

Biodegradation of poly(lactic acid) waste from 3D printing

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Abstract

One of the most widespread applications of poly(lactic acid) (PLA) is as a raw material in the form of filaments for 3D printing. To improve final disposal alternatives and minimize their effects on the environment, the aim of this study is to determine the biodegradability of 3D-printed PLA waste composted in a landfill with leachate soil and garden soil for 90 days and 180 days. The soil characteristics and material properties were evaluated by laboratory analyses. Changes in soil chemical composition and the loss of microorganisms were recorded. The thermal and mechanical properties of PLA did not change significantly, but fungal colonies, encrustation, and changes in the original colour were found, indicating the onset of surface biodegradation of the samples. Controlled conditions or longer periods would be needed to maintain an ecosystem favourable to biodegradation; otherwise, PLA could accumulate in the environment, causing future pollution problems.

Keywords: 3D printing, PLA, biodegradable polymers, waste, sustainability.

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1. Introduction

Petrochemical-based polymers are present in multiple sectors of the economy, as they are relatively cheap and easy to process. However, when discarded, they can take years to decompose in the environment, accumulate in landfills, or even be inappropriately released into nature, generating environmental impacts^[1].

In response to these issues, biodegradable polymers have emerged, produced mostly from renewable resources, such as the raw material sugarcane, corn, wheat and potato, thus avoiding the impacts caused by the extraction and refining of oil^[2,3].

Among the most commercialized biodegradable polymers in the world, poly(lactic acid) (PLA) is produced mainly from natural sources by the fermentation of carbohydrates, where each process varies according to the type of bacteria used^[2,4].

PLA in the form of filaments has been essential for the manufacture of parts in additive manufacturing (AM), i.e., 3D printers, being considered a fast, economical alternative because there is almost no wasted material, and highly complex and lightweight parts can be manufactured due to its high rate of surface hardness, gloss, low toxicity and desirable mechanical properties^[5-7].

Notably, the use of AM technology using PLA became even more widespread during the COVID-19 pandemic. Shortages in the supply of several medical products and interruptions of industries and transportation made this technology essential for the production of basic equipment

such as face masks, visors, testing devices and personal protective equipment (PPE)^[8].

The biodegradation of PLA occurs through hydrolysis, accompanied by the release of lactic acid and, consequently, a decrease in the pH of the medium^[9]. This biodegradation process is considered a type of natural composting because there is no need for the use of specific equipment or energy resources^[10].

The factors that affect biodegradation are related to the microorganisms present by enzymatic action^[11]. For a colony of microorganisms to grow and develop, adequate humidity, temperature, pH, necessary oxygen, and enzymes specific to aerobic or anaerobic conditions are needed^[12]. Humidity is essential, as it provides the environment with favourable conditions for microorganisms to grow and reproduce and contributes to the hydrolysis process, especially since polymers degrade more in humid environments than in low or no humidity conditions. Changes in pH from acidic to basic can also affect the growth of microorganisms and the rate of hydrolysis. Temperature influences the microbial environment and is a parameter that should be controlled because the rate of biodegradation increases with increasing temperature, but at too high a temperature, the microbial activity decreases^[13,14].

The biodegradation process results in changes in the mechanical, optical or electrical properties of the materials, causing the appearance of cracks, fractures, and changes in

surface aspects such as colour fading, chemical transformation, and decreases in viscosity and molar mass, consequently reducing their shelf life^[1,3,15].

As a consequence of the increasing use of PLA, a large amount of this discarded material has been inserted into the management system of urban and industrial solid waste. When PLA is discarded under favourable natural composting conditions, biodegradation occurs. However, its degradation mechanism under certain circumstances is still unclear, raising uncertainties about the conditions of the environment, the influence of microorganisms, the characteristics of the material, and the time needed, among others^[9,14-16].

Thus, to improve alternatives for the final disposal of PLA and minimize its effects on the environment, it is important to know the biodegradability behaviour of this material when discarded, either through the natural composting process that occurs in landfills or if incorrectly discarded in the soil, observing its physical and chemical characteristics, to assist in decision making concerning the use of PLA, in addition to contributing to waste management.

2. Materials and Methods

2.1 Materials and experimental procedure

For this study, we analysed 3D-printed visors made from PLA filaments that were manufactured as personal protective equipment during the COVID-19 pandemic

but then discarded when they deformed during printing (Figure 1a). For the experiment, the PLA material was divided into equivalent parts according to the type of analysis to be carried out, the exposure time in the soil and the type of soil (Figure 1b, 1c).

The biodegradation process was performed with two types of soils:

- Landfill soil with leachate: taken from the landfill of a municipality in Minas Gerais, Brazil, geographical coordinates -20.99321 S and -42.82450 W. The leachate was taken from the garbage collection truck, and approximately 10 mL of leachate was added to the landfill soil to ensure a diversified microbiota, with the purpose of exploiting the natural microorganisms of a final waste disposal site^[17];
- Garden soil used for planting flowers and vegetables was taken from a residence in Minas Gerais, Brazil, geographical coordinates -21.74897 S and -43.36530 W.

Approximately 10 kg of each soil type was collected. Part of the PLA samples were buried in soil for 90 days, a period when the anaerobic process occurs and the aerobic process may occur, known as biostabilization or active degradation. The other samples were buried for 180 days, which is the period when the maturation process occurs, with humification and extraction of organic matter^[18]. Soil temperature, pH and moisture parameters were monitored

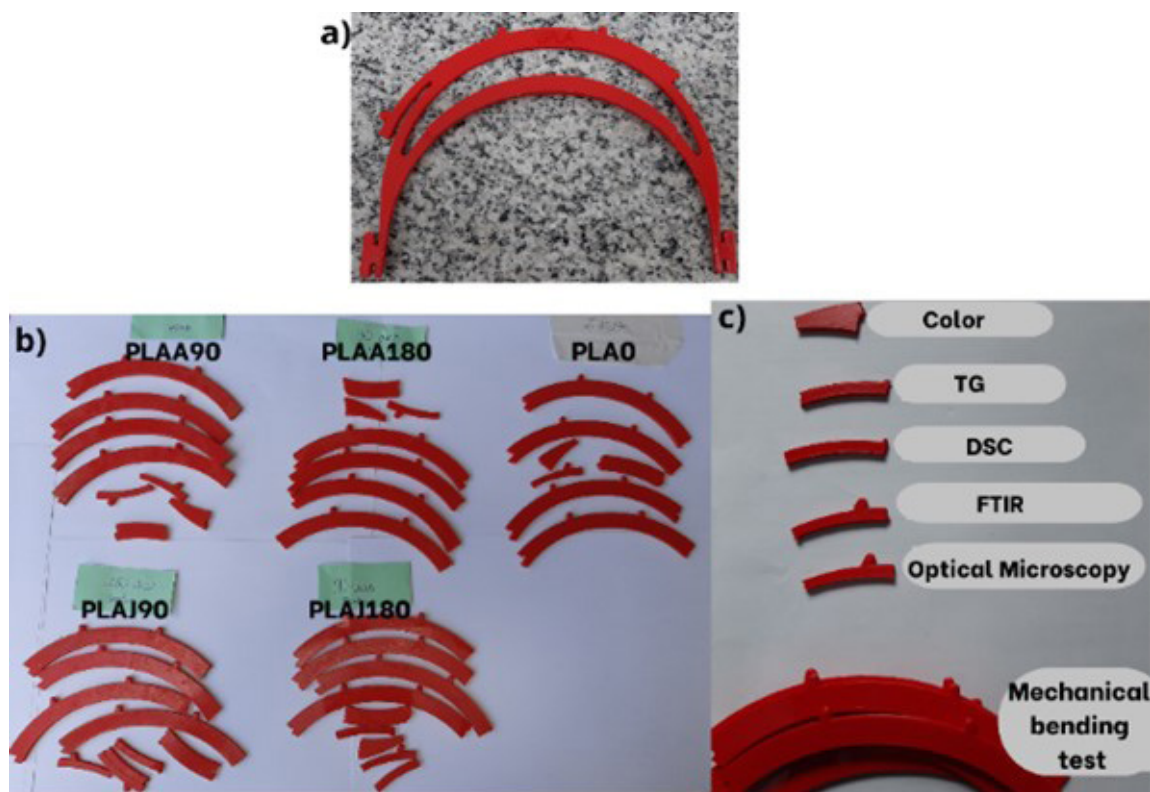


Figure 1. PLA residue printed on the 3D printer.

periodically using a digital measuring equipment model Soil Survey Instrument, CE brand. Water was added whenever necessary to maintain an ideal environment for the microorganisms, with approximately 60% humidity^[19]. At the end of the determined times, 90 days and 180 days, the PLA samples were removed, washed in running water, and stored. The soils were stored in a refrigerated environment for analysis. Table 1 identifies the nomenclature used during the experiment for each type of soil and PLA sample used for analysis:

2.2 Analysis and testing

2.2.1 Analysis of the soil samples

Microbial biomass carbon (MB-C) was determined by the fumigation-extraction method^[20], which consists of oxidation of microbial carbon by K₂Cr₂O₇ in fumigated and nonfumigated soil types by chloroform, and the microbial C of each sample was extracted by K₂SO₄ solution (0.5 mol/L). In the presence of H₂SO₄, the microbial carbon present in the soil type was oxidized, and the residual K₂Cr₂O₇ was quantified by titration with Fe(NH₄)(SO₄)₂·6H₂O. The results were expressed as mg of C per kg of dry soil. Basal respiration (RB) was determined according to the method of capturing and quantifying the CO₂ released by the microbial respiration process over a 72 h incubation period^[21]. Samples of 20 g were taken from each soil type (J0, J180, A0, A180), and these samples were transferred to hermetically sealed bottles. These bottles contained vials with 20 mL of 0.05 mol/L NaOH. The samples were mixed with NaOH (which did not react with CO₂) plus the addition of 5 ml of BaCl₂·2 H₂O (0.5 M) and three drops of the phenolphthalein indicator (0.1%), titrated with HCl (0.5%). The results were expressed as mg C-CO₂ kg dry soil⁻¹ day⁻¹. From the results of microbial biomass carbon and basal respiration, the metabolic quotient (qCO₂) was calculated, representing the amount of C-CO₂ evolved per unit of microbial C^[22]. The results were expressed as mg C-CO₂/mg C-BM.day⁻¹.

2.2.2 Analysis of PLA samples

Three-point bending mechanical tests were performed based on the technical standard ASTM International

D790, Standard Test Method for Bending Properties of Unreinforced Plastics and Electrical Insulation Materials^[23]. An MC universal testing machine was used, model WDW - 20E. The distance between the lower support points was 75 mm, and the speed was 2 mm/minute. Properties such as maximum strength, modulus of elasticity and flexural deformation were determined by means of stress x strain curves using WinWdw-F020 software. The samples were taken in quadruplicate, the means and standard deviation for each sample were determined, and a one-way ANOVA was carried out without repetition, with a significance level of 0.05 (5%) to analyse the variability between the results.

The differential scanning calorimetry (DSC) technique allowed measuring the processes of enthalpy (ΔH) changes and temperatures of the thermal events of the samples. The crystallinity was calculated by Equation 1^[24]:

$$X_c = \left(\frac{(\Delta H_2 - \Delta H_1)}{\Delta H_0} \right) * 100 \quad (1)$$

ΔH₂: is enthalpy referring to the melting peak (T_m); ΔH₁: enthalpy of the cold crystallization peak; ΔH₀: melting enthalpy of 100% crystalline PLA: 93.7 J/g^[21].

A DSC-60 calorimeter was used with a flow rate of 50 mL/min nitrogen, a sample weight of approximately 6.0 mg, and a temperature increase from 25 °C to 210 °C with a heating rate of 10 °C/min.

Fourier transform infrared spectroscopy (FTIR) was used to evaluate the chemical structures of the PLA samples. The spectrum ranged from 4000 to 400 cm⁻¹, the resolution was 2 cm⁻¹, and the number of scans per sample was 32 times/min.

A Motic microscope, model BA210E, was used for the analysis by optical microscopy of several different parts of the PLA samples, providing images magnified at 100x. The software used for generating the images was Moti Connect.

The colour analysis was performed using a Color Muse 9600 spectrophotometric colorimeter. The samples were evaluated as to their visual aspect, following the biodegradation evolution through colour alteration, according to the CIELab 1976 colour evaluation standard, based on the elements luminosity, hue and saturation, where the parameter L indicates the luminosity scale, and the parameters a* and b* are the opposite axes for the colours green to red versus yellow to blue, were automatically calculated by the equipment. E delta and (ΔE) is the difference between the standard colour and the altered colour, calculated using Equation 2^[25,26]. For each type of sample, an average of three readings were taken.

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (2)$$

3. Results and Discussions

The temperature of the soil during the study varied according to the ambient temperature of the region, with an average of 24 °C. This temperature had little influence on the rate of biodegradation of the material. Higher temperatures or

Table 1. Nomenclature used for the soil and PLA samples.

Nomenclature	Description
J0	Garden soil without contact with the PLA samples
J180	Garden soil that was left for 180 days with the PLA samples
A0	Landfill soil with leachate without contact with PLA samples
A180	Landfill soil with leachate that was left for 180 days with the PLA samples
PLA0	PLA samples without contact with soils
PLAJ90	PLA samples buried 90 days in garden soil
PLAJ180	PLA samples buried 180 days in garden soil
PLAA90	PLA samples buried 90 days in landfill soil with leachate
PLAA180	PLA samples buried 180 days in landfill soil with leachate

artificial means of control may have possibly lead to greater biodegradation, as demonstrated in a study that evaluated the degradation of PLA at different temperatures, showing that microorganisms have a direct role in the degradation of PLA only at temperatures close to 50 °C with a humid environment^[9].

For the landfill soil, we found neutral values, starting at pH 7.0, and throughout the experiment, did not become as acidic as those in the garden soil. In the garden soil, pH values started at 6.5, and gradually decreased, reaching a minimum of 5.1. This pH reduction may be related to PLA biodegradation, since biodegradation is accompanied by the release of lactic acid and this result was also observed in studies evaluating PLA biodegradation^[9,27]. Table 2 shows the monthly average (measured daily) of the temperatures and pH obtained over the 90 days and 180 days. The humidity was maintained at 60%.

3.1 Biological indicators of soil quality

According to Moreira and Siqueira^[28], microbial biomass carbon is a sensitive indicator of changes in the ecosystem and is related to the amount of carbon received over a given time. Usually the lowest values are found in degraded areas, and in this case, the lowest values were found in areas that were most disturbed over the time of the experiment: the garden soil that was in contact with PLA samples for 180 days (J180) had half the microbial biomass carbon compared to the PLA-free garden soil (J0). The Landfill soil (A180) could not be evaluated the same way, since leachate was added. Since leachate was not present in the Zero-PLA Landfill (A0), this may have influenced the soil characteristics, changed its microbial composition, resulting in an increase in the biomass carbon^[29]. The results for basal respiration (Table 3) were similar to those found for the biomass carbon tests; again, the Zero-PLA Garden sample (J0), which was not affected by the time of the experiment,

obtained the highest value, while the other samples (J180 and A180) obtained lower values. In other words, the lowest RB values occurred in the samples with the lowest levels of organic carbon. The results of the qCO₂ (Table 3) were higher in the zero-PLA soil samples (J0 and A0), which may be related to the higher organic carbon values.

When microorganisms are in soils exposed to stress for a long period, even at low concentrations, they are not able to maintain biomass stability^[28]. Thus, it was shown that even leaving the soil in favourable conditions was not enough to maintain its natural conditions and that the lack of vegetation cover may also have influenced stability, resulting in a considerable loss of microorganisms in these soil ecosystems.

3.2 Analysis of PLA samples

3.2.1 Mechanical bending test

The flexural strength was slightly higher in the PLA0 samples compared to the others that were exposed to the soil. In^[27], a decrease in the strength of PLA exposed to soil was also found; however, for this study, these were very small changes, with no significant differences. For the elasticity modulus results, we found that the PLA0 samples obtained lower values than the other samples. For the samples that were exposed to soil, we could not see any differences in the elastic modulus values; they all showed very similar results. A feature of PLA is its inability to resist deformation under stress^[30]. In the maximum deformation results (Figure 2), we also found no significant differences between the samples. According to Eutionnat-Diffo et al.^[31], PLA deforms less due to the material's high modulus.

The mechanical bending results were used to check for parameters that could explain the biodegradability of the material; however, no relevant standard values were found;

Table 2. Average results of temperature and pH measured during the experiment.

Time (days)	A90		J90		A180		J180	
	Temperature (°C)	pH	Temperature (°C)	pH	Temperature (°C)	pH	Temperature (°C)	pH
30	21.93	7.00	22.03	6.38	22.22	6.93	21.58	6.46
60	26.43	7.00	25.93	5.90	26.43	7.00	25.60	5.78
90	24.75	6.87	24.77	5.48	24.75	6.90	24.53	5.50
120					24.27	6.87	24.20	5.48
150					27.87	6.78	27.83	5.27
180					27.47	6.60	26.90	5.10
Total	24.37		24.24		24.47		23.91	

Table 3. Results of Microbial Biomass Carbon, Basal Respiration and Metabolic quotient.

Samples	Microbial Biomass Carbon (MB-C) (mg of C kg dry soil ⁻¹)	Basal Respiration (mg of C-CO ₂ kg dry soil ⁻¹ day ⁻¹)	Metabolic quotient (qCO ₂) (mg of C-CO ₂ / mg of MB-C.day ⁻¹)
J0	608.82	78.52	0.129
J180	334.63	9.53	0.028
A0	101.44	13.11	0.129
A180	176.40	4.08	0.023

in general, the mechanical properties of PLA did not change throughout the experiment. These results were confirmed by the single-factor statistical analysis ANOVA, without repetition, significance equal to 0.05 (5%), to compare the variances between the sample means, in which the value of $F = 0.308$ was found, lower than the critical F value = 2.866. In addition, the P value was equal to 0.869, i.e., greater than 0.05, so we no significant difference between the average results found for the samples in the mechanical bending test analyses.

3.2.2 Thermogravimetric analysis (TG)

The results for volatiles at 100 °C were similar and cannot be considered different for this temperature range (Table 4). The samples showed a stage of mass loss close to 270 °C,

with a peak at approximately 360 °C (Figure 3a and 3b). For the onset temperature, the values did not differ since thermogravimetric analysis has a margin of error of 2 °C. An ash content greater than zero is indicative of inorganic contamination (sand, clay, etc.), which was observed mainly in sample PLAJ180, where residues of inorganic soil material may have been adhered to the surfaces of the samples. In general, the TG thermograms obtained showed very similar behaviour for all the samples, and the curves showing the PLA characteristics were also similar to other works^[32].

3.2.3 Differential scanning calorimetry (DSC)

The DSC results, identified by the second heating curves, are shown in Figure 4 and Table 5. The first curve,

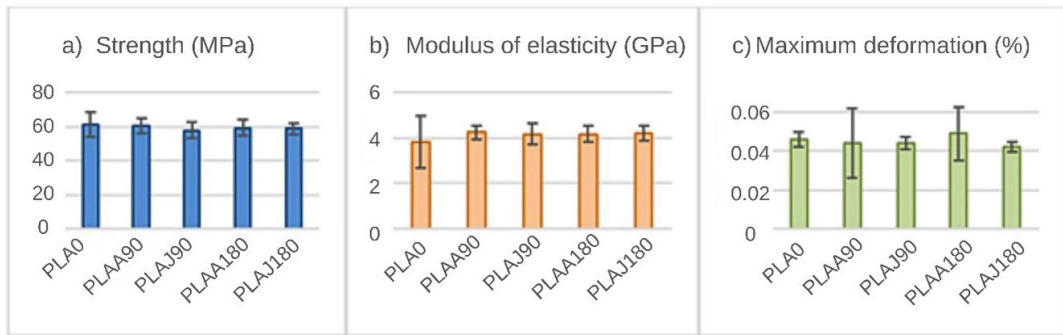


Figure 2. Results of mean and standard deviation for the mechanical tests: (a) Strength; (b) Modulus of elasticity; (c) Maximum deformation.

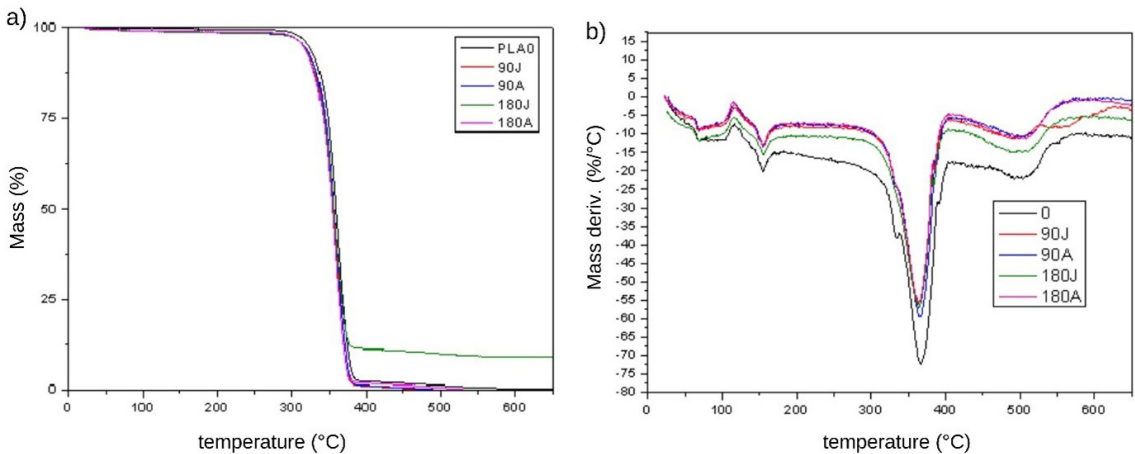


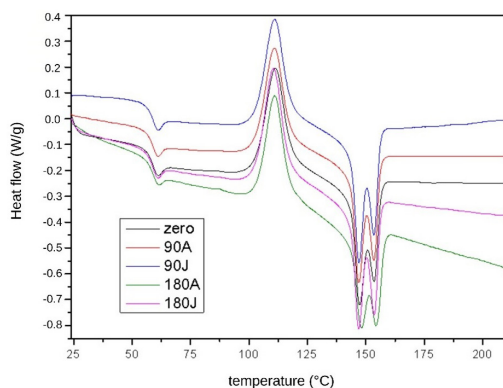
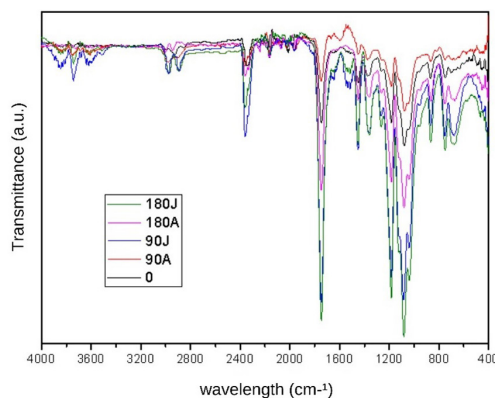
Figure 3. TG (a) and DTG (b) curves.

Table 4. Thermogravimetry results.

Samples	100 °C	Volatiles Residual mass (%)	Ash (600 °C)	T start	T onset °C
PLA0	99.67	99.38	0.431	274	339
PLAA90	99.12	98.37	0	269	337
PLAJ90	99.10	98.38	0	272	335
PLAA180	99.18	98.75	0	271	334
PLAJ180	99.06	98.32	9.285	271	334

Table 5. Differential Scanning Calorimetry results.

Samples	T_g	Crystallization		T_m			Xcrist
	°C	°C	ΔH_1 (J/g)	1 _a °C	2 _a °C	ΔH_2 (J/g)	
PLA0	58.48	111.31	24.41	147.39	153.51	26.93	2.7%
PLAA90	58.47	110.88	23.55	147.07	153.48	25.30	1.9%
PLAJ90	58.25	110.88	23.26	147.05	153.32	26.41	3.4%
PLAA180	59.04	110.97	22.37	148.19	154.41	25.28	3.1%
PLAJ180	58.63	110.53	23.19	147.00	153.78	26.94	4.0%

**Figure 4.** DSC curves for PLA samples.**Figure 5.** FTIR spectra obtained for the different PLA samples.

close to 58 °C, refers to the glass transition temperature (T_g) of the samples. The exothermic peak at approximately 110 °C is attributed to cold crystallization, which is typical of PLA^[33]. Finally, two melting peaks, close to 150 °C, represents two melting peaks due to lamellar crystalline structures (crystallites) with different sizes, which is the characteristic behaviour of PLA. All the DSC curves obtained for the PLA samples showed similarity in their profiles^[6,34].

3.2.4 Fourier transform infrared spectroscopy (FTIR)

The characteristic spectrum of pure PLA is represented in this case by PLA0, the black line (Figure 5). Notably, the effect observed around the 3700 cm^{-1} bands, which refers to the OH bond, in the samples that remained in the soil can be an indicator of degradation through the formation of carboxylic acid, i.e., the degradation of the chain causes an increase in the number of ends of the carboxylic chain, which is a characteristic behaviour of PLA. The soluble lactic acid near the surfaces tends to leach out before complete degradation, and the lactic acid inside is retained and contributes to the autocatalytic effect, which may explain the steeper curves, from 2400 cm^{-1} , in the samples that were exposed to soil compared to the Zero-PLA samples (PLA0). The band near 1750 cm^{-1} is associated with the C=O bond; we observed an increase in the intensity of this band for both garden samples (PLAJ180 and PLA90). The same samples stood out from the others, with a reduction in the intensity of the 1200 cm^{-1} to 1000 cm^{-1} bands, while after 900 cm^{-1} , more pronounced bands were observed. This behaviour has also been observed in other PLA studies^[35].

3.2.5 Optical microscopy (OM)

The use of optical microscopy helped to visualize characteristics such as the formation of fungal colonies and incrustations on the PLA surfaces. The images made it possible to see the areas most affected by the action of the microorganisms. All the samples exposed to the soil for 90 and 180 days (PLAJ90, PLAA90, PLAJ180 and PLAA180) showed the presence of orange and black pigments, typical of bacteria and fungi, as well as surface perforations, irregular and fragmented edges, cracks and superficial peeling, leaving the material with a whitish colour (Figures 6b, c, d, e), while the PLA0 samples maintained uniform and unchanged surfaces (Figure 6a). Regardless of the type of soil and the biodegradation time, the samples did not differ significantly; they all had similar aspects, but the samples that were left in the soil for 180 days (PLAJ180 and PLAA180) had whiter surfaces than the 90-day samples (PLAJ90 and PLAA90). In his research^[23], similar degradations on the surface of PLA were observed.

3.2.6 Colour analysis

A bleaching trend was observed in the PLAA90, PLA90, PLAA180 and PLAJ180 samples, confirmed by the increase in clarity values (L^*); the closer the L^* values were to 100, the more they tended towards absolute white. In addition, there was a decrease in the values of the a^* and b^* coordinates, explained by the reduction in PLA colour pigments. The greater the fading of the sample that remained in the soil, the greater the $\Delta E^{[25]}$, with the highest value observed in the PLAJ180 sample^[26]. In this context, it was possible to observe a variation in the original colour (PLA0) compared to the PLA samples that remained in the different types of soil, as shown in Table 6.

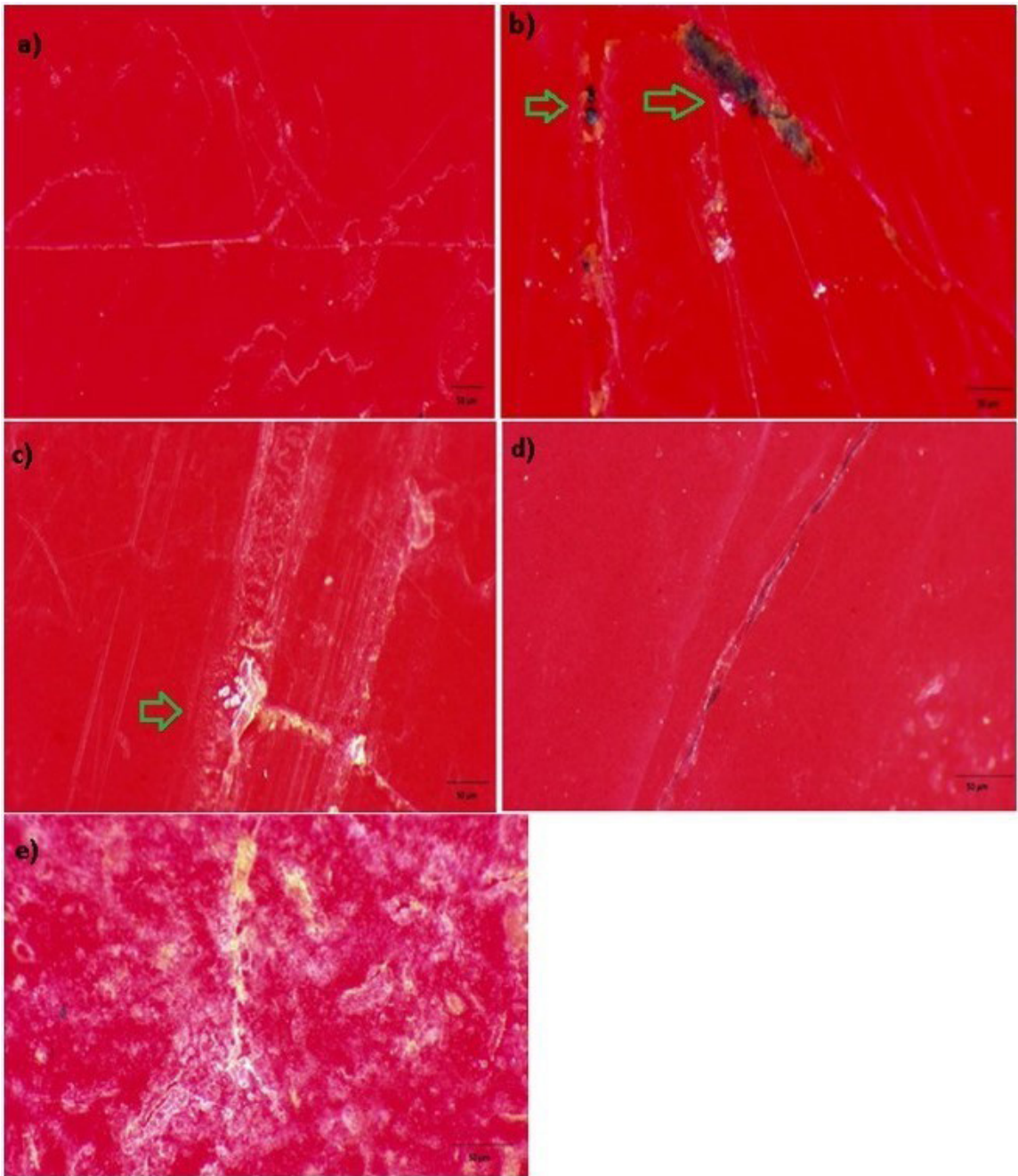


Figure 6. Images obtained by optical microscopy for the PLA samples. (a) PLA0 sample; (b) PLAJ90 sample; (c) PLAA90 sample; (d) PLAJ180 sample; (e) PLAA180 sample. The arrows indicate the points that stood out, indicating fouling, microorganism colony formation, and surface scaling.

Table 6. Mean and standard deviation of delta (L, a, b and E) results.

Amostras	ΔL	Δa	Δb	ΔE
PLAA90	2.53 ± 1.24	-4.00 ± 1.67	-3.10 ± 0.65	5.78 ± 1.59
PLAJ90	1.98 ± 1.98	-2.71 ± 3.80	-2.47 ± 2.74	4.34 ± 4.87
PLAA180	3.41 ± 1.19	-2.15 ± 1.32	-3.89 ± 1.53	5.63 ± 2.24
PLAJ180	3.35 ± 2.85	-4.11 ± 2.52	-4.96 ± 3.97	7.33 ± 5.35

4. Conclusions

The highest rates of biodegradation of PLA in this study were observed on the surfaces of the material through the appearance of fissures, cracks, flaking and colour change, as well as greater functional groups that indicated surface degradation. Within the terms and conditions of this work, samples buried in different soils did not have significant changes in thermal and mechanical properties. This indicates that there was no significant biodegradation in the internal structures of the samples.

Under conditions of an average ambient temperature of 24 °C, humidity of approximately 60%, pH between 5.1 and 7.0 and the types of soil in which the experiment was carried out, greater biodegradation did not occur. Greater degradation may occur under more controlled conditions of temperature and humidity, in the presence of biological indicators of soil quality, or especially, over a longer period.

Therefore, PLA needs favourable composting conditions and a longer period when discarded in landfills or in inappropriate places. As a possible recommendation, recycling or disposal should be carried out in suitable environments; otherwise, PLA waste can accumulate in the environment, causing future pollution problems.

5. Author Contribution

- **Conceptualization** – Alfredo Rodrigues de Sena Neto.
- **Data curation** – Virginia Mendonça Lourenço Benhami; Alfredo Rodrigues de Sena Neto.
- **Formal analysis** – Virginia Mendonça Lourenço Benhami; Alfredo Rodrigues de Sena Neto; Silvia Maria de Oliveira Longatti; Fatima Maria de Souza Moreira.
- **Funding acquisition**: Alfredo Rodrigues de Sena Neto.
- **Investigation** – Virginia Mendonça Lourenço Benhami; Alfredo Rodrigues de Sena Neto.
- **Methodology** – Virginia Mendonça Lourenço Benhami; Alfredo Rodrigues de Sena Neto; Fatima Maria de Souza Moreira; Silvia Maria de Oliveira Longatti.
- **Project administration** – Virginia Mendonça Lourenço Benhami; Alfredo Rodrigues de Sena Neto.
- **Resources** – Virginia Mendonça Lourenço Benhami; Fatima Silvia Maria de Oliveira Longatti; Alfredo Rodrigues de Sena Neto.
- **Software** – Virginia Mendonça Lourenço Benhami; Alfredo Rodrigues de Sena Neto.
- **Supervision** – Virginia Mendonça Lourenço Benhami; Alfredo Rodrigues de Sena Neto.
- **Validation** – Virginia Mendonça Lourenço Benhami; Alfredo Rodrigues de Sena Neto; Silvia Maria de Oliveira Longatti; Fatima Maria de Souza Moreira.
- **Visualization** – Virginia Mendonça Lourenço Benhami; Alfredo Rodrigues de Sena Neto; Silvia Maria de Oliveira Longatti; Fatima Maria de Souza Moreira.
- **Writing – original draft** – Virginia Mendonça Lourenço Benhami.

- **Writing – review & editing** – Virginia Mendonça Lourenço Benhami; Silvia Maria de Oliveira Longatti; Alfredo Rodrigues de Sena Neto.

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