*Biotechnology lactic acid production from hemicellulosic hydrolyzate of Sorghum bicolor by Lactobacillus pentosus*

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ABSTRACT

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| --- |
| Lactic acid is considered a type of produce of wide industrial application; has high reactivity, thus expanding its possibilities of use. Lactic acid synthesis is possible from the fermentation of residual and renewable raw materials, being the economically viable process. **Aims:** This study is aimed to investigate lactic acid production from the sorghum bagasse fermentation, through the conversion of xylose contain in the hemicellulose by *Lactobacillus* *pentosus* using experimental statistical design. **Study design:** Initially, a synthetic adapted medium was evaluated for fermentation assays after cell activation and propagation. The screening for parameters that influence lactic acid SSF production has been done through factorial design. The desirability function was verified and statistically validated. **Place and Duration of Study:** this research was carried out at Department of Biochemical Engineering, School of Chemistry/ Federal University of Rio de Janeiro, Rio de Janeiro, RJ, 21249-900, Brazil. The study will lasted from 2016 to 2018.**Methodology:** For the SSF production, the hemicellulose acid pretreatment conditions were as follows: 1.39% (v/v) H2SO4 concentration, 1:4 solid:liquid ratio, 52 g.mL-1 and 46.7min at 121ºC. Subsequently, 2% (v/v) and 10% (v/v) cells in the hydrolyzate, 50% (v/v) and 100% (v/v), respectively, were added under anaerobic conditions, 120 rpm orbital agitation in a shaken flasks and in a instrumented bioreactor. Then, the statistical analysis was used for following independent variables evaluation: inoculum, xylose concentration and KH2PO4 concentration, add to the complementary hemicellulose hydrolyzate medium.**Results:** The onepot fermentation steps using xylose synthetic medium promoted the production of Lactic Acid, 21 g/L, volumetric productivity (QP) of 0.59 g/L.h and 0.583 g/g. The main results obtained was Lactic Acid, 30g/L, under the condition of 50%, hemicellulose hydrolyzate from sorghum, without detoxification, reaching 1.25 g/L.h volumetric productivity*.***Conclusion:** The results were very promising using an agro-industrial residue. The optimization of cultivation conditions in a chemically defined medium enabled the development of the best growth conditions and reduction the use of potassium phosphate.  |

***Keywords:*** *Lactic Acid, Lactobacillus pentosus, Hemicellulosic hydrolyzate, Sorghum bicolor, SSF.*

1. INTRODUCTION

Lactic acid has a privileged position in the family of carboxylic substances due to its applications in pharmaceuticals, chemicals, cosmetics and food industry (Jarboe et al., 2013). Worldwide LA production is being driven by the production of biodegradable polymers such as PLA (polylate), corroborating the important sector of the chemical industry and being considered one of the top 10 biorefinery chemicals, as stated by the US Department of Energy (Abdel-Rahman & Sonomoto, 2016).

The lact acid could be obtained by bacterial or chemical fermentation; however, a racemic mixture of lactic acid is obtained while the fermentative process allows the formation of isomerically pure acid (John et al., 2007). A biochemical route for lactic acid production uses as the main fermentation agent such as bacteria, which captures nutritional nutrients in amino acids and vitamins for their performance and fermentative activity (Wang et al. 2021; George et al., 2018). The genus *Lactobacillus sp.* was also reclassified into 25 genera (Zheng et al., 2020), representing highly heterogeneous microorganisms with approximately 67 species identified to 2020 (Mejía-Gomez, 2020).

It is worth highlighting that cereals, like sorghum and other grains, are an important part of the global development of food culture, representing over 50% of the global population's primary caloric and protein intake. In relation to the raw material of study, sorghum (Sorghum bicolor L.) is the fifth most important cereal in the world, surpassed only by wheat, rice, corn and barley. It is grown in very dry and/or very hot environmental areas and situations, where the productivity of other cereals is uneconomical (Adebo e Kesa, 2023). This important cereal was used in Africa between 3.000 and 5.000 years ago and later spread to India and China (Fuller e Stevens, 2018). In African countries, for example, South Africa, sorghum is usually grown in drought-prone areas and is regarded as the second most significant cereal crop, been used as a staple food in rural communities, representing their primary source of nutrients and energy (Khoddami et al., 2021). In these countries, cereal accounts for 70% of daily caloric intake, thus playing a key role in food security (Dicko et al., 2006; Mutisya et al., 2009).

In view of the above, cereals provide nutrients, such as macronutrients (starch, protein, and lipids) and micronutrients (minerals and vitamins) and make up the majority of human nutrition and health (Ofori et al., 2022). Some cereals also contain a significant quantity of dietary fiber, coloured varieties and bioactive components with functional properties and high nutritional value (Rawat et al. 2023, Garutti et al., 2022). In relation to the object of study of this work, sorghum is a highly valued cereal crop recognized for its high nutritional value, containing 8–18% protein, 70–80% carbohydrates, 19% dietary fiber, about 3% fiber, and various minerals, as well as bioactive components like vitamin B and fat-soluble vitamins (D, E, and K), micronutrients, macronutrients, and non-nutrients like carotenoids and polyphenols. Due to its versatility and ease of production, it is estimated that sorghum has been used as a staple food for over 500 million people living in developing countries, mainly in Africa and Asia (Mutisya et al., 2009). Currently, more than 35% of sorghum production is directly for human consumption and the remainder is mainly used for animal feed (Awika; Rooney, 2004).

The search for xylose-fermenting microorganisms, the most abundant sugar proven by the hemicellulosic fraction, is one of the biggest challenges of modern biotechnology; since it requires the efficient and integral conversion of necessary carbohydrates from lignocellulosic materials (Brobbey et al. 2025). The development of technologies for the production of lactic acid from the residual biomass of lignocellulosic composition it is quite attractive, considering that Brazil has the largest availability of low-cost biomass in the world from harvesting and processing agricultural crops such as sugar cane, rice, wheat, corn and soybeans (Mujtaba et al., 2023). Furthermore, it is necessary to evaluate the downstream steps integrated into the fermentation process. Pola et al. (2019) evaluated the use of non-lignin residue from kraft black liquor as a renewable source of carboxylic acids. It was found that the formation of carboxylic acids could be maximized and the presence of an oxidizing atmosphere generated a less concentrated, but more purified, stream of acids than that obtained by thermal hydrolysis, simplifying the subsequent downstream processing.

In these terms, there is a gap in studies related to the biotechnology of lactic acid production, which corroborates the importance of this work. In addition, there is currently a trend in the chemical sector to replace technologies for the production of petrochemical derivatives with biochemical technologies that use renewable raw materials, which is in accordance with the principles of Green Chemistry (Sheldon, 2024; Duarte et al., 2022).

Within the context, the aim of this work was to investigate the second-generation lactic acid production from the sorghum bagasse fermentation, through the conversion of xylose from hemicellulose by *Lactobacillus pentosus*.

2. material and methods

**2.1. Preparation of starter cultures**

*Lactobacillus pentosus* strains were obtained from the American Type Culture Collection strain bank, kept in lyophilization, stored in properly sealed, light-free ampoules. The strains were activated by suspending cells in an Manga-Rogosa-Sharpe (MRS) liquid medium (Glucose, 20.0 g/L; Polysorbate 80 1.0 ml; Ammonium Citrate, 2.0 g/L, Sodium Acetate 5.0 g/L, Magnesium Sulphate 0.1 g/L, Manganese Sulphate 0.05 g/L and Dipotassium Phosphate 2.0 g/L - pH: 6.5. Lyophilized cultures were reactivated according to the instructions of the collection from which the culture was obtained (Tabacof et al., 2023; Liu et al., 2016). After breaking the ampoule, 0.5 mL of previously sterilized MRSglucose medium (Table 2) was added. After initial rehydration for 10 to 15 min, the contents were transferred to a test tube containing 5 mL MRS and then incubated. Subsequently, the cells were separated by centrifugation at 8000 rpm for 10 min and resuspended in a mixture of MRS medium with 50% glycerol to preserve cell integrity, and stored in cryotubes within the aseptic chamber kept at -80ºC.

**2.2. Cell Activation, propagation and fermentation**

The culture was activated and propagated in penicillin flasks containing 50 mL MRS medium at 37ºC and 120 rpm for 16 hours under anaerobic conditions. For propagation cell, the flasks containing the suspended cells were keep at 120 rpm, 37°C for 8 hours reaching appropriate cell concentration and metabolic conditions to obtain the inoculum for the fermentation step (Bharti et al., 2025). For fermentation cell production, 10% (v/v) cell concentration from the previous step was injected into 100 mL flasks containing 50mL of reduced sterile MRS medium were using aseptic chamber. Adaptation MRS medium containing xylose (MRSxylose) has the composition described in Table 1, which is based on the standard MRS medium (Man, Rogosa, Sharpe) usually used in the cultivation of lactic acid bacteria. MRSxylose medium has xylose as its main carbon source, unlike standard MRS medium that uses glucose.

**Table 1.** Medium componentes of denominated *MRSglucose* and *MRSxylose.*

|  |  |
| --- | --- |
| ***Component Medium*** | ***Concentration (g/L)*** |
| Peptone | 10 |
| Yeast extract | 5 |
| Beef extract | 10 |
| Glucose/ xylose | 20 |
| Tween 80 | 1 |
| Ammonium citrate | 2 |
| Sodium acetate | 5 |
| Magnesium sulfate | 0.1 |
| Manganese sulfate | 0.05 |
| Potassium phosphate | 2 |

**2.3. Cell mass quantification**

The biomass concentration were quantified by spectrophotometric reading at 600 nm between 0.1-0.8 absorbance units, using distilled water as calibration reference. A standard curve for the microorganism was constructed using the biomass obtained in the inoculum culture after 12 hours of cultivation: different dilutions were made and for each biomass concentration the absorbance at 600nm and dry mass were determined. The dry mass was determined after centrifugation of the fermented medium at 12.000rpm for 10 min, followed by washing the cells with distilled water and further centrifugation and subsequent oven drying at 60°C for 24 hours (until constant weight).

**2.4. Fermentation Assays**

Initially, the Manga-Rogosa-Sharpe medium was used as a synthetic fermentation medium, with slight variations in the initial glucose concentration. For the tests that evaluated “the effect of the xylose concentration”, the composition of the MRS medium was maintained, with glucose being replaced by xylose. Subsequently, the composition of the synthetic medium was modified according to experimental designs to evaluate the effects of MRS medium components for D-Lactic Acid production. The fermentation medium was reduced and sterilized, and a 10% (v/v) inoculum containing the active bacterial cells was injected into the aseptic chamber under 120 rpm at 37°C in 16 hours. For pH control experiments, CaCO3 (5%) was added as neutralizing agent. Cell growth, substrate consumption and products were monitored during the assays (Yang et al., 2015). In all experiments, aliquots were periodically collected under aseptic conditions.

**2.5. Bioreactor fermentation**

Fermentation under controlled conditions was performed using a mechanically shaken instrumented bioreactor (New Brunswick) using a 1.5L reaction vessel containing 1 L of fermentation medium. Batch experiments were automatically controlled at 37°C, 120 rpm agitation, and pH 6.5-7.0 monitored using a sterile pH electrode and controlled by the addition of NaOH (4M), incorporation of N2 at the beginning of the process. The operation was carried out in a single batch with the fermentation medium resulting from the shake flask designs.

**2.6. Onepot D-lactic acid production**

To construct the kinetic profiles of sugar consumption and D-lactic acid production, two assays were performed in instrumented bioreactor using glucose or xylose as substrate. The medium was supplemented with the nutrients and concentrations described in the MRS medium. Activation was done in penicillin flasks and cell propagation in the bioreactor itself. The initial volume of propagation culture was 800 mL, with 720 mL of reaction medium and 80 mL (10% v/v) of activation culture. After the set propagation time, 800 mL of the complementary medium was added to the bioreactor, totaling a final volume of 1.6 L. The initial sugar concentration (glucose or xylose) was 30 g/L; pH and temperature were maintained at 6.5 (HCl/ NaOH) and 37°C, respectively.

**2.7. Hemicellulosic fraction pretreatment**

The sorghum bagasse provided by Monsanto (SP-Brazil) was previously washed, dried and then milled. Initially an acid pretreatment step was performed to disorganize the matrix of lignocellulosic composition in order to remove the hemicellulosic fraction. Therefore, after acid pretreatment under white conditions, according to the methodology described by Barcelos et al. (2016) and detailed below: 1.39% (v/v) H2SO4 concentration, 1:4 solid:liquid ratio, 52 g.mL-1 and 46.7min exposure time at 121ºC in autoclave. Subsequently, 2% (v/v) and 10% (v/v) cells in the hydrolyzate, 50% (v/v) and 100% (v/v), respectively, were added under anaerobic conditions by a gas injection (N2), temperature set to 37oC, pH 7, 120 rpm orbital agitation in a shaken flasks and in a instrumented bioreactor previous described.

**2.8. Analytical Methods**

Samples were centrifuged at 12000 rpm for 15 minutes, the supernatant intended for sugar and product dosages by high performance liquid chromatography (HPLC) on a Waters chromatograph (Model 510 pumping system, Rheodyne injector, refractive index detector) coupled to an Aminex HPX-87P cation exchange column. Identification of the D-lactic and L-lactic isomers were performed by the Chirex® 3126 (D)-penicillamine chiral column under the following conditions: CuSO4 0.001mol/L as mobile phase, 1.0 mL/ min flow, 50ºC, ultra violet detector (UV/VIS) - 254nm and injection volume of 20µL.

**2.9. Statistical analysis**

The analysis of variance (ANOVA) and Response Surface Methodology were carried out using the Design Expert software (version 7.1.6. Stat-Ease, Inc., Minneapolis, USA) with a significance level of 90%.

3. results and discussion

**3.1. Sugars consumption and cell concentration kinetic for activation and propagation stages by *L. pentosus* from chemically defined medium**

The *Lactobacillus pentosus* strain showed high cell growth in a few hours of cultivation, ranging from 9 to 20 hours of process, using MRS synthetic medium at 37°C and 120 rpm, without pH control, in anaerobic condition by N2 injection at the beginning of the assays. Figure 1a and figure 1b shows the activation and propagation kinetic profiles of the *Lactobacillus pentosus* ATCC8041 strain using glucose as substrate. The cell concentration in the activation culture was approximately 1.8 g/L in 24 h and the cell yield was 0.142 g/g. The percentage reduction of substrate in this step was 66%. Regarding the propagation step, the cell concentration was 1.9 g/L in 17 h of culture with a cell yield of 0.172 g/g. The percentage reduction of substrate was similar to the activation step (67%). The kinetic profiles of the activation and propagation culture of *L. pentosus* ATCC8041 strain using xylose as substrate are presented in figure 1c and figure 1d. Cell concentration reached 1.5 g/L, however, the time required to reach this concentration was 24 h in activation culture and 20 h in propagation culture and cell yield was 0.170 g/ L and 0.127 g/g, respectively. The percentage reduction in these stages was 47% and 65%.



**Fig. 1.** Glucose consumption **(A)** and cell concentration **(B)** kinetic of the activation culture **(A)** and propagation culture **(B)**. Xylose consumption **(C)** and cell concentration **(D)** kinetic of the activation culture by *L. pentosus* ATCC8041 strain.

**3.1. LA production from chemically defined medium using the one pot technique in instrumented bioreactor**

Figure 2 shows the kinetic profile of *L. pentosus* ATCC8041 strain, in glucose-containing synthetic medium, in instrumented bioreactor. The propagation and fermentation step was carried out onepot and consecutively in the same reaction vessel. The cell concentration at the end of the propagation step (6 h) was 1.4 g/L when a concentrated glucose solution and a solution with the other nutrients were added to the medium to start the fermentation process. Glucose was fully consumed in 18 h of fermentation, reaching a lactic acid concentration of 31 g/L, which corresponds to a volumetric productivity (QP) of 1.41 g/L.h and a yield in product (YP/S) of 0.848 g/g. The isomeric form D (-) lactic acid represented 61% (19.2 g/L) of the total lactic acid produced. The cell concentration at the end of the fermentation process was 3.8 g/L. Acetic acid was the second main fermentation product, reaching 2.6 g/L.

The kinetic profile of the *L. pentosus* ATCC8041 strain, in xylose-containing synthetic medium, in instrumented bioreactor is shown in Figure 3. The propagation and fermentation steps were also performed in the same bioreactor (onepot) consecutively, and at the end of propagation (8 h), the cell concentration was 0.8 g/L, when a concentrated xylose solution and a solution with the other nutrients were added to the medium to start the fermentation process. The xylose was completely consumed in 36 h of fermentation, demonstrating the ability of this microorganism to assimilate this substrate for acid production. The lactic acid concentration was 21 g/L after 36 hours of fermentation, which corresponds to a volumetric productivity (QP) of 0.59 g/L.h and a product yield (YP/S) of 0.583 g/g. The isomeric form D- lactic acid represented 65% of the total lactic acid produced. The cell concentration at the end of the fermentation process was 2 g/L. Acetic acid was the second main fermentation product, reaching 12 g/L. As performed in this study, Xavier (2011) also demonstrated that MRS medium fermentation, in the absence of xylose, induces the acetic acid production by *L.pentosus* due substrate limitation imposed, having reached a concentration of 4.91 g/L of acetic acid and a biomass concentration around 0.9 g/L.



**Fig. 2.** Kinetic of onepot D- lactic acid production from glucose, evaluating step 1 (Propagation cell) and (step 2 (Fermentation) by *L. pentosus* ATCC8041 strain.

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**Fig. 3.** Kinetic of onepot D- lactic acid production from xylose, evaluating step 1 (Propagation cell) and (step 2 (Fermentation) by *L. pentosus* ATCC8041 strain.

After performing an assay containing both sugars (glucose and xylose), the co-production of acetic acid originated from the metabolism of xylose and arabinose. This means that in the absence of glucose the *L. pentosus* pathway changes from homofermentative to heterofermentative. Metabolism for the degradation of sugars during the heterofermentative pathway follows the phosphoquetolase pathway, so *L. pentosus* can be considered as an optional heterofermentative microorganism, degrading hexoses (like glucose) via Embden-Mayerhof-Parnas and pentoses via Phosphoquetolase (Wischral et al., 2019; Xavier, 2011; Zhu et al., 2007; Bustos et al., 2007).

**3.2. Statistical design assays evaluation parameters**

Among lots of experimental design, factorial design systems stand out because they allow the simultaneous evaluation of the effect of a large number of variables from a small number of experimental trials when compared to univariate processes (Peralta-Zamora et al., 2005). Given the above, such statistical tool was used for the previous evaluation of the following independent variables: inoculum, xylose concentration, as well as KH2PO4 concentration, as highlighted in Table 2. Thus, test 3, which evaluated the addition of 5 g/L of initial inoculum, lower parameter evaluated by the software; associated with the concentration of 6 g/L potassium phosphate, a parameter present at its central level; tied to the xylose concentration at the lower level, 20 g/L, presented the highest lactic acid production, 16 g/L. It is noteworthy that potassium phosphate has a buffering effect, as highlighted in the literature, and is advantageous for fermentation processes that have reduced pH range associated with the production of organic acids (Chuah & Mao, 2020). Additionally, design trials have shown that high concentrations of xylose result in substrate inhibition, as reported in previous cell growth assessments.

The ANOVA indicates the proposed model was significant, as well as the parameters in this study range (Table 2). Thus, all parameters were maintained in the model for next studies. The pure error has non-significance (*P*> .05), demonstrating favorable experimental conduct. The curvature did not present statistical significance (Fisher value at 2.74), which not necessary to have later studies containing the response surface for more detailed analyses.

**Table 2.** Fractional Factorial Design 32-1 evaluating: inoculum (%), initial xylose concentration (g/L), and KH2PO4 concentration (g/L) for the production of lactic acid by the *Lactobacillus pentosus* strain from chemically defined medium.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Run | Block | Factor A | Factor B | Factor C | Response |
| 1 | 1 | 10.00 | 4.00 | 40.00 | 13.55 |
| 2 | 1 | 15.00 | 6.00 | 60.00 | 11.35 |
| 3 | 1 | 5.00 | 6.00 | 20.00 | 16.20 |
| 4 | 1 | 10.00 | 4.00 | 40.00 | 13.60 |
| 5 | 1 | 15.00 | 2.00 | 20.00 | 12.15 |
| 6 | 1 | 5.00 | 2.00 | 60.00 | 12.15 |
| 7 | 1 | 10.00 | 4.00 | 40.00 | 13.00 |

*\*Factor A= Inocullum concentration (g/L), Factor B= potassium phosphate (g/L), Factor C= Xylose (g/L), Response= LA (g/L)*

 **Table 3.** Analysis of variance for LA production from HH by *Lactobacillus pentosus.*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Source | SS | DF | MQ | F Value | *p*-value Prob> F  |
| Model | 14.40 | 3 | 4.80 | 43.31 | .0227 |
| Factor A | 5.88 | 1 | 5.88 | 53.06 | .0183 |
| Factor B | 2.64 | 1 | 2.64 | 23.83 | .00395 |
| Factor C | 5.88 | 1 | 5.88 | 53.06 | .0183 |
| Curvature | 0.30 | 1 | 0.30 | 2.74 | .2397 |
| Pure error | 0.22 | 2 | 0.11 |  |  |
| Cor Total | 14.93 | 6 |  |  |  |

*\*Factor A= Inocullum concentration (g/L), Factor B= potassium phosphate (g/L), Factor C= Xylose (g/L), Response= LA (g/L). SS= Sum of squares, DF= degrees of freedom, MQ= Mean square,*

From a technical and economic point of view, the fermentation process becomes more attractive and viable according as the lactic acid production increases -and culture medium components/ initial cells/ substrate reduction concentrations, regarding scale extrapolation. In turn, the Desirability function was employed in these configurations, with the initial cell concentration and the initial substrate concentration maintained at their lower levels; as well as potassium phosphate levels were minimized; and maximized lactic acid production. The applied criteria and the predicted results for the optimization are presented in Table 4, where the potassium phosphate composition for the optimized medium was 3.5 g/L, with a Desirability coefficient of 0.841, covering around 84% of the joint lactic acid production needs with reduced costs associated with the fermentation medium.

**Table 4.** Optimization conditions for fermentation medium.

|  |  |
| --- | --- |
| Desirability | Real production |
| A | 5 |
| B | 3.46 |
| C | 20 |
| Response | 15.2 |

*\*Factor A= Inocullum concentration (g/L), Factor B= potassium phosphate (g/L), Factor C= Xylose (g/L), Response= LA (g/L).*

It is important to emphasize that the complexity of the fermentation media is a factor increasing the cost of lactic acid production. The minimization of medium components (without significantly influencing the result), as described by Yankov (2022); Gilver Rosero Chasoy et al., (2020), Mejia-Gomez and Balcazar (2020); Gómez-Gómez et al. (2015), contributes to cost reduction and makes the industrial process more attractive. Carbon is an indispensable element and the replacement of costly individual sugars with cheap agricultural and industrial wastes is a step towards decreasing the total cost. Nitrogen is a major component in the anabolic and catabolic processes. Usually, complex nitrogen sources like peptone, yeast extract, and meat extract are used. Alternatives for cheap nitrogen sources are corn steep liquor, hydrolysate of fish waste, and wheat bran extract. DDGS (about 30% protein) is an attractive alternative because its hydrolysis results in carbon sources and nitrogen at a time. The ratio between carbon and nitrogen (C/N) also is very important. An optimal C/N ratio results in a positive effect on lactic acid fermentation. Usually, the optimal C/N ratio is between 3-7.

In this sense, Dashen et al (2024) evaluated microbial succession and effect of fermentation on the proximate composition of sweet potato tubers, leaves and vines and show that fermentation were characterized by positive effect on the proximate composition of the substrates. Gugel et al. (2024) focuses on exploring new, high-value applications for olive leaves waste, utilizing a biotechnological approach with *Lactobacillus casei* for the production of second-generation lactic acid. Small-scale fermentation tests were conducted with and without nutrient supplements, identifying the medium that yielded the highest lactic acid production for scale-up. The scaled-up batch fermentation process achieved an enhanced conversion rate (83.58%) and specific productivity (0.26 g/L.h). The study provides the importance of adding some nutrients and optimizing these compounds to reduce costs, as described in this work.

**3.3. Production of LA from hemicellulosic hydrolyzate**

Searching for the integral utilization of agro-industrial residues with the biotechnological production of substances of industrial interest, the fermentability of the sorghum hemicellulosic hydrolyzate was evaluated by the strain under study. Figures 4 shows the kinetic profile of fermentation from xylose from HH in 50% v/v proportion, with addition of complementary medium, resulting in 30 g/L LA in a instrumented bioreactor.

It is noteworthy that the proportion of 50% (v/v) hemicellulosic hydrolyzate was promising and ideal for the fermentation process; however, the use of 100% (v/v) HH resulted in high residual xylose concentrations (33 g/L) and lactic acid concentration lower than the previous condition. According to Betancur and Pereira (2011), high proportions of acid hydrolyzate promote inhibition by the present toxic compounds such as acetic acid, furfural acid and HMF, which justifies the stagnation/reduction in production when the hydrolysate concentration was doubled from 50% (v/v) to 100% (v/v).

Qiu et al. (2022) achieved similar values, 31.3 g/L of D-lactic acid, using a recombinant strain *of Pediococcus acidilactici*, 126.8 % higher than that of the parental strain (13.8 g/L) using undetoxified corncob prehydrolysate. Furthermore, one-pot SSCF was achieved using undetoxified acid-pretreated corncob slurry, and 61.9 g/L of D-lactic acid was obtained (overall yield of 0.48 g/g dry corncob) with the xylose conversion of 89.9%. Oliveira et al. (2018) investigated the lactic acid production from sugarcane bagasse hydrolysates by *Lactobacillus plantarum* in the presence of furfural and HMF. The strain was capable to assimilate the inhibitors simultaneously with lactic acid production. A decrease of 86% for HMF and 98% for furfural were observed, together with 34.5 g/L lactic acid production. This approach could decrease the cost of the process eliminating the need for detoxification before fermentation.



**Fig. 4.** Kinetic of D- LA production from 50% (v/v) HH added to the complementary medium by *L. pentosus* using a instrumented bioreator.

Cubas-Cano et al. (2019) used *Lactobacillus pentosus* CECT4023T, evolved to improve its xylose fermentation capacity even at acid pH by adaptive laboratory evolution in repeated anaerobic batch cultures at increasing xylose concentration. The resulting strain presented between 1.5 and 2-fold more xylose consumption and lactic acid production than the parental strain in 20 g/L xylose defined media independently of the initial pH value. Thus, the results point to the need for studies involving the use of the hemicellulosic fraction, as development in this word. Parra-Ramírez et al. (2019) evaluated D-lactic acid production from a simulated hydrolysate of corn stover (32 g/L xylose, 42 g/L glucose) with the metabolically engineered *Escherichia coli* strain JU15, reaching a final concentration of 40 g/L and a yield of 0.6 g lactic acid/g sugars. Zhu et al. (2023) studied to reduce byproduct generations, acid pretreatment with high solid loading (solid-liquid ratio 1:7) of garden garbage. Furthermore, semi-hydrolysis with low enzyme loading (10 FPU/g garden garbage cellulase) was conducted to regulate and reduce glucose concentration in the hydrolysate, thereby relieving carbon catabolite repression. During the lactic acid fermentation process, the xylose conversion rate was restored from 48.2% (glucose-oriented hydrolysis) to 85.7%, eventually achieving a 0.49 g/g lactic acid yield of hemicellulose.

In this study, results were very promising, using a residue containing xylose of HH from sorghum, since xylose is the second most abundant sugar in lignocellulose, without detoxification, reaching 1.25 g/L.h volumetric productivity by a native strain of *L. pentosus.* It is worth highlighting the importance of future economic evaluation with regard to the development of future tests aiming at the extrapolation of scale of the bioprocess developed in the present work.

4. Conclusion

The optimization of cultivation conditions in a chemically defined medium enabled the development of the best growth conditions and reduction the use of potassium phosphate. The highlight results obtained in the experimental design for the D- LA production from sorghum HH was 30g/L under the condition of 50% HH and 50% of optimized in a instrumented bioreactor.

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Definitions, Acronyms, Abbreviations

**LA:** Lactic acid

**D-LA:** D-Lactic Acid

**L-LA:** L-Lactic Acid

**MRS:** Man, Rogosa, Sharpe

ANOVA: Analysis of variance