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OPTIMIZATION NUTRITIONAL MEDIUM FOR LACTIC ACID PRODUCTION FROM ORGANIC WASTE THROUGH SSF PROCESS BY DIFFERENT STRAINS OF LACTOBACILLUS SP.

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ABSTRACT

Lactic acid is a commodity product with wide industrial application, which can be generated from different raw materials, making it a target of interest for large companies. On the other hand, most organic acids on the market produced via chemical synthesis generates high levels of pollution. That way, the second-generation organic acids is part of this context, moving towards emerging and future-oriented technologies. The aim of this work was to investigate the lactic acid production from organic waste through *Simultaneous Saccharification and Fermentation* Process by singly *L.helveticus; L.coryniformis; L. delbrueckii lactis* and *L. pentosus.* The fermentability tests on non-hydrolyzed media generated excellent production, yield and productivity results for both strains. *L. coryniformis* produced 25.26g/L of lactic acid, with a productivity of 1.05 g/L.h and a yield of 51%, while *L. pentosus* produced 32.62g/L of lactic acid, with a productivity of 1.36 g/L.h and a yield of 60%.

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INTRODUCTION

Organic waste is a low-cost substrate that could contain cellulosic, starchy and saccharine residues, which can be converted into fermentable sugars, able to obtain various products of commercial interest, like biohydrogen, biopolymers and energy (Villanueva-Galindo *et al*., 2024; Sohani *et al*., 2024). The Brazilian Association of Technical Standards - ABNT, defines waste as "the remains of human activities, considered by the generators to be useless, undesirable or disposable, and may be in solid, semi-solid or liquid form, provided that it is not amenable to conventional treatment" (ABNT - CLASSIFICATION OF SOLID WASTE, 2004). The growing expansion of urban areas has led people to have customs and habits that generate an ever-increasing amount of waste. Currently, almost 87% of the Brazilian population lives in urban areas (IBGE, 2024). In this sense, with the growing productive demand for waste, studies that aiming the biotechnological use of these residues become extremely relevant. In this context, this material contains a significant amount of sugars that can be used as a substrate in microbial fermentation processes, although physical and/or chemical pretreatment is necessary for some specific wastes. A enzymatic hydrolysis on the pretreated material, should be necessary to study processes that allow the material to be fermented under mild operating conditions, as occurs in the the Simultaneous Saccharification and Fermentation (SSF) and Simultaneous Saccharification and CoFermentation (SSCF) strategy (Qiu *et al*., 2023; He *et al*, 2023; Salem *et al*., 2018; Taherzadeh; Karimi, 2007). Biorefinery refers to the chemical and/or biological conversion of materials into a range of valuable products and energy that use minimal waste generation and emissions (Pereira; Couto; Anna, 2008). The biotechnological route offers the possibility of obtaining optically pure D-lactic acid or L-lactic acid or a mixture in different proportions of the two isomers, depending on the species microrganism and the cultivation conditions (Abedi & Hashemi, 2020; Sonomoto, 2013). It is also possible to use low-cost or residual raw materials in the fermentation process. In this context, the biotechnological is a viable and beneficial alternative for lactic acid production. Advances in this area indicate that the use of renewable raw materials, including their residues, will reverse the world's dependence on fossil fuels (López-Gómez, 2019). In addition, Lactic acid is a high value-added and versatile product due to its numerous applications in the food, pharmaceutical, chemical and textile industries. The global consumer organic acids market is estimated to increase to $36.86 billion by 2026 (Liu, 2023; Ning *et al*, 2022; Singh, 2022; John *et al*., 2009). Thus, the production and consumption of LA has expanded considerably due to its applicability in the polymer industry, as a monomer to produce polylactic acid (PLA), which is then used in the manufacture of biodegradable plastics and textile fibers (Lmillmén *et al*., 2007). On this point, for more competitive and profitable bioproducts, the industrial has been compelled to incorporate technological innovations, developing new

production systems and equipment for processes that protect the environment and generate fewer pollutants. (Marzo-Gago *et al*, 2022; Abedi & Hashemi, 2020; Santos, 2012). Based on the information provided, the production of 2nd generation organic acids corroborates current studies on the subject of Biorefinery, towards sustainable and green technologies.

OBJECTIVE

The aim of this work was to investigate the lactic acid production from organic waste through *Simultaneous Saccharification and Fermentation* Process by different strains of *Lactobacillus sp.* from different experimental design strategies.

MATERIAL AND METHODS

Cell Activation, Propagation and maintenance : Different strains of *Lactobacillus sp.: Lactobacillus helveticus; Lactobacillus coryniformis; Lactobacillus delbrueckii lactis* and *Lactobacillus pentosus* used in this work were obtained from the Bioprocess Development Laboratory at Department of Biochemical Engineering, School of Chemistry/ Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, which has an imported collection of lactic acid-producing microorganisms. These strains were obtained from the *American Type Culture Collection (ATCC*), kept in freeze-dried form and stored in sealed vials away from light. The microorganisms were preserved in freeze-dried form. They were activated using BHI (BD Brain Heart Infusion) culture medium (Tab. 1). To carry out the activation, 1.3 ml of each of all strains of *Lactobacillus sp*. were added to Falcon tubes with 13 ml of BHI medium each and incubated at 37ºC for 24 hours. For proper maintenance, the cells were preserved at 4ºC for later use. The cultures cells were incubated in an oven at 37ºC in MRS (Man-Rogosa-Sharpe) culture medium (Tab. 2). The culture media was autoclaved under a pressure of 0.5 kgf/cm^2 and a temperature of 120ºC for 20 minutes. It should be noted that all the glassware used in the experiments was also autoclaved under a pressure of 1 kgf/cm² and a temperature of 120ºC for 15 minutes. The preinoculum was carried out in 15 mL Falcon tubes with a 13 mL volume of BHI activation medium with the addition of 1.3 mL of cells, incubated at 37ºC. 50 mL Falcon tubes were used to prepare the inoculum. The working volume was 45 mL of MRS growth medium, with 10% (v/v) of the total volume of each Falcon tube added to the preinoculum. The inoculum was incubated at 37^o C for 72 hours without stirring.

Table 1. Components of BHI culture medium

Nutrients	Concentration (g/L)
Brain-heart infusion	8
Peptic digest of animal tissue	5
Pancreatic digest of casein	16
Sodium chloride	5
Glucose	2
Disodium hydrogen phosphate	2.5
Agar	13.5

Table 2. Components of MRS culture medium

Nutrients	Concentration (g/L)
Proteose Peptone No. 3	10
Beef Extract	10
Dextrose	20
Yeast Extract	5
Polysorbate 80	1
Ammonium Citrate	2
Sodium Acetate	5
Magnesium Sulfate	0.1
Manganese Sulfate	0.05
Potassium Phosphate	2
Agar	15

Raw material pretreatment: The raw material used in this work is food waste from schools, homes and restaurants, known as HOD (Household Organic Waste), based on food waste from fruits, vegetables, legumes, leguminous plants, meat waste and similar. For example: lettuce, tomatoes, bread, rice, beans, banana peel, mango peel, acerola, carrot, boiled meat, lemon peel, egg shells, potato, coffee, cheese and other. The organic waste was crushed and then beaten so that the food had the greatest possible contact area for the enzymes to use and subjected to two different strategies to obtain the culture medium in which the microorganisms were inoculated, and the fermentation started.

Pretreatment 1: The waste was subjected to enzymatic hydrolysis. The raw material has a mixed composition substrates polymers, which can be difficult for microorganisms access, making it necessary to evaluate the use of enzymes in the fermentation process. This hydrolysis stage allows the materials to be convert into fermentable sugars to make the carbohydrates available to the microorganisms. Thus, one of the strategies was to subject the HOD to enzymatic hydrolysis using the a-amylase, cellulase and glucoamylase enzymes (Ma *et al*., 2008).

Pretreatment 2: The waste was not subjected to enzymatic hydrolysis. The food waste was processed, distributed into 50 mL Falcon tubes and then resuspended in 25 ml distilled water to dissolve the nutrients. The solid and liquid components were separated by filtration. Subsequently, the liquid fraction with fatty components was eliminated by centrifugation (9000 RPM, 4ºC for 15 min), to obtain a clear culture broth. After obtaining the broth, the pH was adjusted to 7 and the medium sterilized by 120°C, 1 ATM for 10 min (Chalón *et al*., 2013).

Fermentation assays: In all the trials, the cells were initially activated in BHI liquid medium and then propagated in MRS liquid medium. Initially, preliminary experiments were carried out, evaluating the performance of the *Lactobacillus spp.* strains using synthetic MRS culture medium, with the nutrient concentrations defined by the experimental design. Subsequently, the performance of the strains in culture medium obtained from food waste was evaluated. The fermentation tests were carried out in 50 mL Falcon tubes containing 45 mL of the corresponding medium and add 5 mL of cells. The inoculum was incubated under agitation at 120 rpm at 37ºC without pH control. Cell growth, substrate consumption and product formation were monitored for as long as necessary (1 mL aliquots were taken 24h and 48h), to check for stability in cell concentration and exhaustion of the carbon source. The samples taken during the fermentation process were centrifuged at 10000 rpm for 10 minutes, with the supernatant being used to measure sugars and products and the sediment being used to quantify the cells.

Experimental design: *Plackett &* Burman (PB) design was carried out to determine the components of the medium with the greatest significance to produce D-lactic acid. PB is an orthogonal design used when the number of independent variables to be used is high, reducing the number of experiments (Yang *et al*. 2025; Fazil *et al*., 2024; Plackett & Burman, 1946). It is worth noting that the components evaluated are similar to those found in the MRS medium, widely used in lactic fermentation. The PB design evaluate the following independent variables: glucose, xylose, yeast extract, meat extract, peptone, sodium acetate, ammonium citrate, manganese sulphate, magnesium sulphate, potassium phosphate and tween addition to the complementary medium for lactic acid production. The SSF optimization was also evaluated using *Desirability* function, according Rodrigues *et al* (2006).

ANALYTICAL METHODS

The cell mass concentration was monitored by spectrophotometry at 600 nm (Pereira; Couto; Anna, 2008) with distilled water as the calibration reference. Previously, a standard curve correlating the weight of the dry mass of the cells with the absorbance was

constructed using the biomass obtained from cultivation in 50 mL Falcon tubes containing 45 mL of MRS medium at 37ºC. After 12 hours of incubation, different dilutions were made and the absorbance at 600nm determined for each biomass concentration. The dry mass was determined after centrifuging the fermented medium at 9000 rpm for 10 minutes, followed by washing the cells with distilled water, centrifuging again and then drying in an oven until constant weight. The organic acids produced concentrations were determined by stationary phase high performance liquid chromatography (HPLC-HPLC), using an ultraviolet detector (UV/VIS) - 254nm and a CuSO mobile phase$_{4,}$ 0.001 mol/L.

Statistical Analysis: The estimated effects of the variables and process conditions on the final lactic acid concentration (response variable) will be analyzed at a 90% confidence interval, using Analysis of Variance (ANOVA) and Response Surface Methodology, using *Design Expert Software* (version 7.1.6. Stat-Ease, Inc., Minneapolis, USA).

RESULTS AND DISCUSSION

PB: screening of additional complementary components to organic waste: The performance of *Lactobacillus sp.*strains were evaluated, considering cell growth, kinetics and lactic acid production during fermentation, in order to select the most suitable strains for lactic acid production for subsequent trials with organic waste. The search for microorganisms that ferment not only glucose, but also xylose, is one of the greatest challenges of modern biotechnology, since it is necessary to efficiently and fully convert the carbohydrates of various origins that make up organic waste. In order to use the strains that showed the best performance in the stages with organic waste, MRS medium tests were carried out with different nutrient conditions to verify the consumption of hexoses and pentoses and optimize the process. These tests evaluated the production of organic acids and the consumption of substrates by *Lactobacillus pentosus*. The PB design responses are shown in Table 3.

Table 3. Trials representing the PB screening of different compounds (g/L). Where: A: glucose; B: xylose;C: yeast extract;D: meat extract; E: peptone; F: sodium acetate;G: ammonium citrate;H: manganese sulphate;I: magnesium sulphate; J: potassium phosphate; K: tween, added to the SSF process for Lactic Acid production (Response, g/L) by *Lactobacillus pentosus*.

Trials	Factor A	Factor B	Factor C	Factor D	Factor E	Factor F	Factor G	Factor H	Factor I	Factor J	Factor K	Response
1	20	0	0	0	10	0	2	0.05	0	2	1	6.4
2	0	20	0	8	10	0	2	0.05	0.2	0	0	5.6
3	0	0	0	8	0	5	2	0	0.2	2	1	0
4	10	10	2	4	5	2.5	1	0.025	0.1	1	0.5	14.8
5	10	10	2	4	5	2.5	1	0.025	0.1	1	0.5	13.7
6	20	20	4	0	0	0	2	0	0.2	2	0	13.2
7	20	20	0	0	0	5	0	0.05	0.2	0	1	5
8	0	0	4	0	10	5	0	0.05	0.2	2	0	0.2
9	20	20	0	8	10	5	0	0	0	2	0	16.32
10	20	0	4	8	10	0	0	0	0.2	0	1	6.8
11	0	20	4	8	0	0	0	0.05	0	2	1	8.1
12	20	0	4	8	0	5	2	0.05	0	0	0	6.5
13	0	20	4	0	10	5	2	0	0	0	1	7.3
14	10	10	2	4	5	2.5	1	0.025	0.1	1	0.5	13.5
15	0	0	0	0	0	0	0	0	0	0	0	0

In this way, trial 9, in which the addition of 20 g/L of both sugars evaluated, parameters at their maximum levels, associated with the maximum concentrations of meat extract and yeast extract, sources rich in nitrogen, as well as sodium acetate and potassium phosphate at their maximum levels, the synthesis of lactic acid occurred at around 16 g/L, the highest achieved using the chemically defined medium. It should be noted that potassium phosphate has a buffering effect, as highlighted in the literature, and is advantageous for fermentation processes that have a reduced pH range associated with the production of organic acids (Guilherme *et al*., 2006). It should be noted that the curvature of the model showed statistical significance, which points to further tests to be evaluated in the design of the rotational central composite design. The table shows priority importance of carbon sources, xylose and glucose, followed by magnesium sulfate, potassium phosphate, manganese sulfate, meat extract, and in turn peptone, yeast extract, tween, sodium acetate, ammonium sulfate. Interactions between the above variables were not evaluated in this design, the aim of which is to *screen* the components of the medium. In addition to the economic interest in reducing the costs of an industrial process, an excess of nutrients can result in an exacerbated increase in cell synthesis and a reduction in organic acid production.

The lactic acid production model is represented by the following equation. Where: A: glucose; B: xylose; C: yeast extract; D: meat extract; E: peptone; F: sodium acetate; G: ammonium citrate; H: manganese sulphate; I: magnesium sulphate; J: potassium phosphate; K: tween, added to the SSF process for Lactic Acid production (LA, g/L) by *Lactobacillus pentosus*.

[LA]= -1,08333 +0,275167[A] +0,296833[B] +0,365833 [C]+0,233750 [D]+0,163667 [E] +0,215000 [G] -39,40000 [H] -11,51667 [I] +1,08500 [J]

From a technical and economic point of view, as the production of lactic acid rises in tandem with a reduction in substrate concentrations, as well as components of the culture medium and initial cells, the fermentation process becomes more attractive and viable in terms of scale extrapolation. In turn, the *Desirability* function was used in these configurations, with sugars being kept at their maximum levels; sodium acetate, manganese sulphate, magnesium sulphate, potassium phosphate, tween at central levels; peptone, meat extract at minimum levels; yeast extract and lactic acid production being maximized. The criteria applied and the results predicted for optimization are shown in Table 5, with a *Desirability* coefficient of 0.931, covering around 93% of the joint needs for lactic acid production with a reduction in the costs associated with the fermentation medium. In this context, the analysis of the results corroborates the reduction in the composition of the cultivation medium due to the proximity of the values achieved in the tests which only included the yeast extract and resulted in 13.2 g/L.

It is important to emphasize the importance of economic evaluation regarding the development of future trials aimed at extrapolating the scale of the bioprocess developed in this work. The contour surfaces generated by the Desirability function indicate that the highest acid production occurred at the highest concentrations of the carbohydrates evaluated, glucose and xylose (Figure 1). The reddish color points to the optimization of the process as these compounds increase. However, previous tests indicate that the strains under study are strongly inhibited by the substrate at concentrations above 75 g/L.

Comparative analysis of different pretreatment strategies: All the strains were inoculated into HOD medium, separately. The best results shows that after 24 hours of fermentation, without hydrolysis (Table

6) was obtained 25.26g/L and 32.62g/L, respectively, using *L. coryniformis* and *L. pentosus*. These results were obtained through analysis by high-performance liquid chromatography, the chromatogram of which is shown below in Figures 3 and 4. Fermentation in HOD medium with the addition of enzymes for hydrolysis obtained lower values, 7.11g/L for *L. coryniformis* (Fig. 3) and 4.82g/L for *L. pentosus* (Fig. 4).

The discrepancy in values was due to production being inhibited by the high amount of substrate available, according He *et al*. (2023) and Qiu *et al*. (2023). Although the enzymes and cells were added simultaneously -with the intention of reducing the possibility of this inhibition- it was found that hydrolysis occurred quickly, in around 1 hour; while fermentation took place over 24 hours, doubling the maximum amount of sugars that these strains are capable of using without inhibition.

Table 4. Analysis of variance of the model generated by *Design Expert Software* for lactic acid production from chemically defined medium by *L. pentosus*.Where: A: glucose; B: xylose; C: yeast extract; D: meat extract; E: peptone; F: sodium acetate; G: ammonium citrate; H: manganese sulphate; I: magnesium sulphate; J: potassium phosphate; SS: Sum of Squares, df: degree of freedom, MS: Mean of Squares.

Source	SS	df	MS	F-value	*p*-value		
Model	263.78	9	29.31	13.77	0.0112	significant	
A	90.86	1	90.86	42.68	0.0028		
B	105.73	1	105.73	49.67	0.0021		
C	6.42	1	6.42	3.02	0.1574		
D	10.49	1	10.49	4.93	0.0906		
E	8.04	1	8.04	3.78	0.1240		
F	0.5547	1	0.5547	0.2606	0.6366		
G	11.64	1	11.64	5.47	0.0795		
H	15.92	1	15.92	7.48	0.0522		
I	14.13	1	14.13	6.64	0.0616		
Curvature	142.85	1	2.13	67.11	0.0012		
Residual	8.51	4	2.13				
Lack of Fit	7.53	2	3.77	7.69	0.1151		
Pure Error	0.9800	2	0.4900				
Cor Total	415.15	14				not significant	

Table 5. Optimization conditions for fermentation medium by *Desirability* function. Where: A: glucose (g/L); B: xylose (g/L); C: yeast extract (g/L); D: meat extract(g/L); E: peptone (g/L); F: sodium acetate(g/L); G: ammonium citrate(g/L); H: manganese sulphate(g/L); I: magnesium sulphate(g/L); J: potassium phosphate(g/L); K: tween(g/L); added to the SSF process for Lactic Acid production (LA, g/L) by *Lactobacillus pentosus*.

A	B	C	D	E	F	G	H	I	J	K	LA	Desirability
20.000	20.000	10.000	0	0	2.500	1.000	0.025	0.100	1.000	0.500	13.178	0.931
16.965	17.960	10.000	0.390	0.333	2.500	1.000	0.026	0.101	1.000	0.501	11.856	0.874
19.021	12.776	10.000	0.195	0.018	2.500	1.000	0.027	0.102	1.000	0.502	10.813	0.864
19.223	18.531	10.000	2.762	0.495	2.500	1.000	0.028	0.103	1.000	0.503	13.255	0.797
8.470	17.225	10.000	1.895	0.767	2.500	1.000	0.029	0.104	1.000	0.504	9.750	0.749
7.587	19.088	10.000	0.932	3.247	2.500	1.000	0.030	0.105	1.000	0.505	10.241	0.721

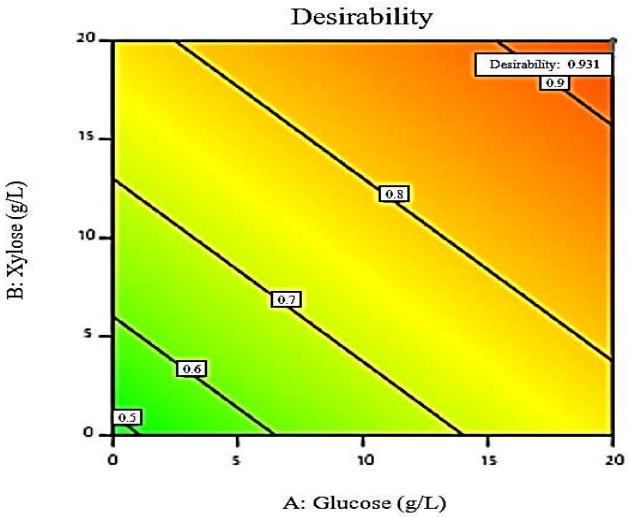

Figure 1. Contour surface evaluating the influence of the interaction between glucose (A) and xylose (B) for LA production

Table 6. Lactic acid production against HOD media with and without hydrolysis by *Lactobacillus sp.* strains. Where: EY= Enzyme hydrolysis, NH= No Enzyme hydrolysis

Responses	*L. coryniformis (EY)*	*L. pentosus (EY)*	*L. coryniformis (NY)*	*L. pentosus (NY)*
Lactic acid (g/L)	7.110	4.820	25.260	32.620
Volumetric productivity (g/L.h)	0.296	0.200	1.053	1.484

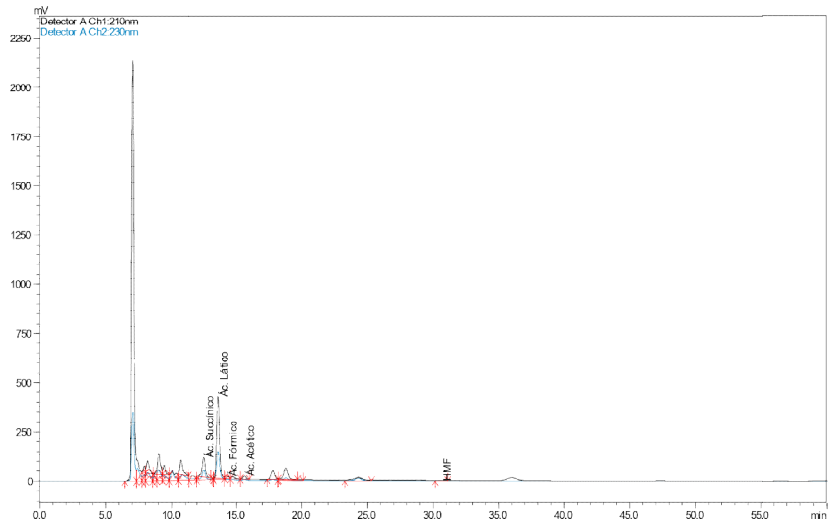

Figure 3. Chromatogram resulting from the analysis of lactic acid production by *L. coryniformis*after 24 hours of fermentation, without hydrolysis

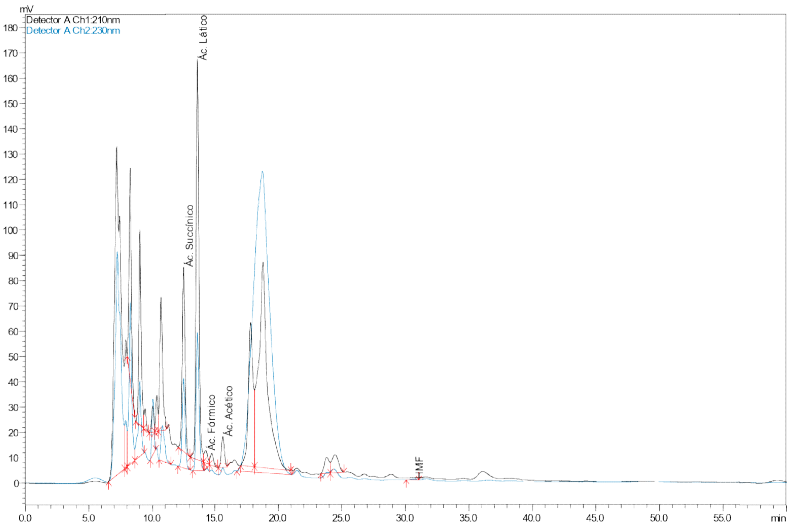

Figure 4. Chromatogram resulting from the analysis of lactic acid production by *L. pentosus*after 24 hours of fermentation, without hydrolysis

Table 7. Total sugars consumed during fermentation. Where: EH= Enzyme hydrolysis, NH= No Enzyme hydrolysis, HOD: Household Organic Waste, TSC: Total sugar consumed, LA: Lacti acid production

	Initial glucose (g/L)	Glucose consumed (g/L)	Initial xylose (g/L)	Xylose consumed (g/L)	TSC (g/L)	LA (g/L)
HOD + *L. coryniformis* (EH)	98.89	16.46	35.99	9.32	25.77	7.11
HOD + L. pentosus (EH)	98.89	13.53	35.99	10.01	23.54	4.82
HOD + *L. coryniformis* (NH)	75.25	16.71	74.5	32.69	49.41	25.26
HOD + *L.pentosus* (NH)	75.25	13.08	74.5	41.35	54.43	32.62

Table 8. Production of organic acids and alcohol. Where: EH= Enzyme hydrolysis, NH= No Enzyme hydrolysis, HOD: Household Organic Waste

HOD samples	Responses (g/L)					
	Succinic acid	Latic acid	Formic acid	Acetic acid	Propionic acid	Ethanol
HOD without inoculum (NH)	11.27	0	0.44	0	5.5	2.47
HOD + *L. coryniformis* (NH)	5.65	25.26	0.64	1.12	0	7.24
HOD + *L. pentosus* (NH)	5.5	32.62	0.36	1.33	0	12.25
HOD without inoculum (EH)	6.01	0	0	0.68	0	1.1
HOD + *L. coryniformis* (EH)	4.4	7.11	0.18	1.08	0	0.96
HOD + *L. pentosus* (EH)	4.43	4.43	0.05	1.22	2.48	1.02

Screening of all synthesized products: Due to the use of non-sterile organic waste as a medium, various other microorganisms are present which can influence the LA production. For this reason, other organic acids and ethanol were analyzed in order to confirm that the production of lactic acid was carried out by *L. coryniformis and L.* pentosus *and* that there was no deviation from the metabolic route by the inoculated strains. To this end, samples of HOD medium with inoculum from the two strains and samples of HOD medium without inoculum were analyzed by high performance liquid chromatography. The HOD samples that did not have inoculated cells, with or without the addition of enzymes for hydrolysis, showed zero values for lactic acid production. From this result, it can be inferred that all the lactic

acid produced came from the lactic fermentation of the *L. coryniformis and L. pentosus* strains. About the other organic acids synthesized as by-products, no acetic acid was found in the HOD medium without enzyme; however, the media without enzyme added with *L. coryniformis* and *L. pentosus* showed values of 1.12g/L and 1.33g/L, respectively. In the HOD medium with enzyme, a concentration of 0.68g/L of acetic acid was found; in the media with enzyme added to *L. coryniformis* and *L. pentosus*, the presence of 1.08g/L *of* this acid was detected for ROD + *L. coryniformis* and 1.22g/L for HOD + *L. pentosus.* The increase in by-product synthesis is due to production via heterofermentation, where acetic acid and ethanol were produced in addition to lactic acid. For this reason, these media showed a considerable increase in ethanol values, with 7.24g/L for HOD + *L. coryniformis* and 6.08g/L for HOD + *L. pentosus* in the media without added enzyme. Unlike lactic and acetic acids, in the HOD medium without enzyme there was already 2.47g/L of ethanol, showing that other microorganisms were present producing part of the final ethanol, which was to be expected since the medium used was not sterilized. Despite the presence of these microorganisms, they had no influence on the main production, which is lactic acid. The HOD medium with added enzymes also produced ethanol, but in smaller quantities. In this medium, *L. coryniformis* produced 0.96g/L, while *L. pentosus* produced 1.02g/L. Succinic and propionic acids were present in the media without enzymes at 11.27g/L and 5.50g/L for *L. coryniformis* and *L. pentosus* respectively, while in the media with enzymes they were present at 6.01g/L for *L. coryniformis* and 0g/L for *L. pentosus*. In turn, succinic acid showed a reduction in concentration with the addition of inoculums, while propionic acid concentrations were zero. Formic acid concentrations varied between 0.05g/L and 0.64g/L. These values do not represent a significant change.

DISCUSSION

When comparing with scientific articles, the present work proved to be very promising,achieving high LA productivity and yieldvalues from waste. In addition, to obtaining excellent results without pretreatment, the use of enzymes or control in bioreactors considerably reduces the cost of production. Several studies in the area suggest the use of residues for the synthesis of LA, due to the issues already described. As an example, as reported by Bernardo *et al*. (2016) that used whey and corn for the synthesis of LA, reaching 57 g/L for *L. rhamnosus.* Sousdaleff *et al*. (2009) used sugarcane molasses as raw material and reached 25.57 g/L of LA for *L. casei.* Nguyen *et al*. (2007) used the species *L. amylophylis,* reaching 43.7 g/L of LA from sweet potato starch. Lopes *et al*. (2008) evaluated the production of LA from supplemented sugarcane juice, reaching 22.57 g/L for *L. delbrueckii*, the same specie that was evaluated in this work. However, it is important to seek the production of LA by waste, thus contributing to sustainability and reducing production costs, as reported by Song *et al*. (2022). Thus, several studies point to the production of LA from waste. Kwan *et al*, (2016) used mixed food waste, mixed food waste powder produced by food waste treatment machine and bakery waste for LA production has been demonstrated with the overall conversion yields of 0.27, 0.25 and 0.23 g/ g, respectively. Results suggest that fungal hydrolysis and fermentation with *L. caseishirota* is an efficient approach for the bioconversion of food waste to LA. In this work, the enzymatic hydrolysis assays showed that the strains had low lactic acid production (4.82g/L for *L. pentosus* and 7.11g/L for *L. coryniformis*), low volumetric productivity (0.20 for *L. pentosus* and 0.30 for *L. coryniformis*) and consequently low yields (20% for *L. pentosus* and 28% for *L. coryniformis*). The fermentability tests on non-hydrolyzed media generated excellent production, yield and productivity results for both strains. *L. coryniformis* strainproduced 25.26g/L of lactic acid, with a productivity of 1.05 and a yield of 51%, while *L. pentosus* produced 32.62g/L of lactic acid, with a productivity of 1.36 and a yield of 60%. The results were similar to achieved by Balakrishnan *et al*. (2018), using *L. delbrueckii* subsp. *delbrueckii* NBRC3202, reaching LA 25.38 g/L; Yp/s, 1.18 g/g; productivity, 0.53 g/L h from cassava fibrous waste, generated as a solid waste by the sago industries in India. The enzymatic action possibly caused the inhibitors release, due to the greater saccharification efficiency (Bahry *et al*., 2019). In addition, the change in pH, among other factors, may have altered the characteristics of the material and impaired fermentation, as studied by Bahry *et al*. (2019), that reported the LA production in immobilized cells from hydrolyzed medium. In this sense, our authors found that the presence of immobilized cells could benefit the process when enzymes (cellulase of 30 FPU/g) are applied for hydrolysis, and can be used for at least five successive cycles. At this point, it is important to adjust the initial concentrations of substrates to avoid interference. However, incomplete utilization of lignocellulose-derived sugars not only reduced the titer, productivity, and yield of cellulosic D-lactic acid, but also resulted in an abundance of residual sugars in the D-lactic acid fermentation broth (He *et al*., 2023). To this end, some studies indicate the use of integrated system of lignocellulose fractionation, saccharification, fermentation, and ex-situ nanofiltration (Okano *et al*., 2017), still aiming to reduce costs. Other recent studies indicates molecular biology techniques to genetically modify *Lactobacillusspp.* strains. The engineered *Lactobacillus plantarum* co-utilized only glucose and xylose with slower xylose consumption rates than glucose, but no data showed the utilization of hemicellulose-derived arabinose, mannose, and galactose, and finally, the lowest titer (22–61.4 g/L) of D-lactic acid was produced from corn stover and sorghum stalks (Zhang *et al*., 2016a, Zhang *et al*., 2016b). *Lactobacillus bulgaricus* after evolutionary engineering utilized only glucose to produce 108 g/L D-lactic acid, but *L. bulgaricus* could not utilize the xylose released from 4.63% (w/w) xylan of pretreated rice straw (Prasad *et al*., 2020). Furthermore, environment optimization has proven to be of utmost importance in reducing costs for industrial production. According to Abedi and Hashemi, (2020),Agro-industrial waste or sub-products with a lower value such as molasses, juices waste, starchy biomass, agricultural residues, forestry residues that are rich in mono and disaccharides, which in some cases need to be hydrolysed by pectinases to enhanced the LA production. To use dairy wastes as a substrate, mainly whey, it is necessary to use an enriched mediums, due to insufficient proteolytic enzyme activity. Worth highlighting that the fermentability tests on non-hydrolyzed media generated excellent productionyield and productivity results in this work using no enzyme and slow cost.

CONCLUSIONS

The strains under study showed very promising abilities in relation to the consumption of different carbon sources, opening up an opportunity to make the most of the different sugars present in organic waste. In terms of completing the steps involving the production of organic acids in MRS medium, the *Lactobacillus coryniformis* strain showed high cell growth and lactic acid production and was selected for further testing in the second stage of the project. In the stages involving the production of lactic acid from xylose, the *Lactobacillus pentosus* strain showed high growth and lactic acid production and was also used in the waste utilization stage. The results of the experimental design point to the priority importance of the carbon sources, xylose and glucose, followed by magnesium sulfate, potassium phosphate, manganese sulfate, meat extract, and in turn, peptone, yeast extract, tween, sodium acetate, ammonium sulfate. The fermentability tests on non-hydrolyzed media generated excellent production, yield and productivity results for both strains. *L. coryniformis* produced 25.26g/L of lactic acid, with a productivity of 1.05 and a yield of 51%, while *L. pentosus* produced 32.62g/L of lactic acid, with a productivity of 1.36 and a yield of 60%. Production was higher without pretreatment, hydrolysis or controlled pH conditions. This shows the possibility of excellent production without additional costs for enzymes, acids and bases, further reducing production costs.

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