Polylactic acid PLA production from alternative substrates submerged fermentation with *Rhizopus oryzae*

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Resumen
Esta investigación tuvo como objetivo obtener un bioplástico a escala de laboratorio a partir de sustratos generados mediante fermentación sumergida, mediante un proceso dividido en dos fases: fermentación y polimerización. Primero, se seleccionó el microorganismo y se formularon medios de cultivo convencionales y alternativos para la fermentación. En estos medios se produjo ácido láctico, materia prima para la polimerización. La fermentación se ejecutó a nivel laboratorio con la cepa seleccionada, y el ácido láctico generado se purificó y caracterizó mediante análisis fisicoquímicos. El experimento se realizó por triplicado en dos medios: PDA y residuos vegetales como sustrato alternativo. Se obtuvo ácido láctico en ambos casos, con concentración máxima de 9.34 g/L y pH promedio de 2.46 en el medio de residuos vegetales. La producción se confirmó mediante espectroscopía UV-Vis e infrarroja. En la segunda fase, se ajustó y llevó a cabo la polimerización, consistiendo en las etapas de preconcentración, oligomerización y polimerización, adaptadas a las condiciones y equipamiento del laboratorio. El bioplástico se caracterizó fisicoquímicamente. Las pruebas infrarrojas identificaron grupos funcionales específicos, mientras que el análisis reológico evidenció un comportamiento pseudoplástico. El peso molecular promedio del polímero fue 16,327.43 g/mol. Además, se realizaron ensayos de biodegradabilidad que resultaron positivos. Finalmente, un análisis económico evidenció la alta viabilidad del proceso para su aplicación en laboratorio.

Palabras Claves: *Fermentación, Residuos Vegetales, Bioplástico, Polimerización, Biodegradabilidad.*

Abstract
The objective of this research was to obtain a laboratory scale bioplastic from substrates obtained by submerged fermentation; a process, which consisted of two parts: a fermentation process and polymerization. First, the microorganism used was selected and the means of conventional and alternative crops for fermentation were formulated, where the material (lactic acid) was obtained that it will be used to get the polymerization; The fermentation process was performed at the laboratory level, in order to produce lactic acid from the microorganism strain, which was purified and characterized by physicochemical form. This was carried out in triplicate for two different culture media PDA and vegetable wastes as alternative substrate, obtaining lactic acid in both media, with a maximum concentration of 9.34 g / L and a pH of 2.46 for the average vegetable waste. To verify the production of lactic acid tests Ultraviolet visible and infrared spectrums were used. In the second step the process suitable polymerization to laboratory conditions as equipment, reagents and time was set; the process contained three steps pre-concentration, oligomerization and polymerization completed bioplastic production stages was characterized by physicochemical form. Infrared tests were performed to identify the functional groups, and observing rheological tests that it has a pseudoplastic behavior, and the calculation of the average molecular weight was 16327.43 g / mol. Besides, biodegradability tests that tested positive were performed. Finally, the economic analysis was obtained feasibility high of application to laboratory level.

Key Words: *Fermentation, Waste Vegetable, bioplastic, Polymerization, Biodegradability*

Introduction

The accumulation of substantial quantities of plastic waste represents a critical environmental challenge, primarily due to the recalcitrant nature of conventional plastics stemming from their non-biodegradable chemical architecture (Elnashar, 2011). Transitioning from petrochemical-derived plastics to biodegradable alternatives offers a viable strategy to mitigate the environmental burden imposed by persistent plastic residues. Biodegradable plastics can be managed as organic waste streams, enabling their disposal in sanitary landfills or controlled composting facilities where microbial-mediated degradation occurs over significantly reduced timeframes. Such bioplastics are typically synthesized from renewable biomass resources of both animal and plant origin (IBAW, 2005).

Polylactic acid (PLA) constitutes one of the most extensively investigated biodegradable polymers, synthesized via the polymerization of lactic acid monomers (García *et al*., 2004). Lactic acid (2-hydroxypropanoic acid), a chiral alpha-hydroxy acid, serves diverse industrial sectors including chemical synthesis, pharmaceuticals, food technology, and bioplastic manufacture, with production routes encompassing both chemical and biotechnological processes. Chemical synthesis methods often yield a racemic mixture containing equimolar amounts of the D- and L- stereoisomers, resulting in optical inactivity—a significant drawback for certain applications (Serna, 2005). Conversely, biotechnological production employs microbial fermentation, enabling the generation of optically pure enantiomers, either D(-) or L(+) forms, depending on the microorganism and fermentation conditions utilized (Hofvendahl & Hägerdal, 2000).

Among lactic acid-producing microorganisms, *Rhizopus oryzae*, a filamentous fungus classified within the *Zygomycetes*, is prominent for its capacity to biosynthesize optically pure L(+)-lactic acid with high yield efficiency. This strain displays metabolic versatility by fermenting diverse lignocellulosic agro-industrial residues, thereby valorizing waste streams while concomitantly producing lactic acid along with ancillary metabolites such as ethanol (Feng-Wei, 2023). Solid waste accumulation represents an additional anthropogenic environmental concern, where plant-derived biomass residues constitute a significant proportion. These plant wastes infiltrate subsurface lithospheric strata, potentially compromising water quality through leachate generation and pollutant dispersion (Eswaran *et al*., 2001).

Plant biomass is inherently rich in complex carbohydrates, rendering it an attractive carbon source for microbial bioprocesses, including the fermentative production of lactic acid by *R. oryzae*. This valorization pathway provides a dual environmental benefit by reducing net CO_2 emissions associated with plant biomass decomposition and generating value-added bioproducts. Therefore, the substitution of persistent synthetic polymers with biodegradable bioplastics derived from renewable carbonaceous feedstocks, such as lignocellulosic plant residues, emerges as a sustainable solution to ameliorate multiple environmental impacts.

The objective of this study is to develop an integrated bioconversion process utilizing submerged fermentation with *R. oryzae* to transform plant waste substrates into bioplastics precursors, thereby contributing to the reduction of environmental contamination linked to both conventional plastic persistence and agricultural residue accumulation.

Materials and Methods

Microorganism

The *R. oryzae* strain utilized in this study was procured from the Venezuelan Center for Microorganism Collection (CVCM), accession number 3031, at the Central University of Venezuela (UCV). Subsequently, the biomass was subjected to lyophilization for preservation.

Reactivation of *R. oryzae* strain

Lyophilized *R. oryzae* strain was reconstituted using sterile deionized water and subsequently inoculated onto Potato Dextrose Agar (PDA) medium. The inoculated plates were incubated under ambient laboratory conditions (approximately 22–25°C) for a period of five days.

Morphological characterization was performed to confirm fungal identification, in accordance with established protocols (Guevara *et al*., 2010; FAO, 1985; Schaad & Jones, 2001).

Inoculum Preparation

Spores were harvested from mature *R. oryzae* cultures cultivated on Potato Dextrose Agar (PDA) plates. The spore suspension was prepared by rinsing the agar surface with 10 mL of 0.1% (w/v) Tween-80® aqueous solution to facilitate spore detachment. The resultant suspension was then subjected to filtration to remove mycelial fragments and agar debris. Spore concentration was quantified using a hemocytometer (Neubauer chamber) under microscopic examination, following established protocols (Sánchez-Rosario & Sánchez, 2011; Khunnonkwaoa *et al*., 2012; Gil-Horán *et al*., 2008; Guevara *et al*., 2010; Cañedo & Ames, 2004).

Culture media

The alternative culture media utilized for submerged fermentation processes consisted of the following raw material concentrations (g/L): potato (200), sweet potato (330), carrot (100), beetroot (300), and cassava (100). This base was further supplemented with (g/L): sodium phosphate (0.40), ammonium sulfate (2.0), magnesium sulfate (0.25), and zinc sulfate (0.40). Vegetables were subjected to washing, mechanical crushing, and drying in accordance with standard COVENIN 1156-79, maintained at 70 °C for a duration of 4 days. The conventional culture medium employed was Potato Dextrose (PD) broth, prepared by boiling 200 g of potato in 1 L of distilled water, followed by filtration through cheesecloth, dilution at a 1:1 ratio with distilled water, and supplementation with glucose at 30 g/L. The pH of all culture media was adjusted to 6.0 prior to inoculation.

Fermentation Process

Bioreactors consisted of 250 mL Erlenmeyer flasks containing 50 mL of either the alternative or conventional fermentation medium. The culture media were sterilized by autoclaving at 121 ºC for 15 minutes, followed by aseptic inoculation with a *R. oryzae* spore suspension at a concentration of 1 × 10^6 spores/ml. The bioreactors were incubated at 30 ºC with agitation at 150 rpm on an orbital shaker for periods of 24, 48, and 72 hours. Fermentation at each time point was terminated by thermal inactivation at 80 ºC for 15 minutes. Subsequently, the fermentation broth was filtered through pre-weighed Whatman No. 5 filter paper to separate the biomass (Bulut *et al*., 2004). Experiments were conducted in triplicate.

Lactic Acid Recovery and Biomass Quantification

Lactic acid produced during fermentation was recovered from the supernatant via a liquid-liquid extraction process. In 250 mL flasks, the fermentation broth was acidified to pH 3 using 1 N hydrochloric acid, followed by the addition of ethyl acetate as the organic solvent. The mixtures were agitated at 150 rpm on an orbital shaker for 30 minutes. The resultant biphasic mixture was transferred to a separating funnel to isolate the organic phase. The collected organic phase underwent simple distillation to evaporate and recover the ethyl acetate, leaving behind purified lactic acid. The final lactic acid solution was stored in sterilized glass containers for subsequent analyses (Nuñes *et al*., 2009).

For biomass quantification, filter papers containing the harvested mycelium were dried at 80 ºC until a constant weight was achieved, and biomass concentration was determined gravimetrically (Cañedo & Ames, 2004). Results are expressed as grams of dry biomass (mycelium) per liter of culture.

Quantification of Lactic Acid

Lactic acid concentration in the filtrates was determined using the ferric chloride colorimetric assay, following the methodologies of Nurbalqis *et al*. (2015) and Orozco & Solarte (2003). An aliquot of 5 mL from each filtrate was combined with 3 mL of 1% (w/v) $FeCl_3$ solution. The solution was then acidified by adding 1 mL of 1N HCl per 100 mL of $FeCl_3$. Absorbance measurements were recorded at 440 nm using a spectrophotometer. Quantification was based on a lactic acid standard calibration curve ranging from 0.000 to 4.056 g/L, adapted from Marquez (2020). All measurements were performed in triplicate. Final lactic acid concentrations were expressed as grams per liter (g/L).

Quantification of Consumed Substrate

Substrate consumption, expressed as reducing sugars, was quantified using the phenol-sulfuric acid method as described by the Nuffield Foundation (1984). To 1 mL of the sample, 1 mL of 5% (w/v) phenol solution and 5 mL of 98.5% sulfuric acid were added. The mixture was incubated for 20 minutes, during which it was gently agitated at 5-minute intervals to facilitate chromophore development. Absorbance was measured at 490 nm. A standard curve was prepared with D-glucose over the concentration range of 0.000 to 0.600 g/L. Results were expressed in grams of substrate consumed per liter (g/L).

Characterization of lactic acid

Quantitative analysis

The characterization of lactic acid was conducted by measuring its refractive index, acidity, relative density, and viscosity. These physicochemical properties were determined according to the procedures established in the COVENIN standards 702:2001, 1116:77, and 658:1997, with the exception of viscosity, which was assessed following the methodology described by Chávez *et al*. (2023).

Qualitative analysis

Fourier transform infrared (FTIR) spectroscopy was employed to identify the functional groups present in lactic acid (Skoog, 2001). A total of six samples, three from the conventional medium and three from the alternative medium, were analyzed directly in the liquid phase without any mixing. Two drops of each sample were placed between two potassium bromide (KBr) plates (Skoog, 2001). Spectral acquisition for each sample was conducted under varying instrumental conditions to achieve optimal resolution, characterized by sharp and intense absorption bands whenever possible. Once the optimal spectrum was obtained for each sample, the spectral scale was calibrated in terms of wavenumber.

Production of polylactic acid

A pre-concentration step was conducted via simple distillation of 100 mL of lactic acid obtained from fermentation. The sample was heated for three hours within a temperature range of 40 to 60 °C. The concentration of lactic acid was quantified by a colorimetric titration using 1 M sodium hydroxide (NaOH) as the titrant and phenolphthalein as the indicator (Jiménez, 2012).

The polymerization process comprised two distinct stages: oligomerization (dehydration) followed by polymerization. During the oligomerization phase, 30 mL of lactic acid was subjected to continuous heating at 150 °C and dehydrated using a vacuum distillation apparatus. The system pressure was progressively lowered to 22 inches Hg vacuum, maintaining these conditions for a duration of 4 hours (Pinzón *et al*., 2006).

Subsequent polymerization was initiated upon attainment of the target vacuum level. Tin chloride ($SnCl_2$) was introduced as a catalyst, and the reaction mixture was maintained at a constant temperature of 170 °C under a steady vacuum of 22 inches Hg. This stage proceeded for an additional 4 hours, during which polymer formation was monitored (Pinzón *et al*., 2006).

Characterization of polylactic acid

Molecular Weight Estimation

Molecular weight was determined by measuring the intrinsic viscosity of polymer solutions prepared in chloroform at concentrations ranging from 0.9 to 2.0% w/v. The measurements were conducted using an Ostwald viscometer maintained at 25°C, following the methodology outlined by Jimenez (2012). The molecular weight was subsequently calculated utilizing the Mark-Houwink-Sakurada equation:

$$n=5.45\times10^{-4}\times\bar{M}^{0.73}$$

Where:

η: intrinsic viscosity, d.u.

$\bar{M}$: molecular weight, g/mol

Structural analysis

A structural assessment of the polymer in solid state was conducted using infrared spectroscopy to characterize its functional groups. For sample preparation, the polymer was finely ground and homogenized with anhydrous, spectroscopic-grade KBr at a 1:100 sample-to-KBr weight ratio using a mortar. The resulting mixture was compressed into a pellet, which was subsequently positioned in the

spectrophotometer sample holder for spectral acquisition.

Degradation of the polymer

A hydrolytic degradation study of the polymer was conducted in two distinct media: acidic and neutral. Buffer compositions were adjusted to maintain a constant ionic strength of 0.15. The protocol involved immersing each sample in 6 mL of buffer solution within test tubes as follows:

Acidic medium: samples were immersed in 6 mL of phosphate buffer at pH 4.5 and incubated in a water bath at 36 °C.

Neutral medium (physiological conditions): samples were immersed in 6 mL of phosphate buffer at pH 7.0 and incubated in a water bath at 36 °C.

Enzymatic degradation was evaluated using Lipase 20 in a phosphate buffer solution at pH 7.0 and 36 °C. Polymer samples were placed in test tubes containing 10 mL of the buffer solution with an enzyme concentration of 1 mg/mL (Bueno, 2012).

Polymer exposure to microorganisms

Biological degradation assays were conducted using the environmental fungus *Aspergillus niger* and the bacterium *Pseudomonas aeruginosa*, both obtained from the National Institute of Agricultural Research (INIA-CENIAP). Sample preparation and dimensions matched those used in hydrolytic and enzymatic degradation tests. Polymer specimens were incubated with these microorganisms for a duration of nine days, following protocols established by Swapnil *et al*. (2015) and Usha *et al*. (2011).

Results

Production of lactic acid

Figures 1 and 2 present the kinetic profiles of fermentations conducted using both conventional and synthetic media. Figure 3 illustrates the lactic acid production yields obtained from fermentations employing conventional versus alternative media formulations.

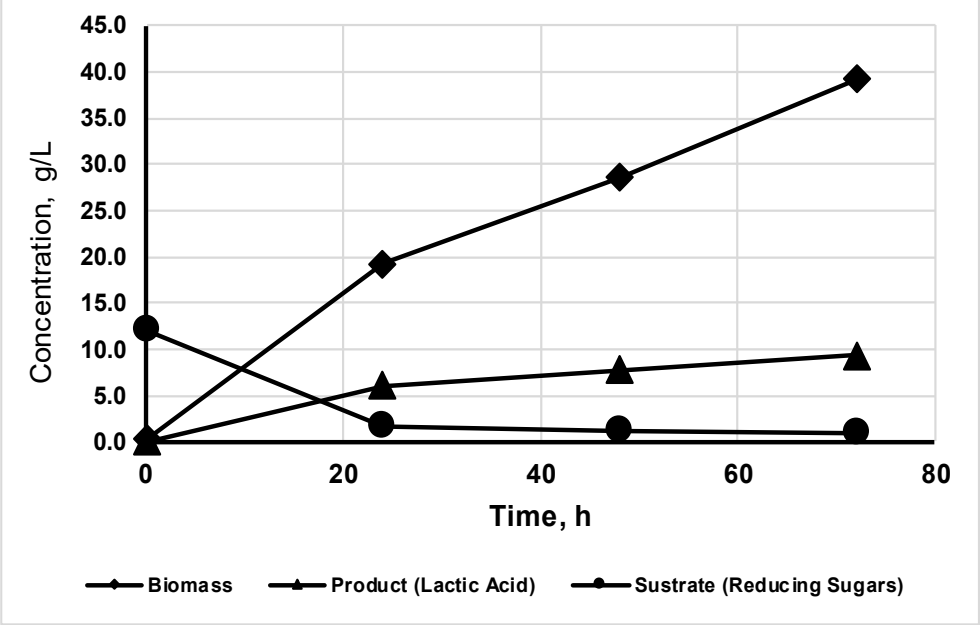


Figure 1. Kinetic analysis of lactic acid biosynthesis employing non-conventional substrate media

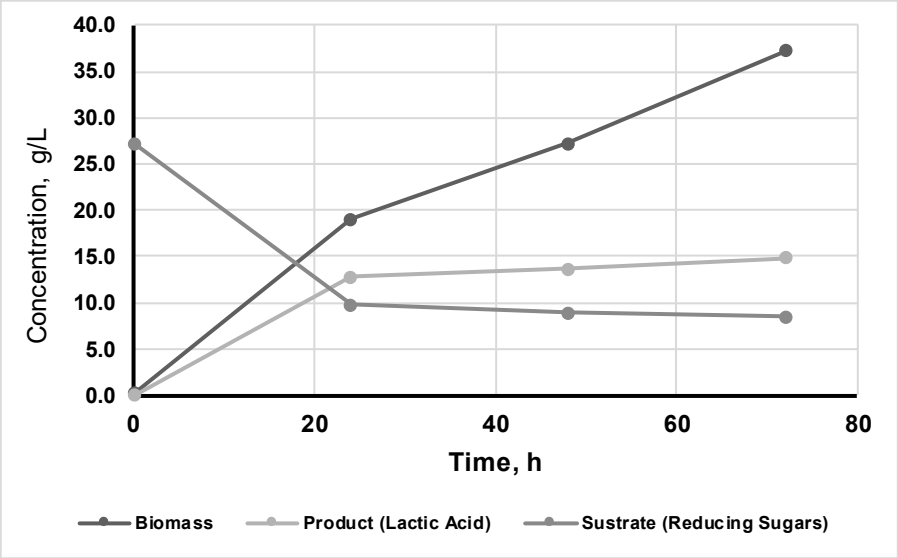


Figure 2. Kinetic analysis of lactic acid biosynthesis employing conventional media

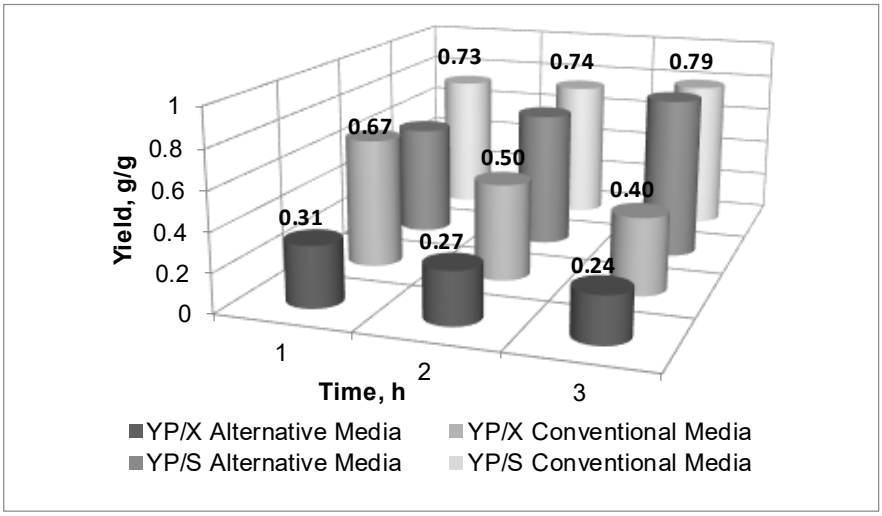


Figure 3. Yields for the production of lactic acid using the conventional and alternative media

Statistical analysis

Figures 3 until 7 show the statistical analysis.

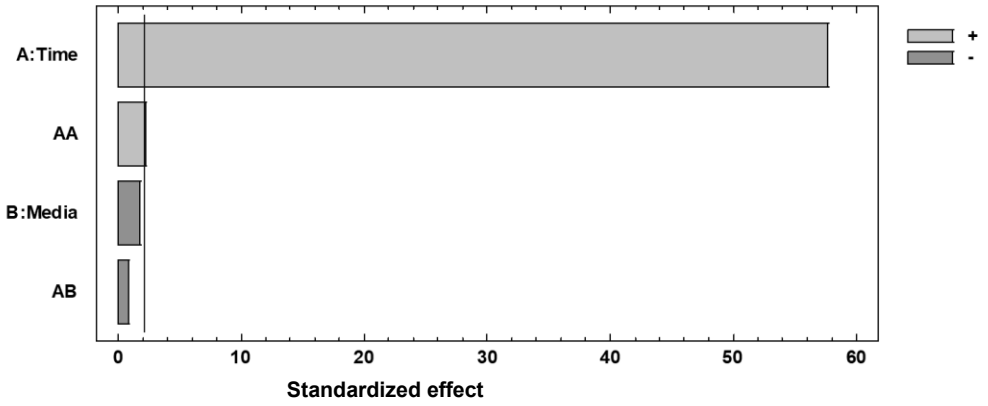


Figure 3. Standardized Pareto diagram for biomass.

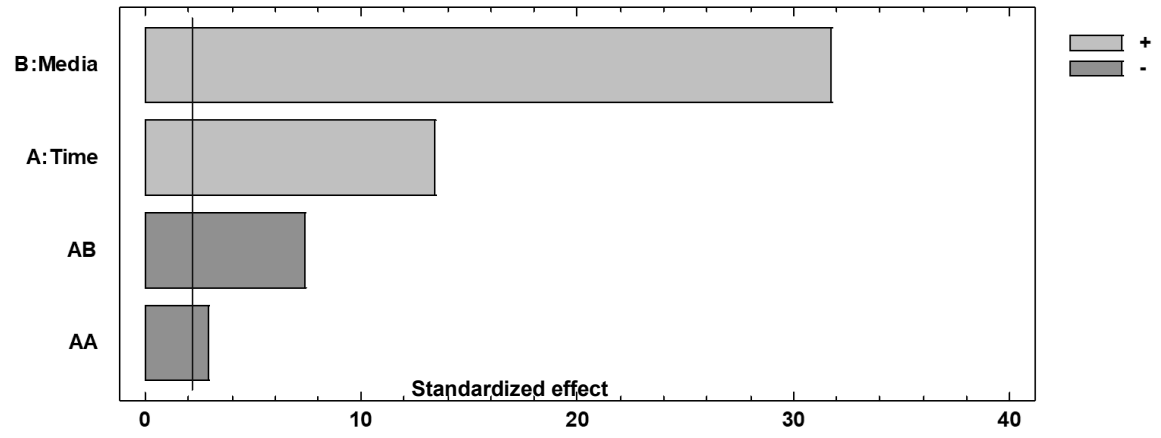


Figure 4. Standardized Pareto diagram for Lactic acid.

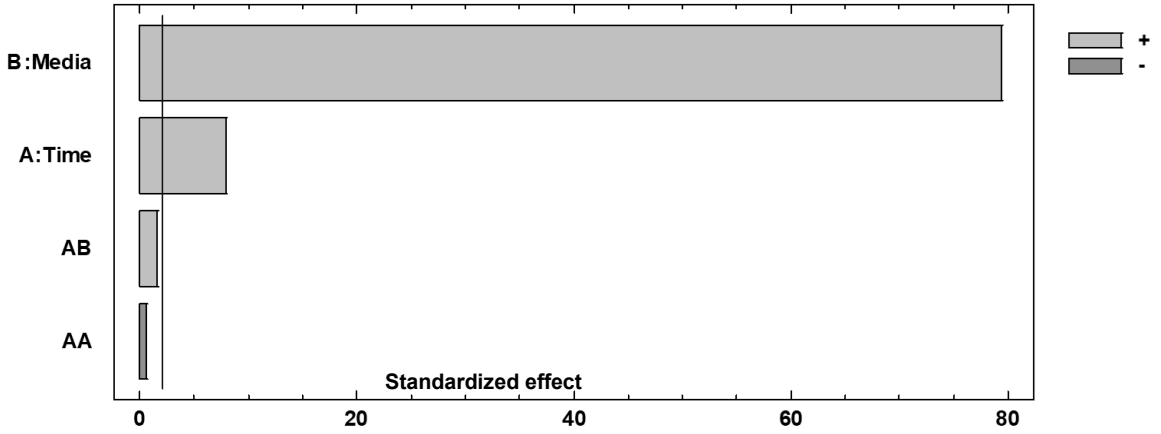


Figure 5. Standardized Pareto diagram for Reducing Sugars.

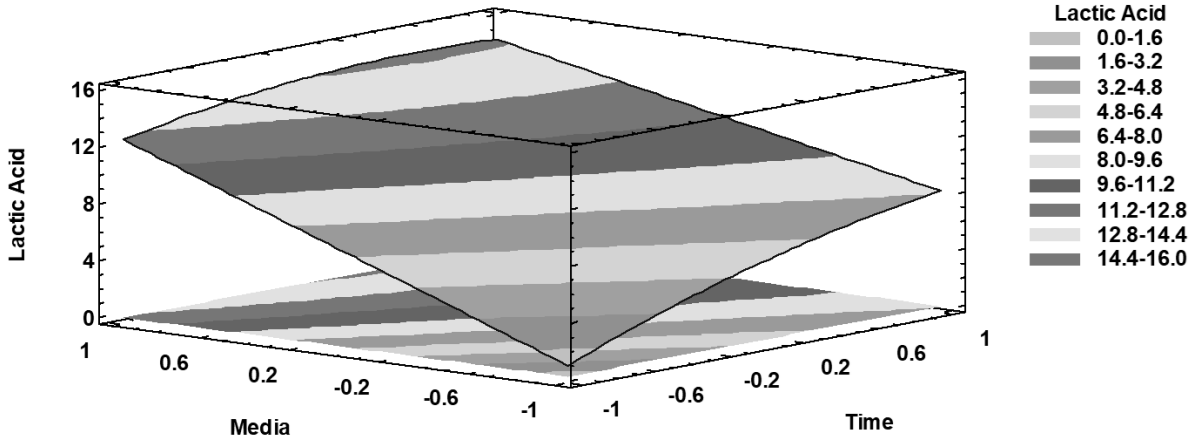
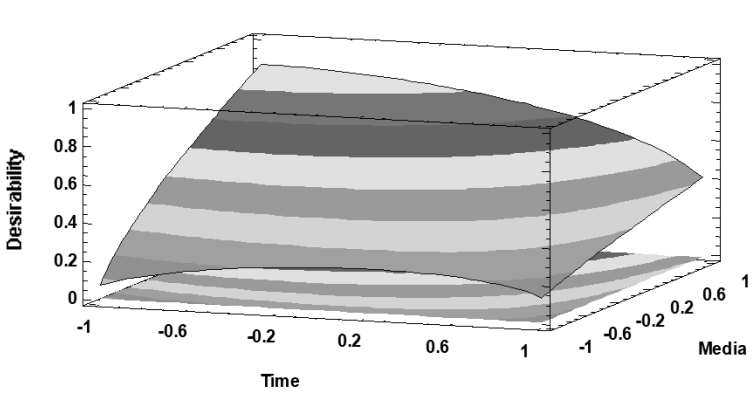
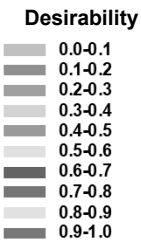


Figure 6. Estimated Response Surface for Lactic Acid.





Response	*Optimum*
Biomass, g/L	18.974
Lactic Acid, g/L	12.4898
Reducing Sugars, g/L	17.8586

Factor	*Low*	*High*	*Optimum*
Time	-1.0	1.0	-1.0
Media	-1.0	1.0	1.0

Figure 7. Response surface determined for desirability function optimizing maximal lactic acid, reducing sugar consumption, and minimal biomass.

Characterization of lactic acid

The results of physic-chemical tests show in Table 1.

Table 1. Properties obtained from lactic acid

Properties	Conventional media	Alternative media	Reference date
Density	984 Kg/m^3	976 Kg/m^3	1174.8 Kg/m^3
Viscosity	9.95x10^{-4} Kg/m.s	9.3x10^{-4} Kg/m.s	28.5x10^{-3} Kg/m.s
pH	2.6	2.4	-
Refraction Index	1.33	1.33	1.44
Titratable acidity	1.323%	1.458%	-

The IR spectrum of the lactic acid obtained by the conventional media is shown in Figure 8 and for the alternative media in Figure 9.

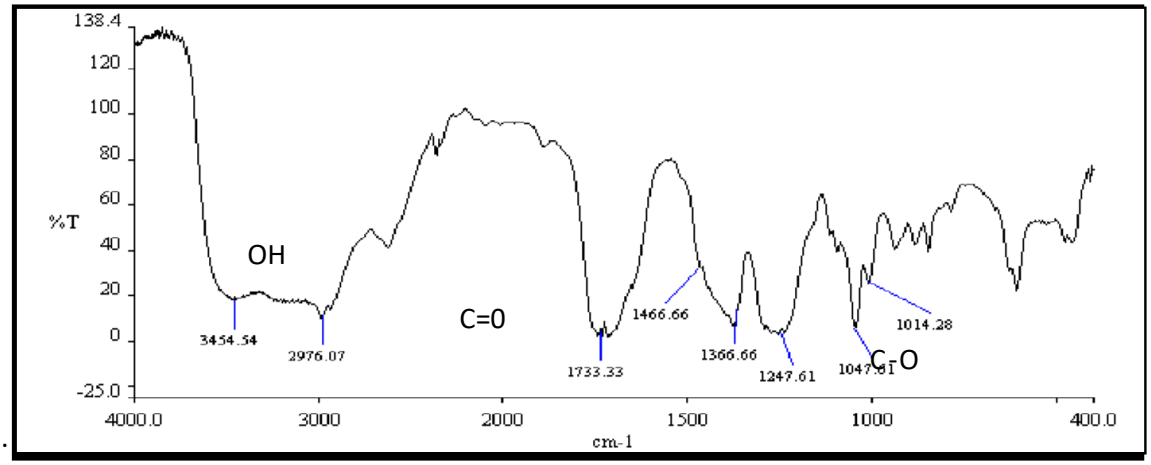


Figure 8. IR spectrum of the lactic acid obtained with the conventional media at 24 hours of fermentation.

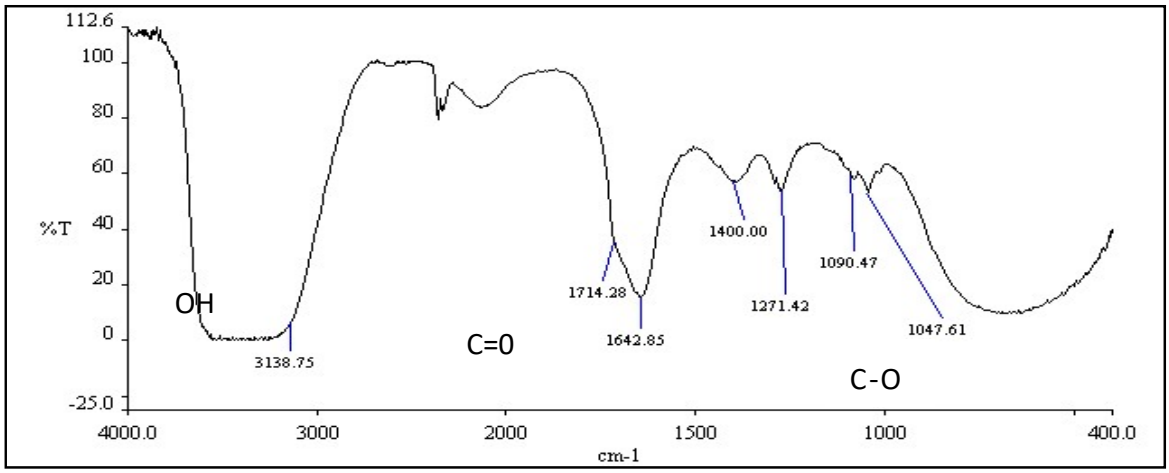


Figure 9. IR spectrum of the lactic acid obtained with the alternative media at 24 hours of fermentation.

Polylactic acid production

The outcomes of lactic acid pre-concentration are presented in Table 2.

Table 2. Pre-concentration of lactic acid

Alternative media		**Conventional media**	
Inicial concentration (g/L)	Final concentration (g/L)	Inicial concentration (g/L)	Final concentration (g/L)
9.33	65.70	14.84	76.50

The data pertaining to polylactic acid production are presented in Table 3.

Table 3. Polylactic acid prodution

Polylactic acid production	
Alternative media (g/L)	Conventional media (g/L)
20.15	20.00

Characterization of polylactic acid

The results of the titratable acidity analysis for polylactic acid are summarized in Table 4.

Table 4. Polylactic acid titratable acidity

Polylactic acid titratable acidity	
Alternative media (% acidity)	Conventional media (% acidity)
0.0054	0.0045

The infrared (IR) spectra of polylactic acid (PLA) synthesized using conventional media and alternative media are presented in Figures 10 and 11, respectively.

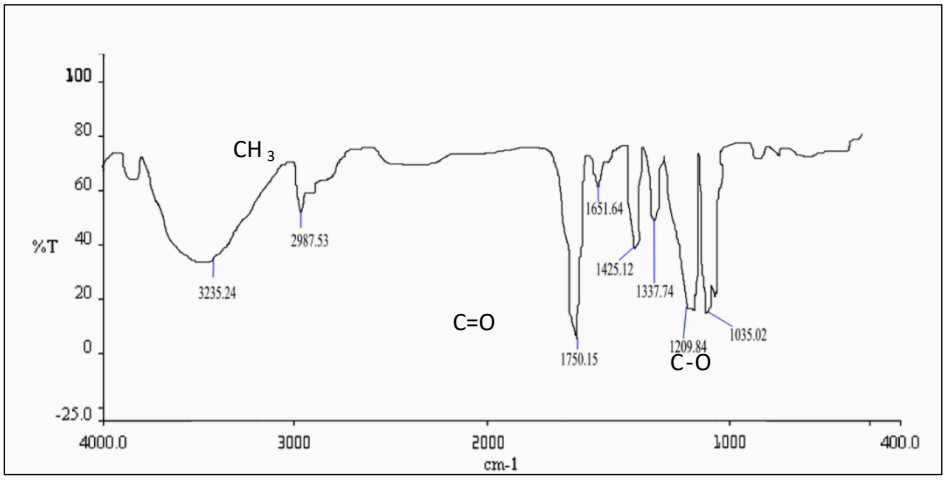


Figure 10. IR spectrum of the polylactic acid obtained with the conventional media.

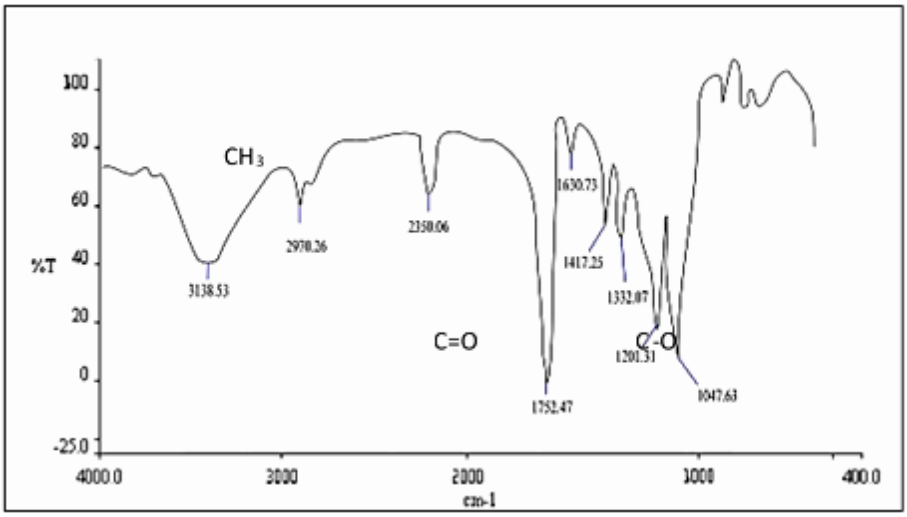


Figure 11. IR spectrum of the polylactic acid obtained with the alternative media.

Figure 12 illustrates the response of the solutions to variations in the fluid cutting speed.

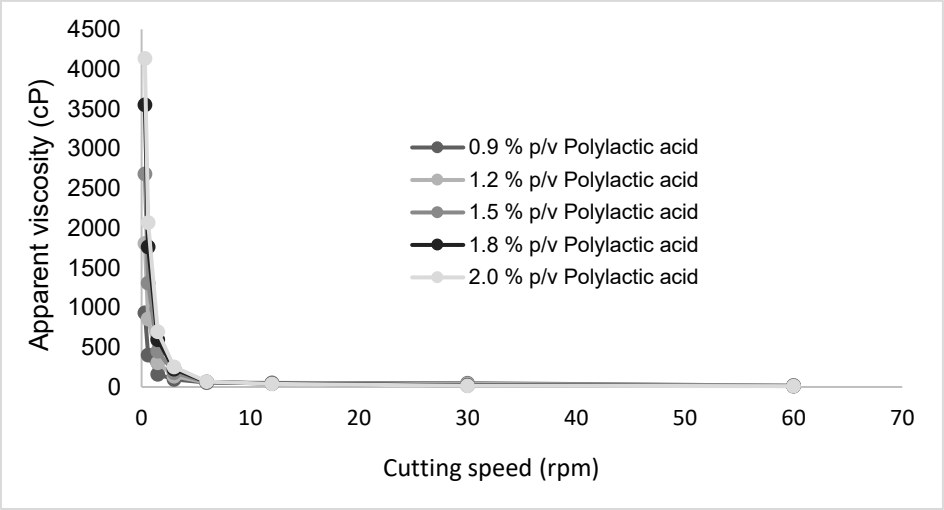


Figure 12. Representation of the viscosity of solutions of different concentration of the polylactic acid versus the cutting speed.

The results of polylactic acid molecular weight show in Table 5.

Table 5. Polylactic acid titratable acidity

Average Molecular Weight of the Biopolymer		**Reference Average Molecular Weight**	
Alternative media (g/mol)	Conventional media (g/mol)	Polycondensation (g/mol)	ROP (g/mol)
16327.43	15695.90	< 1.6x 10^4	2.0x10^4 a 6.8x10^5

Table 6 shows the different pH in polylactic solutions for hydrolytic degradation.

Table 6. Measurement of pH in different media arranged for hydrolytic degradation.

Day	Polylactic acid obtained from conventional media		Polylactic acid obtained from alternative media	
	Acidic medium (pH)	Neutral medium (pH)	Acidic medium (pH)	Neutral medium (pH)
0	4.5	7.0	4.5	7.0
1	3.5	5.0	3.5	5.0
2	3.0	4.0	3.0	4.0
3	2.6	3.0	2.6	3.0
4	2.3	3.0	2.3	3.0
5	2.0	3.0	2.0	3.0
6	2.0	3.0	2.0	3.0

The results of polylactic acid hydrolytic, enzymatic and biological degradation show in Figures13 until 15.

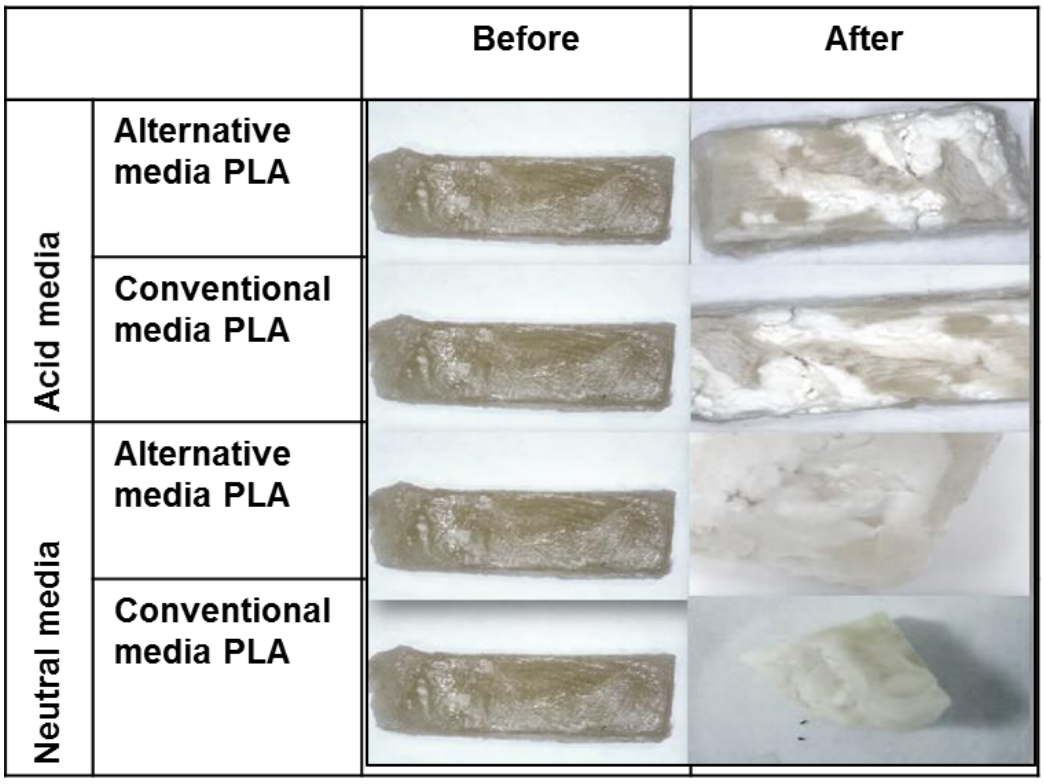


Figure 13. Samples of polylactic acid tested for hydrolytic degradation.

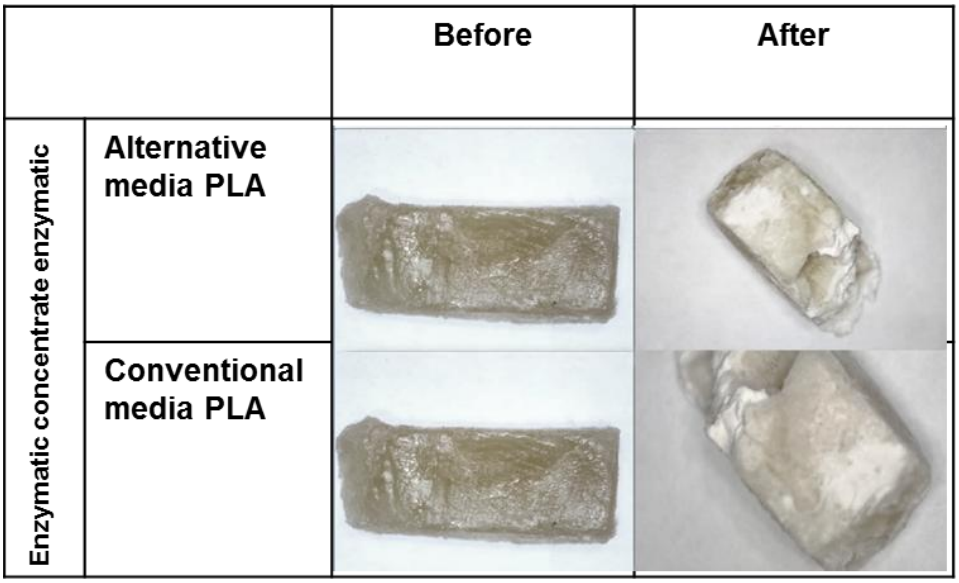


Figure 14. Samples of polylactic acid tested for enzymatic degradation.

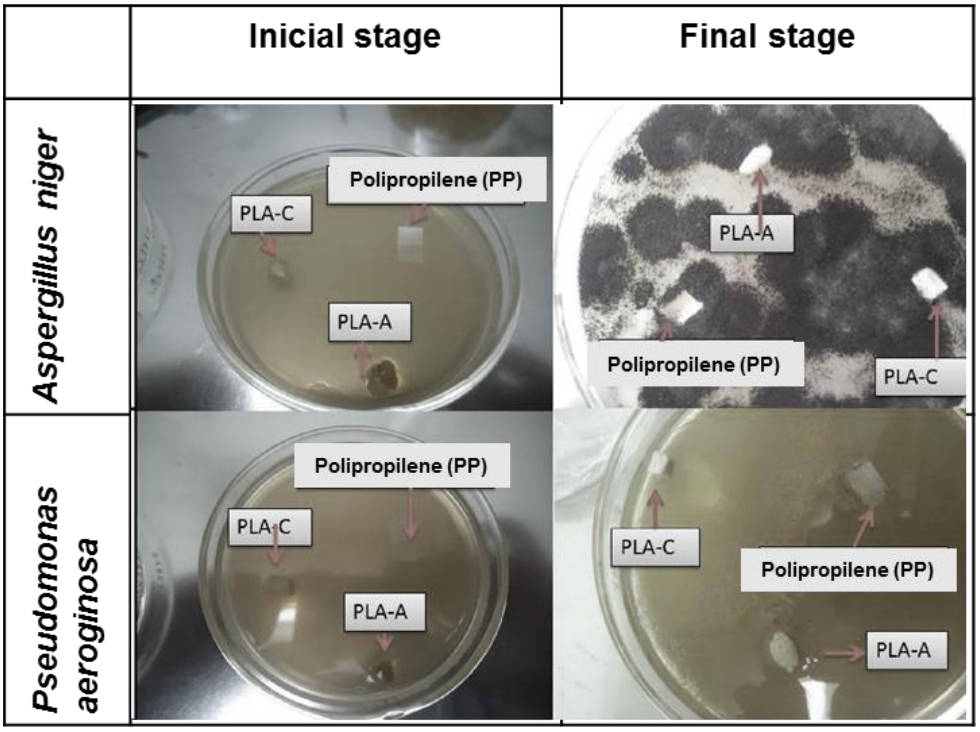


Figure 15. Samples of polylactic acid tested for biologycal degradation.PLA-C: Polyactic acid from conventional media. PLA-A: Polylactic acid from alternative media.

Discussion

Production of lactic acid

Figures 1 and 2 illustrate the characteristic growth phase of *R. oryzae* as previously documented (Xuefeng *et al*., 2011; Gil-Horan *et al*., 2008). During fermentation, both media exhibited similar lactic acid production kinetics, albeit with distinct final concentrations at 72 hours: 9.34 g/L in the conventional medium versus 14.84 g/L in the alternative medium. The conventional medium demonstrated a higher initial concentration of reducing sugars (27.21 g/L) compared to the alternative medium (12.08 g/L). However, substrate utilization efficiency at 72 hours was superior in the alternative

medium, achieving 91.97% consumption relative to 68.93% in the conventional medium, indicative of enhanced fermentative performance in the alternative substrate formulation.

Comparing the results obtained (Table 2) with the research carried out by Xuefeng *et al*. (2010) and Gil-Horan *et al*., (2003) it can be noted that a higher yield was obtained than these (Table 7). In addition, the yield obtained in this case is congruent with other reports; however, for some cases the yield obtained is similar and in others it is greater. Table 7 summarizes the different reports of production of lactic acid by different microorganisms and substrates considering the results of the present study.

Characterization of polylactic acid

The percentage of acidity (Table 4) of the titrated samples is very close between them, that is, it cannot be noticed a notable difference between them, therefore the degree of polymerization in the samples is similar. These values may be because for both monomers used for the polymerization, both the conventional and the alternative media were used at the same concentration, the same polymerization times, as well as the amount of catalyst, were used. Thus, it can be said that the biopolymer obtained from the alternative medium is as acceptable as the one produced from the conventional medium, in addition that the low acidity percentage indicates that the acid used as the monomer reacted mostly in the polymerization process, therefore had a good performance.

The IR spectrum of the polylactic acid obtained from the conventional medium (Figure 10) shows the functional groups characteristic of the structure of this polymer, such as the carbonyl group (C=O) in 1750 cm^{-1}, the strong signal of methyl (CH_3) at 1425cm^{-1} and 2987cm^{-1}, and finally another signal of the CO bond of the carboxyl group at 1035cm^{-1} and 1209cm^{-1} respectively (Pavia *et al*., 2020). Observing the spectrum obtained qualitatively confirms that there is presence of polylactic acid since the functional groups corresponding to it are clearly reflected; The same occurs with the IR spectrum of the polylactic acid obtained from the alternative medium (Figure 11).

In Figure 12, it is observed that the viscosity decreases with increasing the cutting speed of the fluid. Welty *et al.* (2007) says that when viscosity varies according to shear stress, shear rate and / or temperature we are in the presence of a non-Newtonian fluid. In addition, when this fluid decreases its viscosity by increasing the rate of deformation we are in the presence specifically of pseudoplastic fluids. When comparing the results with those expressed by Chatillon (2012), it was found that the rheological study of polylactic acid is of pseudoplastic tendency, equal to that obtained in the present investigation. The values obtained for the molecular weight of the polylactic acid (16327.43 g/mole for the alternative media and 15695.90 g/mole for the conventional media) (Table 5) are congruent with those reported by Zuluaga (2013)

Table 7. Lactic acid production reports.

Author	Microorganism	Substrate	Yield
Tatum & Peterson (1935)	*Steptococccus lactis* *Lactobacillus casei* *Lactobacillus delbrueckii*	Glucose	94.00% 93.00% 95.00%
Pan *et al.* (1940)	*Lactobacillus delbrueckii*	Molasses	87.30%
Cordon *et al*. (1950)	*Lactobacillus delbrueckii Lactobacillus pentosus* 124-2 *Lactobacillus delbrueckii* NRLB--445	Potato starch	61.90% 91.20% 79.40%
Rehm (2009)	*Lactobacillus delbrueckii*	Glucose	90.0%
Xuefeng *et al.* (2011)	*Rhizopus oryzae*	Glucose	78.75%
Gil-Horan *et al*. (2008)	*Rhizopus oryzae*	Solid orange waste	82.00%
Orozco & Solarte (2003)	*Lactobacillus delbrueckii* (NRRL B--763)	Glucose	90.21%

where it is indicated that molecular weight for the synthesis of PLA by polycondensation is 1.6 x 10^4 Da, while the molecular weights synthesized according to the ring-opening polymerization (ROP) method are between 2 x 10^4 to 6.8 x 10^5g/mole (Zuluaga, 2013).

Results for hydrolytic degradation show in Figure 13 where it is observed that where it was observed during the first three days that the samples that were exposed to the media began to turn white and had small cracks in its surface, in addition, it was possible to notice the presence of bubbles in the middle which is indicative of the evolution of dioxide of carbon (CO_2) characteristic of a decomposing material (Meneses *et al*., 2007). The remaining days of the test the samples were completely white, with larger cracks and even small particles detachment. In the same way, the presence of bubbles in the media was maintained; all these changes give evidence of the degenerative process that the samples suffered. These results are congruent with Bueno (2012). In these latter two methods the pH decrease was a characteristic presented as indicative of the degradation (Table 6).

With enzymatic degradation (Figure 14) it was possible to visualize particles detachments on the first day of the sample, some bubbles indicative of CO_2 evolution. On the final day the samples were extracted from the medium observing that it turned white, like those mentioned in the test of hydrolytic degradation, cracking and detachment of particles evidencing the degenerative process that the sample underwent in this test. These results were congruent with those presented by Bueno (2012).

The last method was biological degradation (Figure 15), in which the samples were exposed to two microorganisms, one fungus (*A. niger*) and one bacterium (*P. aeruginosa*), in Petri plates for 9 days. The results are shown in Figure 15 where three samples can be seen on each plate, which are PLA-C (biopolymer obtained from the conventional medium), PLA-A (biopolymer obtained from the alternative medium) and polypropylene Petroleum). The deterioration of the biopolymer samples obtained was evident, showing surface cracking, color change and texture change in both samples. Unlike the PLA-C and PLA-A samples, Polypropylene did not undergo any changes when exposed to microorganisms, which indicates that the degeneration of the other samples shows the effectiveness of the degradation.

Conclusions

The maximum achieved concentration of lactic acid using conventional and alternative media was 14.84 g / L and 9.34 g / L respectively. The yields of lactic acid achieved from both media were 80% and 84% respectively, expressed as grams of lactic acid produced between grams of consumed substrate. The degrees of acidity of conventional and alternative media were 1.323% and 1.458%, respectively. The total polymerization time was 8 hours. The amounts of bioplastic obtained from the lactic acid polymerization of the conventional medium and the alternative media were 20.00 g and 20.15 g respectively. The average molecular weight of the bioplastic obtained from the lactic acid polymerization of the conventional media was 15695.90 g/mole. The average molecular weight of the bioplastic obtained from the lactic acid polymerization of the alternative media was 16327.43 g/mole. The bioplastic obtained from the polymerization of lactic acid from the conventional and alternative media according to various tests is biodegradable.

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