_3$. At the same conditions, the experimental LA production led to a production of 140.1 g/L.

3.1.2. Kinetics of lactic acid production using HPPW

LA production was studied in Erlenmeyer flasks during 96 h (Fig. 3A) with previously optimized conditions. LA is a primary product of cells' metabolism, which is associated with cell growth. Thus, LA was produced in the first 12 h with a high production rate up to 60 h reaching the highest concentration at 96 h (150 g/L) with a productivity of 1.6 g/L.h.

In STR (Fig. 3B), LA production reached its maximum concentration (110 g/L) at 48 h, with a productivity of 2.4 g/L.h and a yield of 83.0%. After this time, LA and TRS concentrations remained constant. So, the higher LA production was observed in Erlenmeyer flasks, while the highest productivity was reached in STR (Table 2). The performance in different reactor systems may vary due to the differences in the reactor configuration and size. According to Crater and Lievense (2018), the scale-up has an impact on fermentation parameters including agitation, aeration and other factors such as sterilization and media nutrients degradation. In addition, the presence of impellers in the STR improves the mixing of the culture medium and mass transfer processes, however, it may increase cell stress affecting some biochemical processes.

The sterilization step is also considered a critical point because it may promote some changes in the composition of the culture medium due to nutrient degradation (Ahmad et al., 2020; Crater and Lievense, 2018), which can be observed, for example, in differences of initial sugar concentration. The heat sterilization, which is carried out in STRs, requires a longer time of contact with higher degradation of nutrients that can affect the performance of fermentation. Even so, the study of LA production in a STR has shown good perspectives for future process scale-up.

It is important to mention that no work was reported before about the re-use of PPW in LA production. In addition, high yields were reached with good perspectives for production at large scale with low costs. Different reports show that LA is majorly produced by different strains of LAB, where high yields can be achieved using simple but expensive carbon sources, such as glucose. Considerable LA yields are reported using glucose as carbon source: 0.948 by L. rhamnosus (Radosavljević et al., 2020) and 0.938 by L. pentosus (Wang et al., 2020). Higher LA productions can also be reached using complex substrates, with or without pretreatment processes. Karp et al. (2011) applied the biorefinery concept utilizing L. agilis bacteria and soybean vinasse as substrate with a final high LA production of 138 g/L. Meng et al. (2012) achieved high concentrations of LA (225 g/L) using an inexpensive substrate (peanut meal) through a Bacillus sp. Thakur et al. (2018) utilized a cell immobilization technique for LA production by L. casei reaching 130 g/L with diluted sugarcane molasses. Paulova et al., 2020 employed degreased chicken feather as substrate after treatment with NaOH solution, achieving 116.5 g/L of LA by L. casei. Dumbrepatil et al. (2008) reached a maximum L-LA (166 g/L) production from 190 g/L of molasses sugar with a productivity of 4.1 g/L.h.

Table 2Summary of the optimization steps of LA production using HPPW as substrate.

Optimization step	LA concentration (g/L)	LA productivity (g/L.h)	Yield
First step optimization	44.0	0.6	88.0%
Second step optimization	135.0	1.9	90.0%
Kinetics in Erlenmeyer flasks	150.0	1.6	93.7%
Kinetics in STR	110.0	2.4	83.0%

3.2. LA recovery and clarification

The first step of LA recovery consisted of bacterial cells and suspended solids separation, including CaCO3 excess, by centrifugation. The yield of this step was 94.5%. After that, the clarification step using PAC was performed to remove color and other components though adsorption. Experiments were conducted under controlled temperature (30 $^{\circ}$ C, 40 $^{\circ}$ C and 50 $^{\circ}$ C), time of contact (5, 15 and 25 min) and PAC concentrations of 5, 10 and 15% with the support of a full factorial experimental design 2(4-0) (data not shown). It was observed a significant difference of recovery yields according to adsorption conditions applied. The clarification yield was calculated considering the initial and final volumes of recovered calcium lactate solution. Lower yields were obtained when using higher amounts of PAC. In the Pareto chart (data not shown) it was possible to see that PAC concentration presented significant influence on calcium lactate adsorption process yield (p < 0.05) with a negative effect. Temperature and agitation did not influence the adsorption process yield in the studied range. Clear differences could be observed related to the color of the calcium lactate solution. Best clarification conditions were defined as 30 °C, 100 rpm, 25 min and 15% of PAC led to a yield of 60% was reached ($R^2=0.94$).

After clarification, precipitation was carried out at low temperature under static conditions providing the recovery of 75% of calcium lactate from the clarified solution.

Then, the conversion of calcium lactate to LA was performed using $\rm H_2SO_4$ at different concentrations (1, 2, 3, 4 and 5 mol/L) and the yield of conversion was analyzed. When the concentration of $\rm H_2SO_4$ was increased, the yield of acidification decreased because the amount of water in reaction decreased, which significantly affects precipitation reaction. The highest LA concentration (495 g/L) was obtained with a yield of 84%, with 5 mol/L $\rm H_2SO_4$. A yield of 100% was obtained using 2 mol/L $\rm H_2SO_4$, but LA concentration was 238.9 g/L due to amount of water present in solution of $\rm H_2SO_4$. With 4 mol/L $\rm H_2SO_4$ a yield of 88% was achieved that was close to that obtained by Min et al. (2011), who achieved 92% of yield using a 1:1 ratio of $\rm Ca(LA)_2/H_2SO_4$ and a pH higher than the pKa value.

LA quality and yield recuperation process is usually very important, where separation and recovery processes are the bottlenecks to turn the LA production economically viable. These steps can represent up to 50% of total costs of LA production (Alexandri et al., 2019; Oliveira et al., 2018). Table 3 presents the partial yields of each step of the LA separation and recovery processes; the final yield of the process was 51%. In this process, clarification and precipitation steps contributed to the low yield. Alternatives can be proposed to increase downstream performance such as the recycling of the supernatant after precipitation process due to presence of 25% of calcium lactate and the recovery of calcium lactate adsorbed in PAC.

In fact, the major challenge is to find an effective downstream process to obtain a product with high chemical purity. For this purpose, various methods have been reported. Kumar et al. (2020) optimized parameters and proposed a downstream process where they found some difficulties in the LA chemical-extraction from a medium fermented by L. *pentosus* due to pH variations. This fact, corroborates with the previously mentioned LA ionic form. As a recovery method, solvents were used (butanol and ammonium sulphate), where 86% yield was achieved with a LA purity of 93%. Beitel et al. (2016) obtained 113 g/L of D

Table 3Yield of different steps of LA separation and recovery.

Process	Condition	Yield
Centrifugation	1800 ×g for 20 min	95%
Clarification	50 °C, 100 rpm, 25 min and 15% of PAC (w/v)	64%
Precipitation	Low temperature and static mode	75%
Acidification	H ₂ SO ₄ 4 mol/L	88%
Total yield of process		51%

(-)-lactic acid by Sporolactobacillus nakayamae, using peanut flour as a nitrogen source and commercial sucrose as a carbon source. Two purification methods were tested; filtration with activated carbon followed by Celite and Amberlite IRA 120 cation resin exchange. A final LA recovery yield of 79% was reached, and 98% of remaining sugars were removed. Coelho et al. (2018) also followed the combination of activate carbon and Celite during filtration and purification using cationic resins. Bacillus coagulans reached 207 g/L of L-(+)-lactic acid using granulated sugar, with pH control. Initially, fermented broth was acidified to pH 5.0 and then it was filtered twice with Celite and powdered activated carbon. The filtered solution was passed through Amberlite IRA 120 cationexchange column, with final pH 3. LA recovery was 86% with a colorless solution. López-Gómez et al. (2020) studied the organic fraction of municipal solid wastes as substrate for LA production by Bacillus coagulans, and used micro and nanofiltration techniques for its purification. Final production of 60 g/L L(-)lactic acid was reached. After purification steps, final solution contained 93 g/L of LA concentration, with 45% of LA overall recovery yield. Authors observed about 60% LA losses during downstream process, 14.3% were lost during microfiltration and 15.5% were during nanofiltration step.

4. Conclusions

The hydrolysate of potato processing waste (HPPW) after acid pretreatment was efficiently employed in lactic acid (LA) production through submerged fermentation by the bacterium *Lactobacillus pentosus*. High productivities and yields were reached both in Erlenmeyer flasks and 7-L STR. LA passed through separation and recovery processes including different steps of centrifugation, adsorption through activated charcoal (PAC), precipitation, filtration and acidification. Relatively low total LA recovery yield of 51% was observed, showing the need of finding alternative solutions to minimize downstream process losses.

CRediT authorship contribution statement

The corresponding author is responsible for ensuring that the descriptions are accurate and agreed by all authors.

The role(s) of all authors are be listed below.

Juliana de Oliveira: She was responsible for production, recovery and formulation of GA_3 . She was completely involved on paper's preparation.

Luciana P. S. Vandenberghe: She is the corresponding author as she supervised the work of Juliana de Oliveira, Priscilla de Oliveira and Ariane Fátima Murawski de Mello. She was also responsible for paper revision

Priscilla Zwiercheczewski de Oliveira: She was also responsible for LA production and recovery. She was completely involved on paper's preparation.

Ariane Fátima Murawski de Mello: She worked in LA production and recovery.

Cristine Rodrigues: She was responsible for the analytical part of the study.

Poonam Singh Nigam: She was also responsible for paper revision and English editing.

Vincenza Faraco: She is the coordinator of European project.

Carlos R. Soccol^a: He is the Head of the Bioprocess Engineering and Biotechnology Laboratory where all experiments were carried out. He has initiated this line of research of lactic acid production and worked in the revision of the final version of the paper.

Declaration of competing interest

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.

Acknowledgements

Authors want to tank CAPES and CNPq for the financial support. This research was also supported by a Marie Curie International Research Staff Exchange Scheme Fellowship within the 7th European Community Framework Programme: "Improvement of technologies and tools, e.g. biosystems and biocatalysts, for waste conversion to develop an assortment of high added value eco-friendly and cost-effective bioproducts" BIOASSORT (contract number 318931).

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