

# Sugarcane bagasse as a material in the production of polylactic acid

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## Original Research

## Abstract:

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Sugarcane bagasse (SCB) is an abundant, renewable, and low-cost lignocellulosic material from sugarcane juice extraction. The SCB was used as a substrate for the production of lactic acid (LA), with *Lactobacillus helveticus*/*Streptococcus thermophilus* as bacteria, in different fermentation conditions: (4, 8, 24, 48, 72) hours, and (37, 40)°C. The highest concentration of LA was 3.56 g/L at 40°C after 72 hours of fermentation. The polymerization of LA was the ring opening method, obtaining 1.15 g of PLA with a yield of 19.29% and 0.12% concerning lactide and SCB. Although the concentration obtained is low concerning other research, the SCB use in countries with high sugar cane production represents an eco-friendly proposal for producing organic acids and biobased polymers with properties similar to the synthetic origin. This research provides an environmental solution to the problem of agribusiness waste and a proposal with the potential to develop new products.

**Keywords:** Acid lactic; Bioplastics; Sugarcane; Waste revaluation

## 1. Introduction

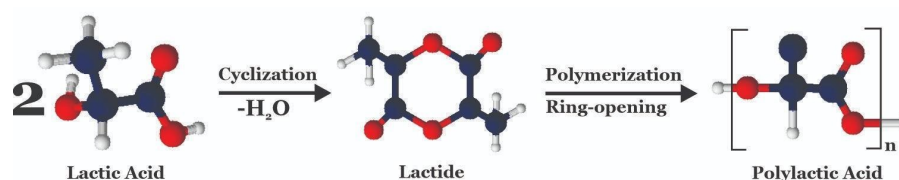
Polylactic acid (PLA) is an aliphatic, biodegradable, biocompatible, and bioabsorbable polyester with mechanical properties similar to those of polyethylene terephthalate (PET), polystyrene (PS) and other common petroleum-derived plastics [1]. Its mechanical, thermal, and rheological properties make it a polymer with diverse applications, among which the medical industry, food packaging, and textiles stand out, among others [2].

PLA reports final properties that depend on the stereoisomers used in the raw materials (lactic acid or lactide) and the conditions used to polymerize them. The synthesis of PLA occurs through lactic acid polymerization through different processes: direct polycondensation, ring opening polycondensation (ROP), azeotropic dehydration, and enzymatic polymerization [3]. The conventional production process of high molecular weight PLA requires high temperatures, low pressures, and a long time. In recent years, ROP and lactic acid polycondensation are the most used to manufacture

high molecular weight PLA in less time with mild reaction conditions [4].

The ring-opening polymerization method (Fig. 1) begins with the oligomerization reaction of lactic acid (LA). The concentration of LA occurs by releasing water molecules during distillation, and the appearance of oligomers begins, which ends in lactide formation. A catalyst is added to improve the polymerization process and increase the molecular weight of the polymer chain [5].

LA is the precursor to PLA and can be synthesized chemically or biotechnologically [6]. For chemical synthesis, ethylene from a petrochemical feedstock is oxidized in a metal catalyst presence to produce acetaldehyde. Reacts in the liquid phase and at high pressure with hydrogen cyanide in the presence of a base to produce lactonitrile. After recovery, the product is distillate to purificate. It is then hydrolyzed with hydrochloric acid or sulfuric acid, producing ammonium salt and lactic acid. LA is esterified with methanol to produce methyl lactate, which is recovered and



**Figure 1.** Ring opening polymerization reaction.

purified by distillation. Methyl lactate is subjected to hydrolyzation with water in the presence of the acid catalyst to produce lactic acid and methanol, the latter being recovered and recycled in the process [7]. The method described produces a racemic mixture of lactic acid (D/L-LA), this being of lower commercial value when compared to the pure isomers because it has a specific use on an industrial scale [8].

Another route for the chemical synthesis of LA consists of oxidizing propene to  $\alpha$ -nitropropionic acid, using nitric acid in the presence of oxygen. Subsequently, the acid obtained is hydrolyzed to convert it into lactic acid [9]. Other chemical routes include the oxidation of propylene glycol, carbon monoxide, and water at high temperature and pressure, hydrolysis of chloropropionic acid, hydrolysis of ester derivatives, decarboxylation of some derivatives of the acid 2-methylmalonic acid, among others [7–10].

The chemical synthesis of LA is neither economically viable nor environmentally sustainable due to the problems involved in raw materials of petrochemical origin. The production of LA through biotechnological processes concerning chemical synthesis requires lower process temperatures, lower energy consumption, and the possibility of using low-cost substrates [11]. Approximately 90% of the lactic acid sold globally comes from microbial fermentation of first-generation substrates, such as sugar cane juice and starch from some cereals [12]. However, its disadvantage is the use of raw materials that compete with the alimentary needs of the population.

Lignocellulosic biomass use represents a promising alternative for acid lactic production due to its wide availability, renewability, low cost, and because it is not food. The most widely used lignocellulosic sources include agricultural waste, forest woody feedstocks, energy crops, industrial waste, and the organic fraction of municipal waste [13]. Previous research describes the synthesis of lactic acid from various agro-industrial waste, such as orange peel and cheese whey, using *Lactobacillus-type* bacteria in the fermentation. Within agricultural biomasses, there is also corn stubble, cereal straw, woody residues, and sugarcane bagasse, among others [8].

Sugar cane (*Saccharum officinarum*) is a biomass of interest due to the large quantities of bagasse that remain as waste after sugar extraction. In Brazil, for example, during the years 2015 and 2016, it is estimated production of more than 666 million tons of sugar cane, with an approximate generation of 250 to 270 kg of bagasse/ton of sugar cane [14]. Ecuador, for the year 2017, 2,203 thousand tons of agro-industrial waste were estimated, of which about half came from the sugar mill [15].

Sugarcane bagasse (SCB) is mainly composed of cellulose, hemicellulose, and lignin, raw material for the production of different bioproducts (adsorbents, nanomaterials, fuels) or platform molecules intended for the production of chemicals such as lactic acid, the precursor of polylactic acid [16–18]. According to the Vasant Institute of India, 50% of the available SCB becomes in sugar platform molecules, working with 70% efficiency in the pretreatment and saccharification process between 40 and 5 million tons of fermentable sugars. If only the glucose obtained through a homofermentative route with 85% efficiency is valued, between 2.4 and 3 million tons of LA can be produced [19].

From this perspective, countries that produce sugar from sugar cane have great potential to produce LA from this biomass. Furthermore, in many rural contexts around the world, waste of this type is often burned in the open for land maintenance or simply as a disposal mechanism [20]. According to the Food and Agriculture Organization of the United Nations, the generation of CH<sub>4</sub> in 2021 from the burning of agricultural waste was 1275.18 kilotons in the year alone [21]. The environmental impact caused by agro-industrial development is visible in the volume generated by agro-industrial byproducts or waste in South American countries with high agricultural activity, such as Colombia, Paraguay, and Ecuador [22].

Considering the economic importance of agriculture in Ecuador, as well as the existing availability of sugarcane bagasse, the use of this waste as a substrate in lactic fermentation to obtain lactic acid and its subsequent polymerization to acid polylactic as it is one of the main applications of LA.

## 2. Experimental

### 2.1 Waste selection

The SCB used comes from the Ecuadorian coast. The waste was washed and dried in a Memmert oven at 100 °C until reaching a constant weight. Then, it was milled in a Tecnal TE-680 Wiley-type mill to reduce its size to 10 Mesh.

### 2.2 Waste characterization

The waste characterization included humidity, pH, ash, volatile matter, fixed carbon, cellulose, lignin, extractives, hemicellulose, and holocellulose. Moisture determination was by weight difference with a PW 254-ADAM thermobalance [23]. With the official AOAC method, 973.41 pH was determined [24]. The content of ash, volatile material, and fixed carbon using BS EN 14775 [25] and BS EN 15148 [26]. Carbon, nitrogen, hydrogen, and sulfur content using BS EN 15104:2011 [27].

The cellulose and lignin content were determined as de-

scribed by Domínguez Domínguez et al. [28]. For the amount of cellulose weighed 1 g of sample, 15 mL of 80% (v/v) acetic acid and 1.5 mL of nitric acid concentrated were added. The solution was stirred for 20 minutes, filtered, and washed with 99.9% (v/v) ethanol. The sample was dried at 100–105 °C for 90 minutes and taken to a balance to record its weight (material A). The sample was then incinerated at 540 °C for 3 hours and weighed again (material B). The Eq. (1), was used to determine cellulose content.

$$\text{Cellulose}(\%) = \frac{\text{material A} - \text{material B}}{\text{weight of the sample}} * 100\% \quad (1)$$

To determine the lignin, 1 g of sample weight and 70 mL of 1.5% (v/v) sulfuric acid solution were added. The mixture was stirred for 2 hours, filtered, and washed with distilled water. A 72% (v/v) sulfuric acid solution was added and stirred for 4 hours. It was washed with plenty of distilled water, filtered, and dried for 120 minutes in the oven at 100–105 °C. It was then weighed (material C) and incinerated at 540 °C for 3 hours. Finally, it was weighed (material D), and the lignin content present in the sample was determined using Eq. (2).

$$\text{Lignin}(\%) = \frac{\text{material C} - \text{material D}}{\text{weight of the sample}} * 100\% \quad (2)$$

The sample was subjected to Soxhlet extraction to determine the extractables Eq. (3). The volume of the balloon filled 3/4 using n-hexane as a solvent. 12 g of the sieved sample was placed in the equipment thimble and left to act for 5 hours. The material free of extractables was weighed and heated at 100 °C for 2 hours. The dried sample was placed in a desiccator for 15 minutes to cool, and its weight was noted [29].

$$\text{Extractables}(\%) = \frac{12 - \text{weight of the sample}}{12} * 100\% \quad (3)$$

Following the methodology proposed by Loja Sánchez [30], holocellulose Eq. (4) and hemicellulose Eq. (5) content were determined. 2 g of the sample free of extractables were weighed and placed in a flask together with 160 mL of distilled water, 1 g of sodium chlorite and 0.2 mL of concentrated acetic acid, and heated in a thermostatic bath between 70 °C and 80 °C. After 1 hour, the same amount of sodium chlorite and acetic acid was added. The process is repeated three times for 3 hours until there is a change in the color of the sample. The sample was then cooled to 10 °C, filtered, and washed with 500 mL of cold distilled water. The waste was heated at 105 °C for 3 hours until reaching a constant weight.

$$\text{Holocelullose}(\%) = \frac{\text{weight of the waste}}{\text{sample weight without extract}} * 100\% \quad (4)$$

$$\text{Hemicelullose}(\%) = \% \text{Holocelullose} - \% \text{Celullose} \quad (5)$$

### 2.3 Lactic fermentation

The sugarcane bagasse was subjected to acid hydrolysis with 2% (v/v) HCl, with a 1:10 (m:v) ratio, for 30 minutes at a temperature of 75 °C [31]. *Lactobacillus helveticus/Streptococcus thermophilus*, from the company CHR

HANSEN, was used in the lactic fermentation, using MRS agar as a culture medium with a streaking technique. The microorganism was made at 37 °C for 24 hours. 10 mL of inoculum prepared in a liquid medium of peptone water enriched with 20% (m/v) sucrose, with the microorganism at a volume of 100 mL at 37 °C for 12 hours [32].

The hydrolysate was filtered and adjusted to pH 5–6 with 5N NaOH, and the sugar content with 20% (m/v) sucrose was regulated. The inoculum with the hydrolysate was placed in hermetically sealed flasks and taken to an oven to evaluate at two operating temperatures (37 and 40) °C, taking samples at 0, 4, 8, 24, 48, and 72 hours.

### 2.4 Determination of lactic acid concentration

For the identification and quantification of LA, an ACCELA high-performance liquid chromatograph from Thermo Fisher Scientific, equipped with a C18 reversed-phase column (5 μm; 4.6 × 100 mm) and a UV-VIS diode array photodetector (PDA). The binary mobile phase used was made up of an aqueous solution of 3 × 10<sup>-2</sup> M of phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) and an HPLC grade acetonitrile (C<sub>2</sub>H<sub>3</sub>N) solution, in a proportion of 88:12 (v/v) respectively, with a constant flow of 1000 μL/min. Detection was at a wavelength of 210 nm, an injection volume of 20 μL, a controlled temperature of 40 °C on the column, and a total run time of 3 minutes [33].

In the HPLC measurement, the fermentate was centrifuged and filtered. It was subjected to microfiltration with a hydrophilic nylon membrane (Biomed Scientific), with a diameter of 13 mm and 0.45 μm pores. The concentration and extraction of lactic acid were done by centrifuging the fermentation broth for 25 minutes at 5000 RPM and vacuum filtering using 125 μm membranes. To reduce the amount of water of the supernatant placed in a thermostatic bath at 70 °C [34]. The amount of biomass was determined by dry weight, drying the residual biomass in an oven at 60 °C until reaching a constant weight [35]. The extraction of the resulting liquid was done with diethyl ether in equal proportions. The supernatant was distilled at 40 °C for approximately 2 hours to evaporate the solvent, extracting and concentrating the LA sample.

### 2.5 Modeling the kinetics of lactic acid production

The kinetics of lactic acid production were evaluated using three mathematical models: the logistic model Eq. (6), simple production Eq. (7), and Luedeking and Piret Eq. (8), as described [36, 37].

$$P = \frac{P_f}{1 + ae^{-kt}} \quad (6)$$

$$\frac{dp}{dt} = \alpha \frac{dX}{dt} \quad (7)$$

$$\frac{dp}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (8)$$

P is the production of the product (lactic acid) (g/L), P<sub>f</sub> is the maximum production of lactic acid, a is the y-intercept, k is the slope of the linearized equation, t is the time (h), X is the biomass (g/L), α is a dimensionless constant, β is the production constant (h<sup>-1</sup>).

The best fit of the experimental data with the mathematical models was determined, with the statistical coefficients reduced Chi-square ( $\chi^2$ ) and root mean square error (RMSE) using Equations (9) and (10).

$$\chi^2 = \frac{\sum_{i=1}^N (P_{\text{exp},i} - P_{\text{pre},i})^2}{N - n} \quad (9)$$

$$\text{RMSE} = \sqrt{\frac{1}{N} \sum_{i=1}^N (P_{\text{exp},i} - P_{\text{pre},i})^2} \quad (10)$$

$P_{\text{exp}}$  is the concentration of the experimental product,  $P_{\text{pre}}$  is the concentration of the predicted product,  $N$  is the number of samples, and  $n$  is the coefficient not associated with growth.

## 2.6 Polymerization of lactic acid by the ring opening method

The lactic acid polymerization made with the ring-opening method produces a cyclic diester called lactide by esterification. A 50 mL sample of LA was taken and mixed with 2.5 mL of 60% (v/v) sulfuric acid. The mixture was heated to 80°C with magnetic stirring at 300 RPM for 35 minutes, using an IKA<sup>®</sup> C-MAG HS 7 heating plate. The temperature was increased to 120°C and held for 2 hours. 2.5 mL of H<sub>2</sub>SO<sub>4</sub> was added, and after 5 hours, a new addition of the acid with the same volume. After 2 hours, lactide was obtained [38].

The polymerization was done in a flask by adding 6 g of lactide, 50 mL of methanol, and 3 g of tin II chloride. The working conditions were adjusted to 60°C and 300 RPM, obtaining a precipitate with the LA. The removal of methanol was by evaporation at a temperature of 78°C. The precipitate was washed with acetone and filtered with a GAST model DOA-P704-AA vacuum pump.

The polylactic acid characterization was a Thermo Scientific brand Nicolet Summit PRO infrared spectrometer. The equipment was backgrounded, adjusting the IR detection limits at 500 to 4000 cm<sup>-1</sup>, and then a sample of the dry polymer was placed in the equipment for analysis [5].

## 2.7 Data processing

The processing of the experimental data and the calculation of the kinetics of LA production was possible with the software Statistica version 10 and OriginPro version 9.6.5.169.

# 3. Results and discussion

## 3.1 Raw material characterization

Table 1 presents the results obtained about moisture, pH, extractables, lignocellulosic composition, and proximal and elemental analysis of sugarcane bagasse.

Determining the most suitable applications for waste requires a complete characterization that includes the measurement of fixed carbon, moisture content, volatile matter, pH, granulometry, and others [39]. The characteristics of sugarcane bagasse vary depending on its origin, given that if the process is in artisanal mills or industrial mills, the transformation practice and the products obtained are different,

**Table 1.** Characterization of sugarcane bagasse.

Variable	Value $\pm \sigma$
Moisture (%) (w/w)	7.94
pH	3.35 $\pm$ 0.09
Extractables (%)	23.16
Cellulose (%) (w/w)	68.37 $\pm$ 0.31
Lignin (%) (w/w)	18.90 $\pm$ 0.35
Hemicellulose (%) (w/w)	3.06 $\pm$ 0.16
Holocellulose (%) (w/w)	71.27 $\pm$ 0.39
Ashes, %(w/w) DB	3.24
Volatile matter %(p/p) DB	82.21
Fixed carbon %(p/p) DB	14.55
Carbon %(w/w) DB	43.82
Nitrogen %(w/w) DB	0.24
Hydrogen %(w/w) DB	6.59
Sulfur %(w/w) DB	0
Oxygen % (w/w) DB	49.35

DB: Dry base

affecting the characteristics of the SCB. The moisture values are higher in the fresh SCB samples (43.94 to 44.42%) compared to the stored SCB samples (37.82 to 39.09%). The percentage of sugar in SCB of artisanal origin triples about SCB of industrial origin. In contrast, the SBC of industrial origin contains more cellulose, hemicellulose, and lignin than the artisanal origin [40].

The moisture of the residue is between 7.26% and 15.41%, as reported in similar investigations [41–43]. A lower moisture content in other results obtained may be due to the analysis procedure used in the experimentation and its ambient temperature [44]. On the other hand, low water activity inhibits the growth of microorganisms, and water activity in plant biomass could increase with moisture content. Therefore, a low moisture content should deactivate microorganisms and inhibit fermentative processes [45].

The pH has an acidic character in the raw material since the sugars present are susceptible to fermentation processes, forming a mixture of carboxylic acids, aldehydes, and ketones [42]. The pH of the biomass later affects the fermentation performance, as it reduces with the decrease in pH [46]. The number of extractables presented higher results than those of Cabrera et al. [47], who indicates that heterogeneity depends on factors such as the variety of sugar cane, the characteristics of the land, or the cultivation methods harvesting and processing, which cause this type of variation.

Ash, holocellulose, cellulose, hemicellulose, and lignin for SBC are between 1–4%, 50–84%, 32–55%, 27–32% and 19–25% respectively. The differences may be because the chemical composition of the lignocellulosic fibers depends on the species and variety of sugar cane from which the bagasse is, the type of soil used and climatic conditions of its cultivation, part of which the fibers used, age of the plant, and others factors [44].

Recent advances demonstrate that pure cellulose and hemicellulose/xylan could be used as raw materials in lactic acid production with satisfactory yield [48]. On the other

hand, low content of proteins, lignin, and hemicellulose in biomass produces unfavorable conditions for lactic acid bacteria [49]. Materials with high cellulose and low lignin content are better candidates for lactic acid synthesis by fermentation because the highest percentage of the fermentable sugars needed by lactic acid bacteria is obtained from cellulose [50]. Materials with high cellulose and lignin content can affect the mechanical properties of polylactic acid for its application in packaging, construction, and other fields [51].

Regarding the elemental analysis, the results obtained are similar to those reported by Xie et al. [52] in their research.  $H^+$  was mainly responsible for the selective fractionation of hemicellulose and cellulose from lignocelluloses to soluble polysaccharide fragments and the subsequent conversion of polysaccharide to monosaccharide [48]. The carbon and nitrogen source used as a substrate in fermentation affects lactic acid production [53]. The proteins present in the waste can be hydrolyzed into nutrients for growth and the production of lactic acid, which translates into a minimum requirement for nitrogen supplements in the simultaneous saccharification and fermentation process [54].

Previous research indicates that the higher the purity of the fermentation substrate, the higher the purity of the final product. Likewise, the appropriate combination of biomass composition, depolymerization method, and microorganism with a metabolism contributes to obtaining glucose monomers and a high yield in lactic acid production [55]. In the polymerization of lactic acid, if the latter has impurities in its composition or during its purification, racemization occurs in the biopolymers. When performing polymerization by the established method (ROP), the lactide obtained may present impurities (acids or oligomers), which are generated during depolymerization or purification and whose presence may affect the yield or molecular weight of the desired polylactic acid, hurting the properties of the said polymer [56].

### 3.2 Kinetics and modeling of lactic acid production

Table 2 shows the results obtained by applying the methodologies described in determining the concentration of lactic acid and the amount of biomass present in each sample.

The best working conditions for lactic acid production were 40 °C, obtaining the highest concentration of 3.56 g/L in 72 hours. Concentrations of 67 g/L were reported for lactic acid production from cellulose extracted from the SCB with *Lactobacillus delbrueckii* and with a simultaneous fermentation and saccharification (SSF) process at 42 °C and 72 hours. SSF in lactic acid production offers the advantage of operating the bioreactor in adequate and optimal conditions for cellulose hydrolysis and microorganism growth [57]. 84.2 g/L of lactic acid after 72 hours of fermentation was obtained by using sugar cane molasses as a carbon source by adding calcium carbonate to the culture medium [58]. This difference may be because molasses can provide better cell growth due to the presence of nitrogen in its composition and its buffering action [53].

On the other hand, the neutralizing agent used affects lactic acid production.  $Ca(OH)_2$  and  $CaCO_3$  are generally ap-

**Table 2.** Experimental results for the kinetics of the fermentation process.

Temperature (°C)	Time (h)	Biomass (g/L)	Concentration LA (g/L)
37	0	0.000	0.72
	4	1.112	2.21
	8	1.262	2.26
	24	1.348	2.38
	48	1.702	2.44
	72	2.784	2.48
40	0	0.000	0.72
	4	2.288	2.06
	8	2.422	2.82
	24	2.46	3.43
	48	3.851	3.50
	72	4.0964	3.56

plied in industrial-scale lactic fermentation because these neutralizing agents make the subsequent process easy and less expensive than other pH-controlling agents.  $Ca(OH)_2$  significantly facilitates lactic acid production compared to  $KOH$  or  $NH_4OH$  when using *Escherichia coli* as the microorganism. Likewise,  $NaOH$ -based fermentation is an environmentally friendly process that avoids the generation of precipitated waste; however, this agent could increase the osmotic pressure of the medium, causing stress to microbial cells [53, 59].

The pH during fermentation was 6.09, and the correct development of the process requires a pH between 5 and 7. The production of lactic acid affects the decrease in pH. Consequently, there is inhibition in the activity of the microorganism, which negatively affects the productivity and performance of the process. pH, unlike temperature, has more influence on the fermentation [60]. In hydrolysates with a pH of 4 and without control, constant yields during lactic fermentation can be achieved, which results in weak hydrolysis under low pH conditions. The highest proportions of lactic acid production expecting in simultaneous saccharification and fermentation processes at pH 5 and 6 [54, 61].

The essential stage for the design of a successful fermentation process is the formulation of the culture medium. Lactic acid bacteria are considered microorganisms demanding in their nutritional requirements since they need complex amino acids and vitamins for correct growth, which are specific for each strain [55]. In this sense, the low concentrations obtained compared to those reported in the bibliographies may be due to variations in the pH and the nutritional conditions of the culture medium, which inhibited the optimal growth of the bacteria.

The large-scale fermenter use is hampered by the low tolerance of strains to process stresses, such as substrate and product toxicity, and other fermentation inhibitors, which affect final product yield and productivity rates. Attempts to improve industrial efficiency include statistical designs of experiments and mathematical techniques such as process modeling, bringing successful results [62].

**Table 3.** Evaluation of mathematical models.

Temperature/Models	$x^2$		RMSE	
	37 °C	40 °C	37 °C	40 °C
Logistic	7.2516	6.9475	2.6929	2.6358
Simple production	0.7877	0.2848	0.8102	0.4871
Luedeking and Piret	0.9846	0.3560	0.8102	0.4871

$x^2$ : Chi Cuadrado Reducido; RMSE: Error Cuadrático medio de la raíz.

In this research, three mathematical models were applied to describe the kinetics of lactic acid production at the established temperatures and times (Figure 2), showing an increase in the concentration of LA at the two fermentation temperatures as time passes.

Kinetic models describe a specific part of the fermentation process due to the complexity of representing certain factors related to cellular metabolism and its environment [63]. Such is the case of the logistic equation, which uses a substrate-independent model to represent the inhibition of cell growth in fermentations. Likewise, the Luedeking-Piret model and the simple production model describe the formation of lactic acid, which depends on the biomass concentration and the growth rate [64]. Table 3 shows the statistical coefficients with which the fit of the experimental data to the mathematical models was evaluated.

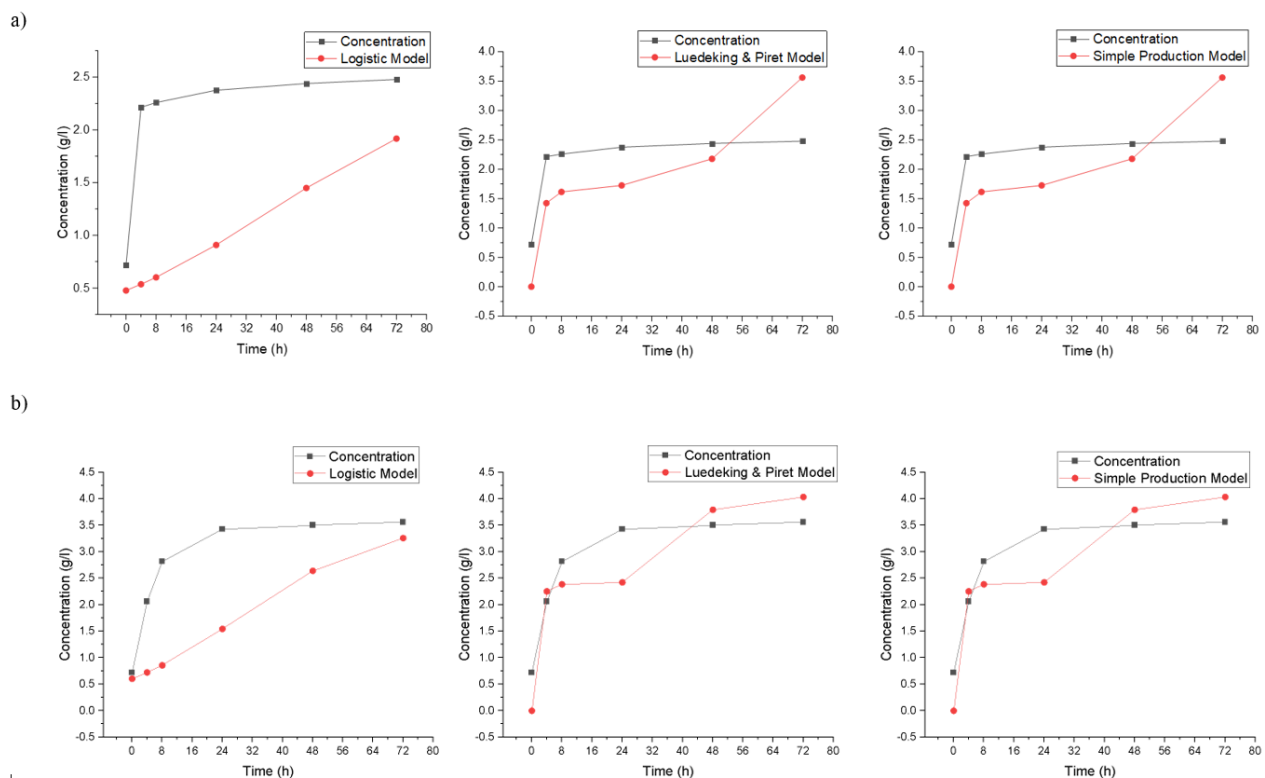
The results indicate that the Luedeking-Piret equation and simple production at temperatures of 37 °C and 40 °C have a better fit, unlike the logistic model. The results at 40 °C for both coefficients are much smaller, this temperature be-

ing the most appropriate when carrying out the fermentation process, given that the smaller these values are, the closer the predicted and observed values are. That happens in the first 8 hours and after 40 or 48 hours.

In research where the production and kinetics of LA were analyzed through batch fermentation, corn residues were used as a substrate, and *Lactobacillus delbrueckii spp. Bulgarian*. Kinetic models proposed for LA production, bacterial growth, and substrate consumption. The Luedeking-Piret and logistic models had the best fit for the experimental data for LA production, substrate consumption, and bacterial growth, respectively [65]. The application of this model would improve the accuracy of bioprocess scale-up to enable industrial-scale production [66].

### 3.3 Ring opening polymerization of lactic acid

After 9 hours of polymerization, the cyclic diester of lactic acid, called lactide, was obtained; its synthesis resulted in a brown viscous fluid. In related research, they achieved similar results when using the same methodology or adaptation



**Figure 2.** Mathematical models applied to the fermentation of sugarcane bagasse with *L. helveticus/Streptococcus thermophilus*. a) 37 °C, b) 40 °C.

of it [67, 68]. This coloration may be due to the presence of  $\text{H}_2\text{SO}_4$  and its reaction with lactic acid. In research where sulfuric acid was used as a catalyst for the esterification reaction, it was evident that the viscosity and color darkened as the concentration of  $\text{H}_2\text{SO}_4$  increased [69].

Although 18 g of lactide was obtained in the pre-polymerization, 6 g of the compound was taken for the polymerization reaction, producing 1.16 g of the polymer after 1 hour. The polymerization yield was 19.29% about g of PLA/g of lactide and 0.12% about g of PLA/g of raw material. Nyiavuevang et al. achieved a yield of 49.04% at 180 °C using the ring opening method. In similar works, percentages near 95% were obtained by applying the same ROP method at temperatures of 180 and 150 °C [56, 70]. In another work in which commercial lactic acid with 88% purity was used as a precursor, a yield of 73% was achieved using the same method [5]. The differences between the results obtained from this research and those mentioned may be due to the time spent in polymerization. The efficiency of the polymerization process depends on the purity of the LA. Process temperature, reaction time, and pH affect polymer synthesis. On the other hand, the presence of residual sugars in lactic acid can significantly affect performance [70].

In the infrared spectrum (FTIR) that was used to identify the polymer (Figure 3), the different peaks of the functional groups that make up the polylactic acid. It is comparable to the spectrum of PLA synthesized from lactic acid obtained from whey fermentation [34]. The characteristic FTIR spectrum of a polymer lies between values greater than or equal to  $1500\text{ cm}^{-1}$  and can be used to recognize and characterize the material [71]. There are characteristic groups of polymers such as PLA; the -OH group is found in band ranges of 3700 – 3020, followed by the -CH- bonds between 3000 – 2890, the carbonyl group (C=O) between the bands 1800 – 1680 and the -CH<sub>3</sub> between 1360 – 1390 [72].

To demonstrate the presence of polylactic acid, two of the four functional groups mentioned are necessary, these being the carbonyl group and the -OH group [5, 67]. In the PLA spectrum, it is possible to see peaks at 1668.12 (-C=O) and

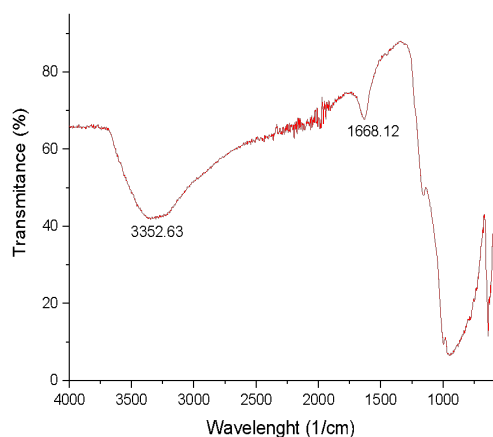


Figure 3. Spectrum of the PLA obtained.

3352.63 (-OH). These quantities are within the ranges of the bands. The functional groups detected within the range  $1300$  to  $1800\text{ cm}^{-1}$  are characteristic of PLA polyester [73], confirming the presence of the polymer in the analyzed sample.

Previous research where PLA was synthesized from commercial lactic acid presented spectra shifted slightly more to the right [5, 67, 74]. This difference is due to the temperature and polymerization times compared to this research. Impurities from the fermentation and polymerization processes also impact the characteristics of the resulting polymer. The operating conditions and the methods to purify both the polylactic acid and its precursor are factors that affect the purity of the product and directly affect when carrying out the respective analyses [72]. On the other hand, the composition of the polymer affects its morphology, in addition to its thermal and mechanical behavior [75].

## 4. Conclusion

The best results were obtained at a temperature of 40°C and a time of 72 h, with the simple production model being the one that best fits the fermentation process. Although LA and PLA were obtained, their performance was low for the established operational conditions compared to what was obtained in related research, making their scaling to an industrial level unfeasible. Factors such as purity, pH, temperature, and reaction time affect the performance of the synthesis process. Other materials with lignocellulosic characteristics could be explored for the production of LA and subsequent synthesis of PLA, evaluating the biodegradable and mechanical characteristics of the polymer obtained to explore the possibilities to improve the properties of PLA.

### Authors contributions

All authors contributed equally in performing experiments or calculations, data analysis, and preparing the manuscript.

### Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### Conflict of interests

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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