

Kinetic parameters of *Lactobacillus acidophilus* growth in the lactic fermentation of non-centrifugal cane sugar agroindustry wastes

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Abstract: The use of sugarcane for the production of non-centrifugal cane sugar is one of the main economic activities in some areas of the central region of the state of Veracruz, México, however, this sector faces different social, techno-economic and environmental challenges. One of the most important problems affecting this agribusiness is the lack of adequate management of the waste generated in the process, mainly sugarcane scum (SCS) and mold wash water (MWW). Lactic fermentation is proposed as an alternative for the utilization of these wastes, since this process reduces the concentration of carbohydrates, producing lactic acid (LA) and increasing the nutrient content. An important aspect of the fermentation process is the knowledge of the kinetic parameters, since with these it is possible to carry out the scaling up. In the present work, the lactic fermentation of SCS and MWW was studied using the bacterium *Lactobacillus acidophilus* and the kinetic parameters were obtained with the Gompertz model and the Logistic model. The physicochemical characterization of the residues was carried out and the parameters of substrate consumption, lactic acid production and cell density were evaluated during fermentation of a 150 g SCS/L solution in a 0.5 L reactor. After 72 h of fermentation, a maximum growth of 7.63 log CFU/mL, a 50.32% carbohydrate consumption, and a maximum production of 7.56 g LA/L were obtained. For the Gompertz model, the parameters obtained were $\mu_{\max} = 1.2420 \text{ h}^{-1}$, $\lambda = 20.46 \text{ h}$ and $A = 7.585 \text{ log CFU/mL}$, whereas for the Logistic model they were $\mu_{\max} = 0.3214 \text{ h}^{-1}$, $\lambda = 25.39 \text{ h}$ and $A = 7.584 \text{ log CFU/mL}$. It was observed that both residues promote the development of the microorganism *L. acidophilus*, however, the kinetic parameters of $\mu_{\max}$ and λ indicates that it needs more time to adapt to the residues, so it will be necessary to implement strategies to optimize these values.

Keywords: Lactic fermentation; Non-centrifugal cane sugar agroindustry; *Lactobacillus acidophilus*; Sugar cane scum.

Introduction

In the agroindustrial sector, a wide variety of wastes are generated from agricultural, livestock, forestry, and related processes; most of these wastes are not treated, and are not used, being incinerated or improperly disposed of, generating environmental pollution problems (Azelee *et al.*, 2020). In Mexico, the state of Veracruz is the largest supplier of agroindustrial products, which during processing generate 7 million tons of waste per year, with sugarcane cultivation generating the largest amount with 5.3 million tons per year (Nava-Pacheco *et al.*, 2019).

Within the sugar agroindustry, non-centrifuged sugar, or non-centrifugal cane sugar, is a sweetener obtained from sugarcane juice, and manufactured in small agroindustries in developing countries (Velásquez *et al.*, 2019). Non-centrifugal cane sugar production participates with 2.3% of the national production of sweeteners from sugarcane, with an average of 115 thousand tons per year (Juárez *et al.*, 2018).

The typical non-centrifugal cane sugar production process includes a juice clarification stage that generates a black colloidal liquid residue, called cachaza, which traditionally has poor or no management practices, generating bad odors, and ecological risk (Mendieta *et al.*, 2020a). In addition, approximately 500 L of wastewater are generated per ton of non-centrifugal cane sugar during the washing of molds, tables, and floors of the beating, and molding room; this water contains about 0.5% dissolved solids, mostly sugars that favor the growth of microorganisms that cause the eutrophication of bodies of water (García *et al.*, 2007).

Different treatment alternatives for these wastes have been proposed, such as anaerobic digestion, co-digestion with sugarcane crop residues, bioethanol, hydrogen, and cellulose production (Mendieta *et al.*, 2020a; Mendieta *et al.*, 2020b; Sánchez *et al.*, 2017; Sánchez *et al.*, 2019; Khattak *et al.*, 2015).

Anaerobic digestion and co-digestion have the disadvantage of requiring long times for the adaptation of microorganisms to the substrate, the application of treatment to the by-products generated in the process, and the generation of greenhouse gases (Náthia-Neves *et al.*, 2018). While for the production of ethanol, hydrogen, and cellulose it is necessary to apply a pretreatment to the residue, to have greater control over the operating conditions of the process, and the use of chemicals at different stages of the process (Carrillo-Nieves *et al.*, 2019; Zhang *et al.*, 2021).

In order to generate value-added products from organic wastes, the use of emerging technologies involving the application of anaerobic microorganisms to carry out microbial fermentation using the carbohydrates present as a carbon source to produce lactic acid, ethanol, volatile fatty acids, and hydrogen has increased (De Groof *et al.*, 2021). Microbial fermentation is a process in which the strain, culture medium, reactor, and operating conditions combine, and interact (Romero-Mota *et al.*, 2023).

Among the anaerobic processes is lactic fermentation, which increases the nutritional properties, and functional value of substrates, increases their shelf life, and microbiological safety, and improves sensory characteristics, while decreasing the concentration of non-nutritional compounds, and sugar content (Jamnik *et al.*, 2022). Lactic fermentation is carried out by a group of microorganisms called lactic acid bacteria (LAB), which grow in a pH range of 3.5 – 10, and a temperature of 5 - 45 °C, and are known for their ability to ferment carbohydrates (hexoses and pentoses) to lactic acid (Raj *et al.*, 2022). One of the LAB species is the *Lactobacillus acidophilus* strain, which is one of the most recognized species for its commercialization in different foods, and supplements containing probiotics (Ozogul *et al.*, 2022). This species ferments carbon sources such as glucose, lactose, maltose, and sucrose, making it have an affinity for substrates rich in these components (Estrada-García *et al.*, 2023).

A very important aspect in the study of fermentation processes is the application of kinetic models, since they are important because they allow the implementation of strategies for process design, cost reduction, and optimization of operating conditions to obtain a desired product (Ajala *et al.*, 2021). Thus, this work presents the study of the lactic fermentation process of SCS and MWW, residues generated during the production of non-centrifugal cane sugar, by using of *L. acidophilus* and the obtaining of the kinetic parameters of growth of this strain.

Materials and Methods

Collection of the residues

The SCS and MWW were collected from a mill located in the town of Zentla (19°05'24.9 "N 96°48'04.3 "W), municipality of Zentla, Veracruz. Subsequently, a solution of 150 g SCS/L was prepared using MWW as solvent. This concentration was established on the basis of reports by Gonzalez-Del Rosario *et al.* (2023), they found no differences in LA production during fermentation of 50, 100 and 150 g SCS/L solutions. This solution was then kept refrigerated at 4°C until use.

Physicochemical characterization of the residues

For SCS, MWW, and SCS solution, pH was determined using a Science Med model SM-3BW potentiometer, Total Solids (TS), and Total Volatile Solids (TVS) according to Standards Methods (APHA, WPCF, AWWA, 2017), carbohydrates by the anthrone-sulfuric colorimetric method with a Thermo Scientific spectrophotometer model Genesys 20 at a wavelength of 620 nm, total nitrogen and proteins by the micro Kjeldahl method using a conversion factor of 6. 25. All determinations were performed according to standard procedures.

Propagation of the Lactobacillus acidophilus strain in SCS solution.

The *Lactobacillus acidophilus* strain was obtained from the commercial product called Lyofast LA 3 (SACCO System), which is in lyophilized form. This strain is considered a probiotic and has been used in other fermentation processes. Luciano *et al.* (2018) studied the effect of the addition of *L. acidophilus* LA 3 on physicochemical parameters, sugar and organic acid content, phenolic profile, and sensory characteristics of mango and acai smoothies. In addition, it has the advantage of being easy to acquire and less expensive than strains from microbial collections. 1 g of the product was weighed and added to a flask with 100 mL of MRS broth (Man, Rogosa, and Sharpe) previously sterilized at 121 °C for

15 min and incubated at 37 ± 0.5 °C for 24 h. This flask was referred to as the seed flask. Then, in order to acclimate the strain to the substrate, 4 mL were taken from the seed flask and added to 36 mL of SCS solution, leaving it to incubate at the same temperature and time as the seed flask. This volume was later used to perform the lactic fermentation kinetics.

Lactic fermentation kinetics

A 0.5 L reactor with heating jacket was set up with a useful volume of 0.4 L. It was operated at a temperature of 37 ± 0.5 °C, continuous stirring at 120 rpm by using of a magnetic stirrer, the inoculum was added in a 1/10 ratio, and the fermentation time was 72 h (Gonzalez-Del Rosario *et al.*, 2023; Romero-Mota *et al.*, 2023). Samples were taken every 2 h during the first hours of fermentation and every 4 h during the rest of the process. For each sample, pH, carbohydrates (according to the described techniques for waste characterization), and cell density was determined using the pour plate technique on MRS agar (Peng *et al.*, 2021), and titratable acidity according to the methodology described in the Mexican standard (COFOCALEC, 2014).

Obtaining the kinetic parameters

Cell growth kinetic parameters were determined with the modified Gompertz model described in Equation 1 and the Logistic model described in Equation 2 (Sharma *et al.*, 2014):

$$y = A * \exp \left\{ -\exp \left[\frac{\mu_{max}}{A} (\lambda - t) + 1 \right] \right\} \quad (1)$$

$$y = \frac{A}{1 + \exp \left[2 + \left(\frac{4\mu_{max}}{A} \right) (\lambda - t) \right]} \quad (2)$$

where y is $\log \frac{N}{N_0}$, μ_{max} is the maximum specific growth rate of the microorganisms, A is the logarithmic increase of the population and λ is the lag phase. The fit of the model with the experimental data was evaluated from the correlation coefficient (R^2) and the least squares error (MSE) percentage.

Results and Discussion

Physicochemical characterization of the residues

The composition of the residues generated during the production of non-centrifugal cane sugar is affected by different factors. In the case of the SCS, its composition is affected by the skill and experience of the personnel in charge of performing the clarification activity. While the MWW is affected by the type of washing process (spray or immersion), making the composition of the residues different from one mill to another (Gonzalez-Del Rosario *et al.*, 2023). Table 1 shows the results obtained from the physicochemical characterization of the residues used in this study.

Table 1. Physicochemical characterization of SCS, MWW and 150 g SCS/L solution.

Parameter [Unit]	SCS	MWW	150 g SCS/L solution
pH [-]	6.80±0.86	5.60±0.75	6.70±0.28
Total Solids [%]	29.81±0.89	1.93±0.47	5.12±0.21
Total Volatile Solids [%]	90.85±3.50	74.6±3.38	89.61±0.77
Carbohydrates [g/L]	172.42±10.90	12.48±1.92	48.31±5.82
Total Nitrogen [%]	1.33±0.14	NP	1.05±0.17
Proteins [%]	8.35±0.92	NP	6.56±0.93

NP=No presence

The pH is a key factor that influences the fermentation process and its value depends on the type of microorganism used; in the case of the *Lactobacillus* genus, they develop at pH values in the range of 5 to 7 (Da Silva *et al.*, 2021). It is observed that for the SCS, MWW and SCS solutions, the pH shows an acid trend, with values of 6.80, 5.60 and 6.70, respectively, being in the range for the development of the microorganism *L. acidophilus*. With respect to the TS content, when high values (19–25%) are present, mass transfer is affected by the low water content, causing an accumulation of by-products that inhibit the fermentation process (Ghimire *et al.*, 2017). In the waste under study, SCS has a high content of TS (29.81%), so it is not convenient to ferment the waste without an adjustment in this parameter, thus, in the solution of 150 g SCS/L the content decreased to 5.12% due to the dilution process. As for the volatile solids content (TVS), which is indicative of the amount of organic matter in the waste, values above 75% were observed, mainly due to the origin of the waste, with 90.85% for SCS, 74.60% for MWW, and 89.61% for the 150 g SCS/L solution.

The SCS and MWW, being residues obtained from sugar cane processing, are rich in carbohydrates; thus, for SCS the content was 172.42 g/L and for MWW 12.48 g/L. In the case of the SCS solution, the carbohydrate content decreased due to the dilution carried out, having a value of 48.31 g/L. These values show that the residues are viable to be used as substrate to carry out lactic fermentation, since environments rich in carbohydrates are ideal for the development of LAB (Vinicius De Melo Pereira *et al.*, 2020). Regarding the nitrogen and protein content, for SCS it is 1.33% and 8.35%, respectively while in MWW it is null, which may be because the facility from which the residue was obtained uses potable water for the mold washing operation and nitrogen, in the form of nitrites and nitrates, should be low. The presence of nitrogen in the substrate to be fermented is important because in the metabolism of microorganisms it acts as the main component of catabolic and anabolic processes (Rawoof *et al.*, 2021).

Lactic fermentation kinetics

Figure 1 shows the data of substrate consumption, LA production and cell density obtained during the lactic fermentation of the 150 g SCS/L solution. It can be observed that the highest consumption occurred during the first 24 h of the process. This represents a consumption of about 37% of the initial carbohydrates showing that the microorganism adapted to the SCS and MWW to perform the anaerobic bioconversion.

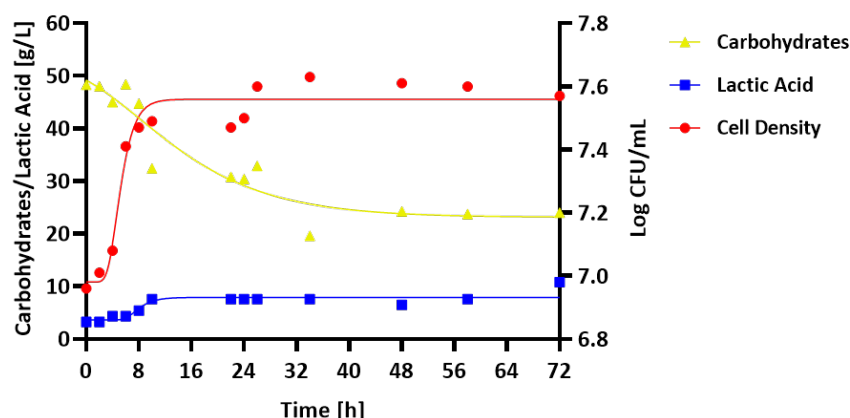


Figure 1. Substrate consumption, lactic acid production and cell growth during lactic fermentation of 150 g SCS/L solution.

For the lactic acid production parameter, it is observed that it begins to increase around 10 h, reaching its maximum value of 7.56 g LA/L. This value is similar to that reported by Guo *et al.* (2019), where they obtained a maximum production of 10.6 g LA/L during the study of glucose fermentation by applying nanobubble water. While Macedo *et al.* (2020) reported a production of 66.90 g LA/L during the fermentation of previously hydrolyzed cassava bagasse. The above values when compared with those obtained by other strains of the *Lactobacillus* genus are lower (Abedi *et al.*, 2020). A possible explanation may be to the low nitrogen content present in the substrates, since *L. acidophilus* bacteria require significant amounts of amino acids since they do not follow the metabolic pathway via pentose-phosphate in a fully functional way, obtaining other by-products (Solval *et al.*, 2019).

Regarding cell density, it was observed that the microorganism showed affinity for the substrate, mainly due to the high carbohydrate content. Microbial growth occurred around 8 h of the process, increasing from 6.96 to 7.47 log

CFU/mL, then during the rest of the fermentation it remained stable, with an average value of 7.56 log CFU/mL. Romero-Mota *et al.* (2023) report values of 7.80 and 8.20 log CFU/mL, in the first 10 h during fermentation of sugarcane residues at 100 and 150 rpm, concluding that microbial growth and process yield is affected by agitation speed. As it is possible to observe, the cell density values are similar, since in the present study the agitation speed used was 120 rpm.

In relation to the kinetic parameters, both models presented a good fit to the experimental data, being better in the Logistic model with a value of $R^2 = 0.9082$ (Table 2), likewise, a value of $\mu_{max} = 1.24 \text{ h}^{-1}$ was reached with the Gompertz model, while with the Logistic model a value of $\mu_{max} = 0.32 \text{ h}^{-1}$ was obtained. These values are higher than those reported by Estrada-García *et al.* (2023), who reported specific growth rates between $0.0568 - 0.1689 \text{ h}^{-1}$ during the bioconversion of swine waste with *L. acidophilus* at different agitation speeds. On the other hand, Romero - Mota *et al.* (2023) reported values between $0.04 - 0.23 \text{ h}^{-1}$ in the anaerobic fermentation of agricultural waste using the same microorganism. This is due to the fact that SCS and MWW have a high carbohydrate content, and are solubilized, resulting in increased growth of *L. acidophilus*. Despite the above, it can be observed that the lag phase is high, 20.46 h for Gompertz and 25.39 h for Logistic, which indicates a long period of adaptation of the microorganism to the substrate. It is possible that this behavior is due to the low content of other nutrients that are essential for the development of the microorganism. Similar results were obtained by Akdemir *et al.* (2021) during the study of the survival of *L. acidophilus* in three different media, where the strain presented a higher specific growth rate (0.58 h^{-1}) in white rice milk, but a lower lag phase (10.94 h) due to the low nutrient content of the medium.

Table 2. Kinetic parameters of *L. acidophilus* growth during lactic fermentation of 150 g SCS/L solution.

Model	$\mu_{max}[\text{h}^{-1}]$	$\lambda[\text{h}]$	$A[\text{Log cel/mL}]$	SS	R^2
Gompertz	1.2420	20.46	7.585	0.0628	0.9074
Logistic	0.3214	25.39	7.584	0.0623	0.9082

Conclusions

The physicochemical characterization of SCS and MWW shows that these wastes possess carbohydrates and nitrogen, vital nutrients for the development of LAB. Since SCS has a high content of TS, it is necessary to carry out a dilution before the fermentation process, so the use of MWW as a diluent is useful for reducing the environmental impact of this waste. In the monitoring of the process, it was observed that both wastes promote the development of the microorganism *L. acidophilus*, having a maximum growth of 7.63 log CFU/mL, a removal of 50.32% of carbohydrates and a maximum production of 7.56 g LA/L. However, the kinetic parameters of cell growth were higher than those reported by other studies, indicating that the microorganism *L. acidophilus* needs more time to adapt to the residues generated in the production of non-centrifugal cane sugar, so it is necessary to explore strategies to reduce the value of these parameters in order to optimize the fermentation process. With this, lactic fermentation emerges as a viable option to be implemented as a new strategy for the utilization of the residues generated in the production of non-centrifugal cane sugar, allowing to valorize them and reduce their impact on the environment.

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