



Enhanced biodegradation of polylactic acid by *Aspergillus oryzae* lipase: Toward sustainable plastic end-of-life solutions

Christina N. Economou^{a,*}, Sine Mandrup Bertozzi^b, Martina Nardi^a, Uttam C. Paul^a, Fabrizio Fiorentini^a, Giorgia Ferrari^a, Marco Contardi^{c,a}, Andrea Armirotti^b, Despina Fragouli^a, Athanassia Athanassiou^{a,*}

^a Smart Materials, Istituto Italiano di Tecnologia (IIT), Via Morego 30, Genoa 16163, Italy

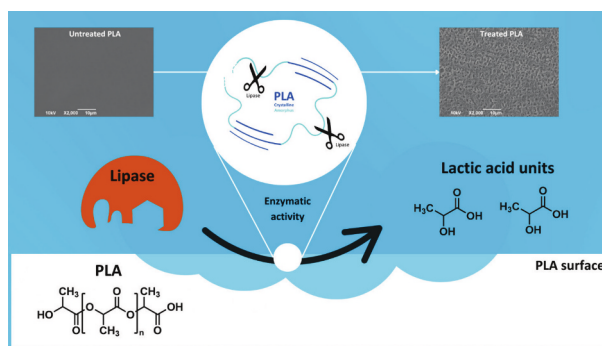
^b Analytical Chemistry Facility, Istituto Italiano di Tecnologia (IIT), Via Morego 30, Genoa 16163, Italy

^c University of Milano-Bicocca, Department of Earth and Environmental Sciences (DISAT), Piazza della Scienza, Milan 20126, Italy

HIGHLIGHTS

- Effective enzymatic degradation of polylactic acid (PLA) films and microparticles.
- Treated materials exhibited changes in surface morphology and chemical structure.
- Lipase degraded the amorphous region of PLA leading to an increased crystallinity.
- Lactic acid monomers production during the biodegradation process was confirmed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

Poly-L-lactide acid
Films
Microparticles
Enzymatic hydrolysis
Lactic acid
Bioplastics

ABSTRACT

This study investigates the enhanced biodegradation of polylactic acid (PLA) films and microparticles using a commercial lipase enzyme from *Aspergillus oryzae*. To evaluate the effect of pH on the bioprocess efficiency, the enzymatic hydrolysis of PLA films was initially examined at pH values ranging from 7.0 to 8.5, at 37°C, for 28 days, using an enzyme activity of 2,425 U/mL. Changes in the surface morphology and chemical structure of the films were more pronounced at pH 8.0, while it was found that the lipase preferentially targets the amorphous regions of PLA, leaving its crystalline structures intact during the treatment period. The treated PLA films exhibited significant alterations in their surface morphology, with enhanced roughness and increased hydrophilicity compared to the untreated films. Using pH 8.0 as the optimum condition, the effect of higher lipase enzyme activities on the biodegradation of both PLA films and microparticles was studied, showing a clear acceleration in enzymatic hydrolysis. Additionally, the production of lactic acid during the degradation process was confirmed through high-performance liquid chromatography. These findings highlight the potential of enzymatic approaches to efficiently degrade PLA-based materials, enabling their bioconversion into valuable lactic acid

* Corresponding authors.

E-mail addresses: christina.oikonomou@iit.it (C.N. Economou), athanassia.athanassiou@iit.it (A. Athanassiou).

<https://doi.org/10.1016/j.biortech.2025.132807>

Received 10 February 2025; Received in revised form 22 May 2025; Accepted 10 June 2025

Available online 11 June 2025

0960-8524/© 2025 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

monomers. By addressing the end-of-life challenges of PLA, this work demonstrates its viability as a sustainable alternative to conventional plastics, contributing to a circular economy and reducing environmental impact.

1. Introduction

Plastic pollution has become one of the most important environmental problems worldwide, mainly due to the non-biodegradable nature of conventional plastics (Gilani et al., 2023). As an alternative, biodegradable and compostable polymeric components from renewable resources are currently being explored to combat plastic pollution issues. To this end, poly(lactic acid) (PLA), a thermoplastic aliphatic polyester of lactic acid, derived from microbial fermentation of renewable carbon resources such as sugarcane and corn (Karamanlioglu et al., 2014; Mo et al., 2023) is a valuable alternative of certain conventional polymers, since it can be completely converted to carbon dioxide (CO₂) and water under certain conditions (Lee et al., 2014; Xu et al., 2022). PLA has found numerous applications in agriculture, textile, and packaging industry and it represents an ideal alternative to petroleum-based plastic materials due to its distinctive mechanical and thermal properties (Satti et al., 2018). On the top, the non-toxic nature of PLA-based materials and their degradation products make them also suitable candidates for drug delivery systems (Abu Hajleh et al., 2020). For all the above reasons, PLA is currently the most widely used biobased polymer accounting for 31 % of all biodegradable plastics produced in 2023, while its global production capacity is expected to triple to 3,237 million tonnes by 2028 (European bioplastics, 2023).

Since lactic acid exists in two optically active forms D- and L-, PLA can be synthesized in three stereochemical forms called poly-L-lactide (PLLA), poly-DL-lactide (PDLLA), and poly-D-lactide (PDLA) depending on the polymerization conditions applied, which endows different physicochemical properties (Qi et al., 2017; Zaaba and Jaafar, 2020). PLA degradation occurs through the cleavage of the ester bonds of the polymer backbone and depends on the polymer's intrinsic properties such as crystallinity and molecular weight, on its final composition, shape and size, as well as on the degradation conditions such as temperature, humidity, and pH among others (Araújo et al., 2014; Casalini et al., 2019; Cui et al., 2022). Between the different degradation processes that have been explored so far, i.e., chemical, thermal, biological and photodegradation (Velghe et al., 2023; Zaaba and Jaafar, 2020), the biological is the most interesting and complicated to study owed to the great variety of conditions and microorganisms present in the environment that can perform such action (Teixeira et al., 2021). In practice, at their end-of-life, PLA products can be degraded under industrial composting conditions at temperatures close to or higher than the glass transition temperature (T_g) of the polymer i.e., 60-65°C, high relative humidity (~60 %) and in the presence of oxygen (Kulikowska et al., 2020). Although PLA has achieved OK industrial compost certificates, the composting industry remains skeptical about introducing bioplastics in their facilities due to the distinct degradation pathways required compared to natural components. Currently, the composting method of PLA has a relatively slow biodegradation rate (up to 12 weeks), and monomer recovery cannot be achieved (Rezvani Ghomi et al., 2021). Therefore, methods to enhance and accelerate PLA biodegradation could provide a compelling solution, encouraging the composting industry to incorporate bioplastics by offering a viable end-of-life pathway. Furthermore, if chemical recycling proves preferable in certain contexts, methods are needed for the recovery of the building block, lactic acid, which can be reused to synthesize new PLA or other products, thus supporting a circular economy approach. Indeed, the primary end-of-life challenges for PLA-based materials is enhancing composting process efficiency, minimizing process duration, and enabling the recovery of lactic acid, thereby integrating PLA more effectively into the circular economy for sustainable bioplastic use.

Enzyme-mediated biodegradation, specifically targeting desired

polymeric chains, represents an efficient and environmentally friendly novel approach to tackle these issues (Ellis et al., 2021). Specifically, extracellular enzymes, excreted from microorganisms, can attack the long polymer chains and break them down into monomer or oligomer subunits of lower molecular weight to be assimilated by the cells (Karimi-Avargani et al., 2020; Noor et al., 2020). The PLA degrading enzymes reported in the literature belong principally to the family of hydrolases. Serine proteases were mainly used due to the similarity in the molecular structure of L-lactic acid units and silk fibroin proteins, a natural substrate of serine proteases (Cui et al., 2022; Luo et al., 2024; Penkhrue et al., 2015), whereas esterases, lipases and cutinases were also studied (Hajighasemi et al., 2016; Nakajima-Kambe et al., 2012; Noor et al., 2020; Wang et al., 2011). Lipases constitute a subclass of esterase enzymes which catalyze the hydrolysis, esterification and interesterification/transesterification of ester bonds (Chandra et al., 2020), while many species of fungi, bacteria and microalgae found in terrestrial or marine habitats have been identified as potential lipase producers (Chandra et al., 2020; Spier et al., 2020). Although microbial lipases have been studied for the biodegradation of plastics such as poly(ethylene terephthalate) (PET) (Carniel et al., 2017; de Castro et al., 2017; Safdar et al., 2024) and polyurethane (PU) (Khan et al., 2017), only a limited number of studies involve them in PLA degradation (Lee et al., 2014; Meza Huaman et al., 2024; Nakajima-Kambe et al., 2012). Specifically, a lipase enzyme from *Candida cylindracea* induced some changes in the surface morphology of PLA nonwovens after 21 days in buffer solution at 40°C (Lee et al., 2014). A decrease in turbidity of an emulsified PLA solution was observed after 3 days at 37°C when using a purified lipase from *Aspergillus niger* (Nakajima-Kambe et al., 2012), and a chemically modified lipase from *Candida antarctica* was able to degrade PLA cup fragments into lactic acid monomers within 24 h at 90°C in ionic liquids (Meza Huaman et al., 2024). However, a comprehensive and detailed study on enzymatic degradation of PLA materials under mild and environmentally friendly conditions without using toxic substances and ensuring lower energy consumption has not yet been explored in the literature.

The aim of this study is to investigate the depolymerization of PLA films and microparticles without being subjected to any pretreatment using a commercial lipase enzyme from *Aspergillus oryzae* under neutral and alkaline conditions, at temperature lower than the T_g of PLA. *Aspergillus oryzae* is an edible filamentous fungus, mainly used in the food industry to produce fermented food thanks to its ability to secrete high concentration of extracellular enzymes, including lipases. The non-toxic nature of this fungus is a significant advantage for scale-up applications, as risks associated with microbial source toxicity or pathogenicity are reduced. Additionally, *A. oryzae* is a well-studied fungus with established genetic manipulation techniques, allowing for the enhancement of lipases properties such as stability, activity or substrate specificity through genetic engineering (Daba et al., 2021). Herein, as a first step, the effect of different pH values and enzymatic activities on the bioprocess efficiency was examined. Detailed studies on the morphological, structural and chemical changes of the PLA materials were conducted indicating high efficiency of the degradation process, while the production of PLA oligomer components was proved and monitored through high-performance liquid chromatography (HPLC). To the best of our knowledge, this is the first report on the enzymatic degradation of PLA components using lipase from *A. oryzae*, paving the way for accelerated biodegradation and demonstrating that composting can serve as a viable end-of-life solution for bioplastics. Moreover, lactic acid recovery enables chemical recycling for new PLA synthesis, advancing circular economy efforts.

2. Materials and methods

2.1. Materials

A commercial lipase enzyme produced from *Aspergillus oryzae*, chemicals for preparing culture media, enzyme assay, and the other analyses were purchased from Sigma–Aldrich. All chemicals are of analytical grade and used as received. Deionized water was obtained from Milli-Q Advantage A10 purification system. Poly-L-lactic acid (PLLA) pellets Ingeo 4043D (Mw = 160 kDa) were purchased from Nature Works and were converted into 100 µm thick film samples through melt extrusion process (Minicast, Eur.Ex.Ma S.r.l.). The profile of temperature had a range between 165 °C to 195 °C, with the speed of the screws of about 35 rpm. The film came out from the extruder die and collected on the heated roll at a temperature of 45 °C each. The collecting system ended with a final collecting roll set at 10 % of its speed. PLLA microparticles of two size ranges, i.e. 2.00–3.00 mm and 0.30–0.75 mm were also prepared through mechanical grinding. For both cases, PLLA pellets (4.00 mm) were ground using Dry mill – IKA – PILOTINA MC equipped with a 3.00 mm pilotina sieve, operating at 1500 rpm. The particles with a size range between 2.00 and 3.00 mm were then manually collected for the experiments. To achieve particle sizes between 0.30 mm and 0.75 mm, a subsequent sieving cycle with a 0.75 mm electrical sieve was then performed, and the obtained grains were subjected to a final sieved using an electrical sieve (VWR Test Sieve 200 × 50 mm) with a 0.30 mm mesh size. The material that did not pass through the sieve with the size range 0.30–0.75 mm was collected and employed in the subsequent phases of the study. Even though this study focused on the degradation of PLLA, the term PLA is used throughout the text to maintain unity in terminology.

2.2. Experimental conditions and design

Pieces of PLA films (1 × 1 cm²) weighing about 16 mg each were placed in tubes containing 3 mL Tris-HCl buffer solution 20 mM with different pH values (7.0, 7.5, 8.0 and 8.5) and lipase enzyme activity of 2,425 ± 35 U/mL. The pH values were measured with a pH meter (Hanna Edge instrument). Only the alkaline pH values were considered since the water absorption into the polymer matrix is higher in alkaline conditions compared to acidic ones due to the difference in osmotic pressure of the alkaline buffer solution and carboxylic end groups formed inside the polymer (Li and McCarthy, 1999). Additionally, lipase enzymes are stable in a pH range between 6 and 9 (Kumar et al., 2020). Two higher lipase enzymatic activities of 4,650 ± 71 and 11,250 ± 177 U/mL were also tested at the optimum pH value (Table 1) to evaluate if the efficiency of degradation could be increased. Experiments with PLA microparticles of two different sizes (2.00–3.00 mm and 0.30–0.75 mm) were also performed at the optimum pH value using lipase activities of 2,425 ± 35, 4,650 ± 71 and 11,250 ± 177 U/mL and keeping the same experimental conditions (~16 mg of material per each tube containing 3 mL Tris-HCl buffer solution 20 mM) in order to study the impact of particle size in the biodegradation process. Note that before adding lipase enzyme, the mixture of PLA and buffer solution was sterilized

Table 1

Enzymatic activity of lipase, protein content of enzyme, specific lipase enzyme activity and mg of protein per g of PLA material used in this study.

Enzyme activity (U/mL)	Protein content (mg/mL)	Specific enzyme activity (U/mg protein)	mg protein/g PLA
2,425 ± 35	0.55 ± 0.01	4,441 ± 65	102.43 ± 2.61
4,650 ± 71	1.11 ± 0.03	4,189 ± 64	208.14 ± 4.93
11,250 ± 177	3.17 ± 0.12	3,513 ± 56	593.99 ± 22.15

under UV light in a biohazard hood (VBH 48, ANGELANTONI LIFE SCIENCE Srl) for 1 h to avoid any action from microorganisms during the experiments. Experiments were performed in triplicate at 37 °C in a rotary incubator (100 rpm) for 28 days. Control experiments (without lipase enzyme addition) were conducted at the same conditions used in each experiment. At sampling times, PLA samples were removed from the tubes, rinsed thoroughly with deionized water, and then dried at 37 °C until a constant weight was achieved. The weight loss % was calculated by subtracting the final weight from the initial weight of the film divided by the initial weight and multiplied 100 %.

2.3. Enzymatic activity and protein content analysis

The lipase (EC 3.1.1.3) activity was determined by titration of free fatty acids according to the Sigma lipase assay (Sigma in-house procedure). The reaction mixture consisted of olive oil (3 mL), Tris-HCl buffer (1.0 mL, 200 mM, pH 7.2), deionized water (2.5 mL) and lipase enzyme solution (1 mL). The mixture was incubated for 30 min at 37 °C in a rotary incubator. The reaction was stopped by the addition of 95 % (v/v) ethanol (3 mL), and then 4 drops of 0.9 % (w/v) thymolphthalein indicator solution were added. The liberated fatty acids were determined by titration with NaOH solution (50 mM) until the pH of the solution reached 10.0. One unit (U) of lipase activity is defined as the hydrolysis of 1.0 microequivalent of fatty acid from a triglyceride in 30 min at pH 7.2 at 37 °C. Protein concentration was determined according to the Bradford method by reference to a standard curve for bovine serum albumin (BSA) (Bradford, 1976).

2.4. Fourier transform infrared spectroscopy analysis

Chemical characterization of dried PLA films and microparticles before and after lipase enzyme treatment was performed by Fourier transform infrared (FTIR) spectroscopy (VERTEX 70v, FT-IR, Bruker). The instrument had an attenuated total reflection (ATR) accessory (MIRacle ATR, PIKE Technologies) scanning over the wavenumber range of 4000–600 cm⁻¹ at a resolution of 2 cm⁻¹ and 64 scans. For all samples, the mean spectrum line of triplicate measurements (three measurements of three different samples) is presented. All peaks were normalized to the reference peak of 1454 cm⁻¹ which was assigned to the CH₃ asymmetric deformation modes (δ_{as}CH₃) and used as internal standard for PLA (Kister et al., 1998; Oliveira et al., 2016). Spectral analysis was performed with OriginPro 2022 software.

2.5. Nuclear magnetic resonance

Proton Nuclear Magnetic Resonance (¹H NMR) analysis of initial and treated PLA films was conducted at room temperature using 5 mm tubes on an Advance III 400 MHz spectrometer, Bruker, equipped with a Broadband Inverse probe. Chemical shifts are reported in ppm and were determined in reference to Tetramethylsilane (TMS). Quantitative ¹H NMR analysis was recorded with NS = 16 scans and D1 = 30 s. The samples were prepared by dissolving about 5–6 mg of PLA film (treated and untreated) in 0.5 mL of CDCl₃. The spectra were processed and analyzed with TOPSPIN software.

2.6. X-ray diffraction (XRD)

XRD measurements of PLA film and microparticles were performed via XRD analysis by a Malvern PANalytical Empyrean diffractometer equipped with a 1.8 kW Cu Kα ceramic X-ray tube and a PIXcel3D 2 × 2 area detector, operating at 45 kV and 40 mA. The degree of crystallinity was calculated dividing the crystalline area regions (absolute area of peaks positioned at 2θ ~ 16.7° and ~ 19.0°) by the total area as follows (Rahmayetty et al., 2018):

$$\text{Crystallinity}(\%) = \frac{\text{Crystalline area}(8 - 50^\circ)}{\text{Total area}(8 - 50^\circ)} \times 100$$

The absolute total and crystalline area regions were estimated by using OriginPro 2022 software.

2.7. Scanning electron microscopy

Dried samples before and after lipase enzyme treatment were attached to aluminum stubs by using carbon tape and coated with 10 nm gold by sputtering. The surface morphology of the samples was investigated by scanning electron microscopy (SEM) in a JEOL JSM-6490LA microscope using the secondary electrons detector. The images were acquired with 10 kV accelerating voltage, load current of 78 μ A and a magnification of 2000 \times .

2.8. Profilometer

Surface roughness of the PLA films before and after enzymatic treatment was measured by a 3D Optical profilometer Zeta-20 by Zeta Instruments complies with ISO 4287–1997 for the calculation of the roughness parameters.

2.9. Wettability study

Static water contact angles of dried film samples were measured at room temperature by using a contact angle goniometer OCA-20 (Data-Physics Instruments GmbH, Filderstadt, Germany). Deionized water droplets of 5 μ L were deposited on the film surface and the contact angle was calculated from the side view with the help of the built-in software. Five measurements on different areas of each sample were acquired to ensure repeatability.

The contact angles values of enzymatically treated PLA films were also theoretically calculated according to the Wenzel equation (Wenzel, 1936):

$$\cos\theta_r = r \times \cos\theta_y \quad (1)$$

where θ_r is the contact angle of a rough surface, θ_y is the Young's contact angle determined on a flat surface of the same material, and r is the surface roughness defined as the ratio of the actual wetted surface area over the geometric (projected) surface area of the material.

2.10. High-performance liquid chromatography (HPLC)

The depolymerization of PLA was analyzed and quantified by high-performance liquid chromatography with refractive index detection (HPLC-RID). The analyses were performed on an Agilent 1260 Infinity HPLC system consisting of a diode array detector (DAD) and a refractive index detector (RID). The analyses were run on an Agilent Hi-Plex H PL1170-6830 (300 \times 7.7 mm ID, particle size 8 μ M), using 5 mM H₂SO₄ in H₂O as mobile phase at 0.6 mL/min in isocratic mode. 10 v/v% 1 M KOH was added to all calibrators, qualitative controls and samples prior to analysis.

3. Results and Discussion

3.1. Modifications of PLA films upon enzymatic treatment

In enzymatic degradation of polymers, enzymes initially attack the surfaces and gradually alter their structure and morphology (Lee et al., 2014). In this study, the surface morphology of PLA films after enzymatic treatment at different pH values (7.0, 7.5, 8.0 and 8.5) and time points (7, 14, 21 and 28 days) with enzyme activity of 2,425 U/mL was studied using SEM. The SEM images in Fig. S1 show that after 7 days for all cases, the smooth surface of the films becomes rougher, with the

changes being more intense at pH values of 8.0 and 8.5. Specifically, the effect of the enzymatic treatment on the PLA becomes evident after 7 days with the creation of rough structures on the surface of the films, while these changes become more intense over time until the end of the treatment at 28 days (Fig. 1A). As expected, no noticeable modifications were observed in the control experiments conducted at different pH values in the absence of the lipase enzyme. Indeed, it is well-known that PLA hydrolysis is favored under strongly alkaline conditions (i.e., at pH 13) and not in moderate alkaline or neutral conditions at 30°C, used herein (Jung et al., 2006). In fact, Tsuji and Ikada (Tsuji and Ikada, 2000) observed changes in the surface morphology of PLA films only after 150 days of alkaline treatment using a NaOH solution with pH 11.0 at 37°C. However, this process can be significantly accelerated at temperatures above the T_g, which favor the chain scission. In fact, as proved by Rodriguez et al. (Rodriguez et al., 2016), intense changes in the surface of a PLA film were observed after only 5 days of alkaline hydrolysis at 70°C in a medium with pH 12.0. Instead, in the presence of the enzyme, similar with our case, changes in the polymer surface morphology have been reported after 21 days when a commercial lipase enzyme from *Candida cylindracea* (powder form) was used at a concentration of 10 % (weight of enzyme to weight of material) for enzymatic treatment of PLA fibers in Tris buffer solution with pH 8.0 at 40°C (Lee et al., 2014). Penkhrue et al. (Penkhrue et al., 2015) also found that protease, esterase and lipase enzymes produced from the bacterium *Amycolatopsis* sp. changed the surface of PLA films after 7 days of treatment at 30°C in a liquid medium with pH 7.0. In our work, pH 8.0 was selected as the optimum condition to study further the bioprocess, as enzymatic activity remained largely stable over 28 days of treatment. Specifically, the lipase activity was reduced by approximately 12 % at pH 8, compared to a reduction of 26 % in the experiments at the other pH values after the same period. As illustrated in Fig. 1A, the surface of the PLA films was severely modified after 28 days at pH 8.0, in the presence of the enzyme, with the pores' size clearly increasing when higher activities of lipase enzyme (4,650 and 11,250 U/mL) were used. The roughness of the untreated and enzymatically-treated PLA films was also measured by optical profilometry to quantify the modification on the surface roughness, observed in the SEM analysis, and is shown in Table 2. In all cases, the roughness increased significantly, between 2.3 and 3.2 times, after enzymatic treatment of 28 days.

It is well known that both the surface roughness and chemistry affect the wettability of a solid surface (Wenzel, 1936). Thus, the water contact angles (θ), of untreated and treated PLA films were also measured and compared. As shown in Fig. 1B, the PLA films are slightly hydrophobic with θ values of $87.55 \pm 0.26^\circ$. After 28 days of treatment with lipase of 2,425 U/mL, the θ values of the films decreased to $56.39 \pm 2.85^\circ$, $57.83 \pm 3.75^\circ$, $38.01 \pm 3.18^\circ$ and $30.11 \pm 4.83^\circ$, at pH values 7.0, 7.5, 8.0 and 8.5, respectively, demonstrating that the enzymatic treatment increases the hydrophilicity of PLA surface in more alkaline environments. By introducing the contact angle of the flat PLA film ($\theta_y = 87.55^\circ$) along with the measured surface roughness of the films into the Wenzel model (Equation (1)), which predicts how surface roughness affects the apparent water contact angle on a surface, the theoretical water contact angles of the treated/rough PLA films were calculated (Fig. 1B), and compared with the experimentally measured values (Athanasios et al., 2006). As shown in Fig. 1B, the experimental contact angles deviate significantly from the theoretical predictions. This discrepancy suggests that, in addition to changes in surface roughness, the surface chemistry of the films was notably altered after enzymatic treatment, as the Wenzel model predicts the water contact angle of rough surfaces assuming their chemistry is identical to that of the corresponding flat surface. This is in accordance with previous findings that report the creation of hydrophilic functional groups on polymer surfaces due to the enzymatic interaction with the medium (Srikanth et al., 2022), as well as the transformation of PET fabrics to hydrophilic materials after the treatment with a lipase enzyme from *Pichia pastoris* (Gao et al., 2017). On the other hand, the θ values of the PLA films remained practically unaltered

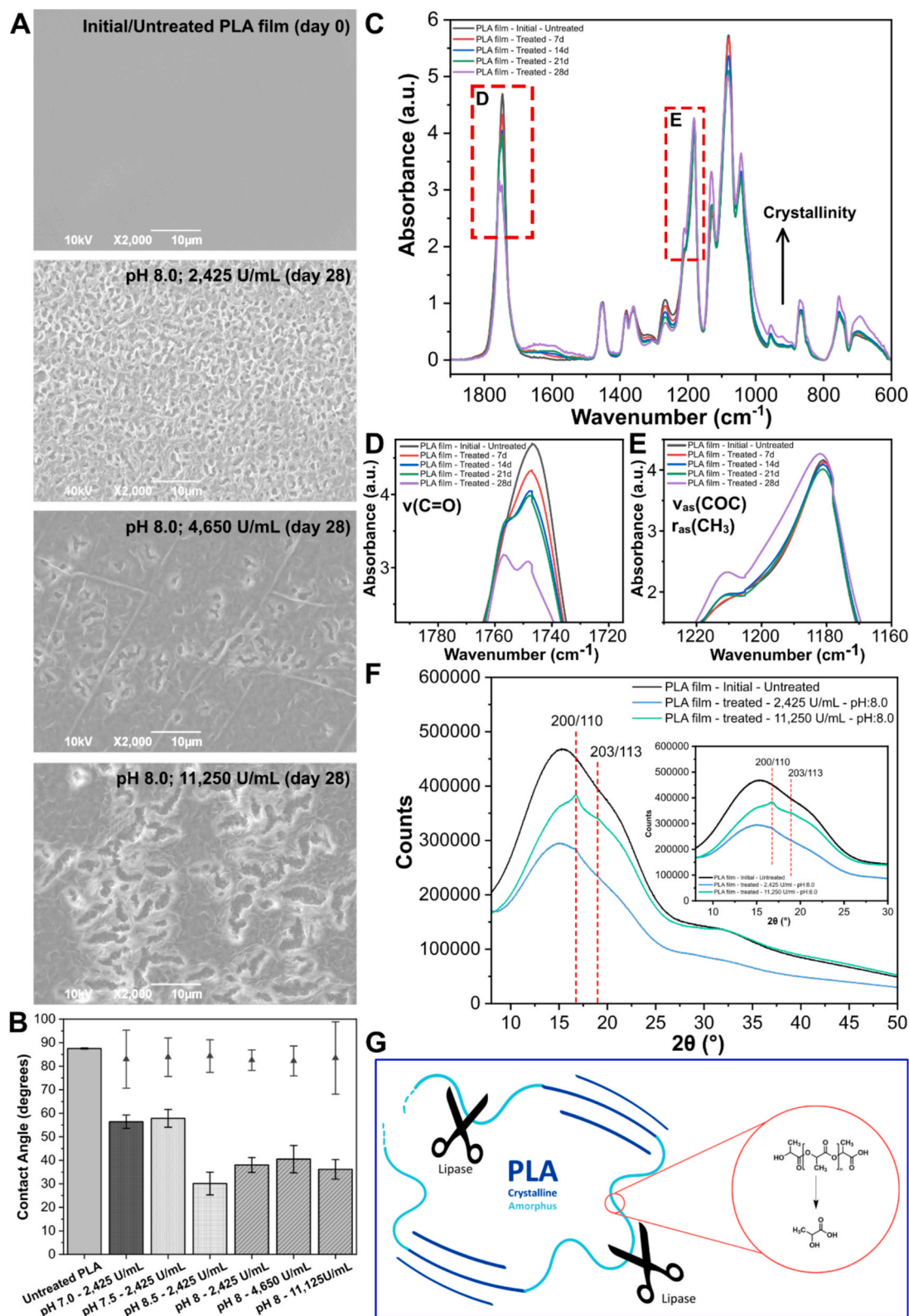


Fig. 1. Morphological, physical, chemical and crystallographic modifications of PLA films. A) SEM images of the surface of untreated PLA films and enzymatically treated PLA films with different lipase enzyme activities at pH 8.0 after 28 days. B) Water contact angles of untreated and enzymatically treated PLA films with different lipase enzyme activities and pH values after 28 days. Triangle symbols represent the respective theoretically predicted contact angle values using the Wenzel equation. C-E) ATR-FTIR spectra of untreated and enzymatically treated PLA films with lipase enzyme activity of 2,425 U/mL at different time points at pH 8.0. F) XRD curves of PLA films untreated and treated with lipase of enzymatic activity 2,425 U/mL and 11,250 U/mL, at pH 8.0, for 28 days. G) Schematic representation of lipase enzyme action on PLA.

Table 2

Average roughness (Sa) and root mean square roughness (Sq) measurements of untreated and enzymatically treated PLA films obtained with 3D optical profilometer.

Sample	Sa ($\mu\text{m} \pm \mu\text{m}$)	Sq ($\mu\text{m} \pm \mu\text{m}$)
Untreated PLA film	0.0475 \pm 0.0043	0.0604 \pm 0.0056
pH 7.0 – lipase: 2,425 U/mL	0.1360 \pm 0.0202	0.1679 \pm 0.0228
pH 7.5 – lipase: 2,425 U/mL	0.1188 \pm 0.0116	0.1523 \pm 0.015
pH 8.0 – lipase: 2,425 U/mL	0.1107 \pm 0.0091	0.1408 \pm 0.0105
pH 8.5 – lipase: 2,425 U/mL	0.1437 \pm 0.0076	0.1850 \pm 0.0150
pH 8.0 – lipase: 4,650 U/mL	0.1508 \pm 0.0117	0.1913 \pm 0.0166
pH 8.0 – lipase: 11,250 U/mL	0.1261 \pm 0.0232	0.1647 \pm 0.0307

in the control experiments after 28 days (Fig. S2), confirming the effective action of lipase enzyme on the surface of the PLA. It is worth noting that the surface roughness and the wettability did not significantly change when higher activities of lipase enzyme were used (4,650 and 11,250 U/mL, pH 8.0) (Table 2 and Fig. 1B) even if the surface after 28 days of treatment presented larger pores (Fig. 1A).

Notably, the transparent PLA films became whitish after enzymatic treatment in all the conditions tested, while the films in control experiments remained transparent after 28 days in the buffer solutions (Fig. S3). In general, transparency is associated with nonporous and/or amorphous polymer films, while porous or semi-crystalline/crystalline polymer films tend to appear opaque or translucent. This is due to light scattering caused either by the presence of pores, when they are close to the size of the light's wavelengths, or by variations in the refractive index between crystalline and amorphous regions, respectively (Yu et al., 2023). Thus, the herein observed phenomenon can be attributed both to the creation of pores on the films' surface after enzymatic treatment, as well as to possible changes of crystallinity after biotreatment. Similar modifications in the transparency of PLA films during biodegradation in compost have been also correlated with a decrease in molecular weight of the polymer (Araújo et al., 2014). According to the literature, the molecular weight, composition, and matrix dimensions of PLA play the most important role in the biodegradation studies (Lin and Anseth, 2013; Von Burkersroda et al., 2002), while there is an induction period until a significant weight loss is observed during hydrolysis of PLA (Tsuji and Ikada, 2000) accompanied with a drastically reduced molecular weight (Rodriguez et al., 2016). In our study, a small weight loss of about 3 % (data not shown) was recorded at the end of the biotreatment, for all cases of enzymatic treatment studied. In fact, similar results, with no significant weight loss, have also been reported in other enzymatic biodegradation studies of PLA using the lipase enzyme for a limited treatment period. Specifically, Lee et al. (Lee et al., 2014) observed less than 5 % weight loss after 21 days of treatment of PLA nonwovens at 40°C, using Tris buffer solution at pH 8. Similarly, the weight loss of PLA films treated with the fungus *Trichoderma viride*, which is characterized by lignocellulosic enzymatic activities, was about 1 % after 21 days cultivation at 28°C (Lipsa et al., 2016). Longer treatment periods have been associated with higher weight losses, as in the case of the chemical hydrolysis, with loss ranging between 10 and 40 % after 3 years (phosphate buffer solution, pH 7.4 at 37°C) (Tsuji and Ikada, 2000). In contrast, complete hydrolysis (100 % weight loss) has been reported to occur within 40 days when higher temperatures e.g., 60 °C are applied at the same experimental conditions (Li and Mccarthy, 1999). Alternatively, it was shown that the weight loss of PLA films after biotreatment with the fungus *Trichoderma viride* can reach 18 % after 21 days at 28°C when epoxidized soybean oil is introduced as plasticizer in the PLA formulation (Lipsa et al., 2016). Similarly, in another study, epoxidized cardanol oleate was used as a chain extender in the melting process of PLA, and the weight loss of PLA films reached about 70 % after four days of enzymatic treatment with proteinase K enzyme in Tris-HCl buffer solution, pH 8.0 at 50 °C (Luo et al., 2024).

The chemical changes of the PLA films induced by the enzymatic treatment were analyzed by ATR-FTIR spectroscopy as shown in Figs. 1C

and S4–S6, with major changes observed in the range of 1900–600 cm^{-1} . Similarly to the other treatment conditions, at pH 8.0 and lipase enzyme activity of 2,425 U/mL, the band observed at 1746 cm^{-1} was assigned to C=O stretching ($\nu\text{C=O}$) and associated with lactic acid esters of PLA. This region is sensitive to the morphology and conformation of PLA structure as the ester groups are affected by hydrolysis reactions (Kister et al., 1998; Wang et al., 2013) and can split into four conformational bands of tt, tg, gt, and gg (Meaurio et al., 2009; Zhu et al., 2022). According to Figs. 1D and S4–S6, the band at 1746 cm^{-1} was split into double bands at 1756 and 1748 cm^{-1} during the enzymatic treatment, corresponding to gt and tt conformers, respectively (Meaurio et al., 2009). The gt conformation has the lowest energy and corresponds to a 10_3 helix representing the crystalline and amorphous conformers, while the peak at 1748 cm^{-1} corresponds to the tt conformations of amorphous components (Meaurio et al., 2009; Zhu et al., 2022). This trend was first observed after 14 days of enzymatic treatment at pH 8.0 and 8.5 (Figs. 1D and S6) and after 21 days at pH 7.5 (Fig. S5), while it was clear in all experiments after 28 days of biotreatment (Figs. 1D and S4–S6). Similar observations on the splitting in the C=O stretching band have been reported in the literature during biodegradation of PLA under composting conditions at 58°C (Kulikowska et al., 2020). The splitting in the C=O stretching band is also a common phenomenon during the PLA crystallization process (Zhu et al., 2022). Therefore, the above changes in the ATR-FTIR spectra indicate that enzymatic treatment causes conformational changes in the PLA material.

Furthermore, a distinct increase in intensity was observed in the 1680–1550 cm^{-1} region, which was attributed to the formation of non-esterified carboxyl groups (COO^-) during enzymatic treatment, also confirming the hydrolysis of lactic esters (Monsoor et al., 2001; Painter et al., 1983). The bands within the region of 1300–1000 cm^{-1} are assigned to the C–O–C stretching vibrations of ester groups (Krikorian and Pochan, 2005). Specifically, the bands at 1265 cm^{-1} and 1210–1180 cm^{-1} assigned to the vibrations of $\delta\text{CH} + \nu\text{COC}$ and $\nu_{\text{as}}\text{COC} + \nu_{\text{as}}\text{CH}_3$, respectively, reflect the crystallinity in PLA structures (Kister et al., 1998; Urayama et al., 2003). The small peak at 1210 cm^{-1} was detected in enzymatically treated PLA films. This peak was observed after 14 days in media with pH 8.0 and 8.5 (Figs. 1E and S6) and after 21 and 28 days in media with pH 7.5 and 7.0, respectively (Figs. S4 and S5). A decrease in the intensity of the 1265 cm^{-1} peak was also observed over time in all experiments conducted, except for PLA films in media with pH 7.0 (Fig. 1C). These observations at the above bands most plausibly indicate a modification of the crystalline configuration of the PLA structure during enzymatic treatment.

This hypothesis is also supported by a new absorption band that appears at 920 cm^{-1} during the enzymatic treatment, which is assigned to the C–C backbone stretching vibrations mixed with CH_3 rocking modes ($\nu\text{CH}_3 + \nu\text{CC}$). In general, the region from 970 to 850 cm^{-1} reflects the crystallinity of PLA and the type of helical conformation (Beltrán et al., 2016; Zhang et al., 2005). As can be seen in Figs. 1C and S4–S6, the band at 920 cm^{-1} was more intense in PLA films treated at pH from 7.5 to 8.5 at the end of experiments. According to the literature, the presence of this peak indicates that the PLA films used in this study crystallize in 10_3 helical conformation after enzymatic treatment (Meaurio et al., 2009; Zhang et al., 2005). The above change suggests an increase in crystallinity in the PLA films after enzymatic treatment due to the degradation of the amorphous phase (Beltrán et al., 2016; Zhang et al., 2005; Zhu et al., 2022). No changes were observed at the bands at 866 cm^{-1} , assigned to the $\nu\text{C-COO}$ stretching mode (Krikorian and Pochan, 2005), and 754 and 705 cm^{-1} which correspond to $\delta\text{C=O}$ in-plane bending and $\gamma\text{C=O}$ out-of-plane bending, respectively (Babichuk et al., 2022). It is worth mentioning that none of the above chemical changes were observed in the control experiments (PLA films in buffer solution without lipase enzyme) (Fig. S7a–d).

Moreover, the effect of higher lipase enzyme activities i.e., 4,650 and 11,250 U/mL on the biotreatment process was evaluated at optimum pH conditions (pH 8.0) to investigate if the degradation time could be

shortened, since it has been proved that the rate of polymer degradation increases with increasing enzyme concentration (Cui et al., 2022). As shown in Figs. S8 and S9, the same chemical changes in all characteristic absorption bands were observed, but in shorter periods of treatment. For example, the splitting of the band at 1746 cm^{-1} into double band at 1756 and 1748 cm^{-1} was clearly observed after 21 and 14 days in the experiments conducted with 4,650 and 11,250 U/mL, respectively, instead of 28 days in the experiments with lower enzymatic activity. Also, a higher peak intensity at 920 cm^{-1} was detected at the experiments with the highest enzymatic activity after 28 days of treatment (Fig. S8 and S9). At the same time, it was observed that the intensity of the band at 955 cm^{-1} , which is attributed to amorphous vibrations of PLA decreased during the treatment process (Meaurio et al., 2009; Zhang et al., 2005).

XRD analysis of the untreated and enzymatically treated PLA films confirms the conclusions reached by the ATR-FTIR analysis. Specifically, as shown in Fig. 1F, the XRD pattern of the untreated PLA films did not show any characteristic crystalline peaks, indicating a predominantly amorphous structure, according to the ICDD 00-064-1625 database. In contrast, two dominant diffraction peaks at $2\theta \sim 16.7^\circ$ (200/110 plane) and $\sim 19.0^\circ$ (203/113 plane), corresponding to reflections of the semi-crystalline PLA α -phase (Babichuk et al., 2022), were detected in the enzymatically treated PLA films. Notably, these peaks were more intense in the treated PLA films with the highest enzymatic activity. These changes demonstrate that the lipase enzyme can degrade the amorphous region of PLA, revealing the α -crystalline phase of PLA (Fig. 1G).

To further explore the chemical modifications of the PLA after the enzymatic treatment, ^1H NMR analyses were carried out. The spectrum of the pristine PLA showed the typical quadruplet of CH protons in the range 5.19–5.13 ppm and the doublet of CH_3 at 1.59–1.52 ppm (Fig. S10), in agreement with what is reported in the literature (Carrasco et al., 2010; Oliveira et al., 2016). The ratio between the areas of the two signals CH_3/CH was 3.19, close to the theoretical value of 3. The ^1H NMR spectra of the treated PLA films resulted in unchanged chemical shifts and similar proton intensity ratios CH_3/CH (3.11–3.19) (Table S1). These findings confirm that the degradation of PLA occurred through scission of ester bonds, as expected for a lipase enzyme mechanism of action, without the participation of other side reactions as pyrolytic elimination (which forms $\text{CH}_2=\text{CH}$ from $\text{CH}-\text{CH}_3$) (Oliveira et al., 2016). Based on the literature, the active site of the lipase enzyme from *Aspergillus* is characterized by a catalytic triad (Ser-His-Asp), similar to serine proteases and carbonyl esterases. This active pocket typically has a high specificity for monoacylglycerol and diacylglycerol substrates and catalyses the hydrolysis of ester bonds. The hydrolysis mechanism firstly involves the nucleophilic attack of serine on the carbonyl carbon of the ester bond, forming an acyl-aminoacid intermediate by releasing an alcohol. In this step, histidine and aspartic acid stabilize the serine hydroxy group and help the formation of the acyl-enzyme adduct. A second nucleophile attack by water on the acyl-enzyme adduct completes the hydrolysis and forms the carboxylic acid (Mehta et al., 2017). Having a PLA ester bond as an oleophilic substrate, a similar enzymatic mechanism capable of exploiting its functional activity in degrading this polymer could be hypothesized.

As further characterization, the byproducts of the enzymatic treatment of the PLA films were identified and quantified by using HPLC after 28 days of treatment at pH 8.0 for different enzymatic activities. Until now, there is a limited number of studies reporting on degradation products in liquid broth after biological or enzymatic treatment of PLA materials (Hajjighasemi et al., 2016; Meza Huaman et al., 2024; Noor et al., 2020). In accordance with the physicochemical changes observed on the treated PLA films so far, the quantity of the produced by-products identified as lactic acid, was higher when higher lipase enzyme activity was applied for the biodegradation process. In particular, the average lactic acid concentration was 160, 171 and $473\text{ }\mu\text{g/mL}$, after biotreatment with lipase enzyme activity of 2,425, 4,650 and 11,250 U/mL, respectively. The obtained results indicate that the bioconversion of PLA films to lactic acid ranges from approximately 3 % to 9 % after 28 days of

enzymatic treatment, at pH 8 and 37°C , in a buffer solution (Fig. S12). The discrepancy between weight loss (about 3 % in all cases) and lactic acid production could be due to errors introduced by using a standard laboratory balance to weigh light samples. It is worth mentioning that lactic acid was not detected in the control experiments (without lipase enzyme). Using a temperature of 90°C , higher bioconversion efficiencies of PLA to lactic acid monomers have been reported in the literature e.g., 75 % after 40 h when ionic liquids were used alone, while the complete conversion of PLA was achieved under the same conditions by using a chemically modified lipase enzyme (with a concentration of 12 mg/mL) from the fungus *Candida antarctica*. This approach involved a protein surface modification technique to solubilize and stabilize the lipase enzyme in ionic liquids and high temperatures (Meza Huaman et al., 2024). Immobilization techniques that protect the enzyme from high temperatures can also improve the thermal stability of lipases (Panyachanakul et al., 2024), an important factor for efficient PLA degradation as well as the reusability of enzyme (Silva Almeida et al., 2024). Consequently, enzyme engineering could boost their efficiency and thermostability at temperatures close to or above the glass transition temperature of a polymer enhancing the biodegradation efficiency (Bell et al., 2022).

3.2. Modifications of PLA microparticles upon enzymatic treatment

Aiming to investigate the impact of particle size in the biodegradation process, experiments were also conducted using PLA microparticles of two size ranges 2.00–3.00 mm and 0.30–0.75 mm. These experiments were performed at pH 8, using various lipase activities and maintaining the same experimental conditions. The morphology of the initial/untreated and of the enzymatically-treated microparticles at pH 8.0 with lipase enzymatic activities of 2,425, 4,650 and 11,250 U/mL was analyzed by SEM (Fig. 2A for microparticles in the range 2.00–3.00 mm and 3A for microparticles in the range 0.30–0.75 and S11). Fig. 2A, 3A and S11 reveal that the lipase enzyme significantly affected the morphology of the microparticles after 28 days of treatment creating pores on their surface. Larger pores were observed in the case of the smaller microparticles and when higher lipase activity was used (Fig. 3A and Fig. S11).

Chemical changes of the microparticles during enzymatic treatment were also investigated by ATR-FTIR. Figs. 2B and 3B show respectively the spectra of PLA microparticles of 2.00–3.00 mm and 0.30–0.75 mm of diameter, untreated, and treated for 28 days with lipase activities of 2,425, 4,650 and 11,250 U/mL, at pH 8.0. Both types of PLA microparticles showed the typical absorption bands corresponding to the C=O stretching of the lactic acid esters at 1746 cm^{-1} , the CH_3 asymmetric deformation modes at 1454 cm^{-1} and the C—O—C stretching vibrations of the ester groups in the region of $1300\text{--}1000\text{ cm}^{-1}$ (Kister et al., 1998; Urayama et al., 2003). The main differences between the untreated PLA microparticles and films were found in the spectral bands of 1640 and 1560 cm^{-1} , which are assigned to non-esterified carboxyl groups (COO^-) (Monsoor et al., 2001), and 920 cm^{-1} , which reflects the crystallinity of PLA (Beltrán et al., 2016). All the above differences indicate that during the microparticles preparation through mechanical grinding (See “Materials” Section), the polymer’s molecular arrangement is modified, becoming more crystalline. This can be attributed to the mechanical forces that induce local increase of the temperature during grinding that can lead to the reorganization of the molecular chains and other chemical transformations (Jung et al., 2023; Park and Peterson, 2023). The chemical structure of PLA microparticles was monitored every 7 days during enzymatic treatment (data not shown), however clear differences in the FTIR spectra were observed after 28 days (Figs. 2B and 3B). According to Figs. 2B and 3B, the main changes were detected in the bands of 1640 and 1560 cm^{-1} where an increased intensity was observed after enzymatic treatment, indicating increased numbers of carboxyl groups. Also, the appearance of a small shoulder at 1210 cm^{-1} was observed, reflecting the increased crystallinity (Kister

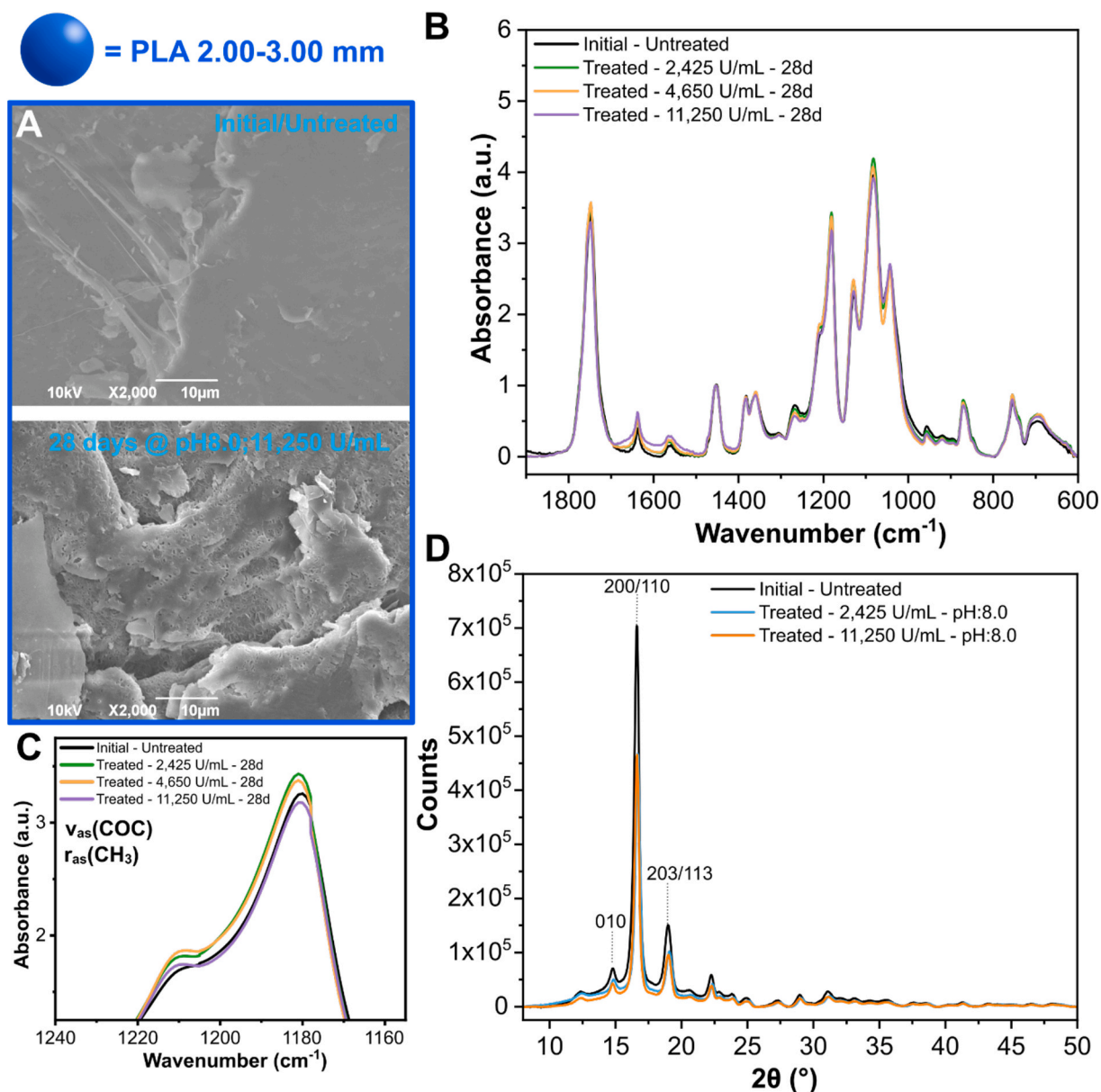


Fig. 2. Morphological, chemical and crystallographic modifications of PLA microparticles with size 2.00–3.00 mm. A) SEM images of the surface of untreated and enzymatically-treated PLA microparticles of 2.00–3.00 mm with lipase enzyme activity of 11,250 U/mL at pH 8.0 after 28 days. B, C) ATR-FTIR spectra of untreated and enzymatically-treated PLA microparticles of 2.00–3.00 mm with different lipase enzyme activities at pH 8.0 for 28 days. D) XRD curves of untreated and enzymatically-treated PLA microparticles of 2.00–3.00 mm with lipase enzyme activity of 2,425 U/mL and 11,250 U/mL at pH 8.0 after 28 days.

et al., 1998) (Figs. 2C and 3C). The above changes demonstrate the modification of the PLA microparticles' chemical structure after the enzymatic treatment. However, the effect of enzymatic hydrolysis was less pronounced compared to the PLA films.

The crystallinity of the PLA microparticles was measured to gain a deeper insight into the effect of the enzymatic hydrolysis on the microplastics. Crystallinity was investigated both for untreated PLA microparticles and for microparticles treated with enzyme activities of 2,425, and 11,250 U/mL at pH 8.0. As shown in Figs. 2D and 3D, microparticles of both size ranges show the characteristic sharp peak at $2\theta \sim 16.7^\circ$ (200/110 plane), the peak at $2\theta \sim 15.0^\circ$ (010 plane), the peak at $2\theta \sim 19.0^\circ$ (203/113 plane) and some other weak diffraction peaks, assigned to the reflection of the semi-crystalline PLA α -phase according to the ICDD 00-064-1624 database. As can be seen in Figs. 2D and 3D, all the diffraction peaks were still present after 28 days of lipase treatment. Additionally, the degree of crystallinity of PLA microparticles before enzymatic degradation was determined to be $38.87 \pm 0.10\%$ and

$28.99 \pm 0.21\%$ for sizes of 2.00–3.00 mm and 0.30–0.75 mm, respectively. In general, the crystallinity of semi-crystalline PLA increases during the degradation since water/enzyme penetrate the amorphous parts of polymer matrix and hydrolyze the ester bonds (De Jong et al., 2001). After this stage, the degradation rate decreases due to the higher crystallinity of the polymer. On the other hand, the increased number of carboxylic acid chain ends in the core of the polymer matrix results in autocatalytic hydrolytic reactions which accelerate the polymer degradation (Lin and Anseth, 2013). Herein, in the case of PLA microparticles of 2.00–3.00 mm, the crystallinity degree remained almost constant (36–38%) after enzymatic treatment, while increased up to $\sim 36\%$ in the case of 0.30–0.75 mm microparticles in both enzymatic activities used. It seems that lipase enzyme degrades more efficiently the microparticles with lower crystallinity degree and particle size, a result that totally agrees with the related literature. Specifically, Hino et al (Hino et al., 2023) who studied the effect of particle size on enzymatic hydrolysis and seawater biodegradation of PLA found that each

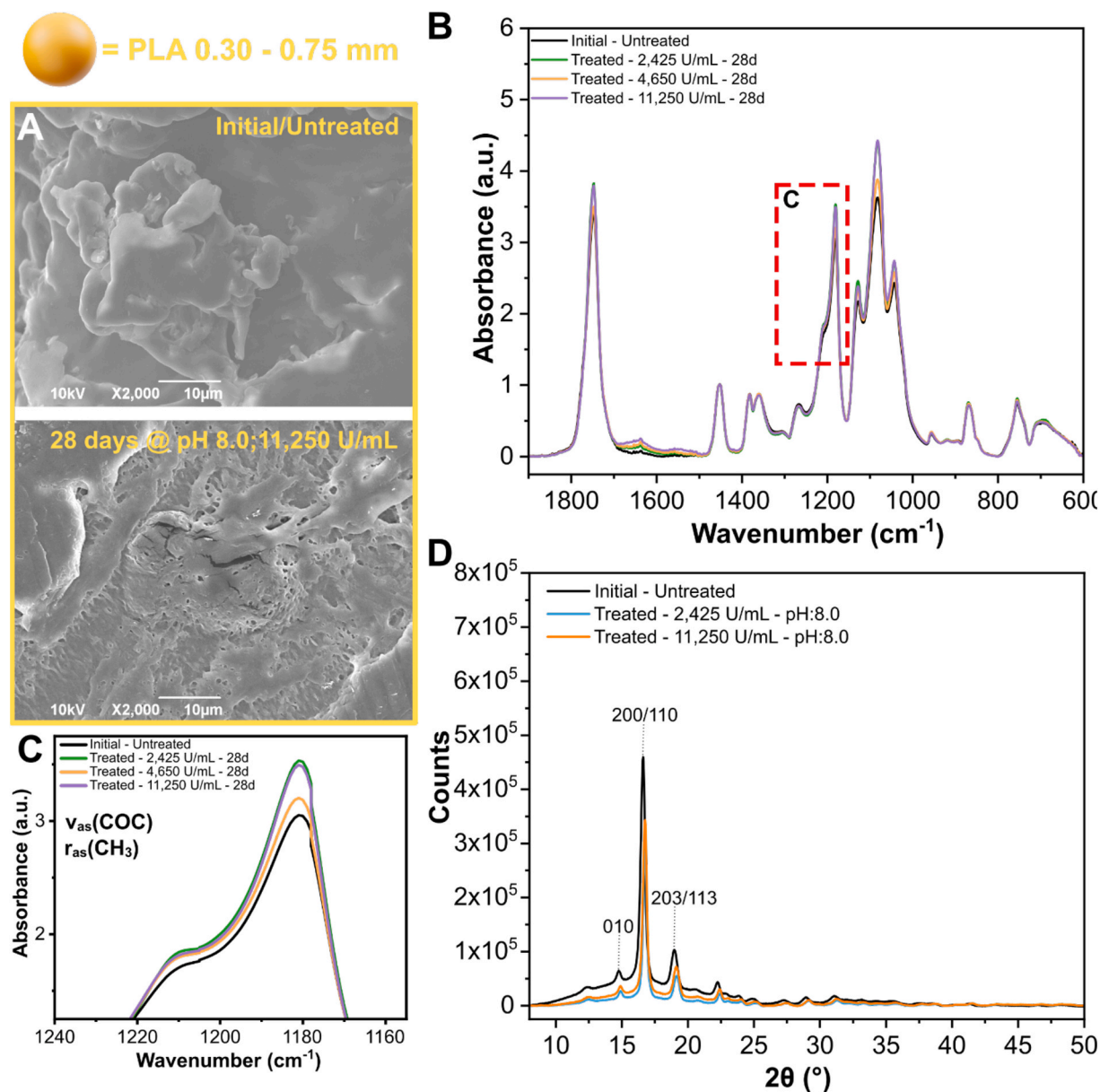


Fig. 3. Morphological, chemical and crystallographic modifications of PLA microparticles of 0.30–0.75 mm. A) SEM images of the surface of untreated and enzymatically-treated PLA microparticles of 0.30–0.75 mm with lipase enzyme activity of 11,250 U/mL at pH 8.0 after 28 days. B, C) ATR-FTIR spectra of untreated and enzymatically-treated PLA microparticles of 0.30–0.75 mm with different lipase enzyme activities at pH 8.0 for 28 days. D) XRD curves of untreated and enzymatically-treated PLA microparticles of 0.30–0.75 mm with lipase enzyme activity of 2,425 U/mL and 11,250 U/mL at pH 8.0 after 28 days.

biodegradation process was accelerated when smaller particle size was used, since the smaller size favored the contact between the enzymes/microbes and tested materials. In addition, it has been demonstrated that a small reduction in the crystallinity degree improves the enzymatic accessibility to a polymer increasing the degradation efficiency (Giraldo-Narcizo et al., 2023). The analysis of liquid broth by using HPLC-RID for lactic acid identification was also performed for the treated PLA microparticles with the highest enzymatic activity i.e., 11,250 U/mL at pH 8.0 after 28 days of treatment. In those experiments, lactic acid was not detected, thus demonstrating that the initial crystallinity of the material plays an important role in bioprocess efficiency.

4. Conclusions

This work represents a comprehensive study for morphological, physicochemical and crystallographic changes during the enzymatic biodegradation of PLA materials revealing that the use of microbial

enzymes could provide an effective and eco-friendly solution for addressing the end-of-life challenges of bioplastics. We demonstrate that lipase enzyme from *Aspergillus oryzae* is able to depolymerize PLA materials without any pretreatment method at temperature of 37°C. Changes in surface morphology of the PLA films as well as increased hydrophilicity of PLA surface were observed after enzymatic hydrolysis. ATR-FTIR analyses also proved the hydrolysis of lactic ester bonds of PLA after 28 days whereas all the chemical modifications occurred led to an increased crystallinity of the PLA films after enzymatic treatment due to the degradation of the amorphous phase. Moreover, we saw that the conversion of PLA to lactic acid increases when higher lipase enzyme activity is applied for biotreatment with conversion efficiencies ranging from 3–9 %. The enzymatic degradation of two different size ranges of PLA microparticles was also confirmed. Our results offer new insights into chemical recycling technology regarding the bioconversion of polymeric wastes into their building blocks and provide sustainable solutions for bioplastics incorporation into composting process.

CRediT authorship contribution statement

Christina N. Economou: Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Sine Mandrup Bertozzi:** Methodology, Investigation, Data curation. **Martina Nardi:** Methodology, Investigation, Data curation. **Uttam C. Paul:** Investigation. **Fabrizio Fiorentini:** Visualization, Investigation. **Giorgia Ferrari:** Investigation. **Marco Contardi:** Visualization, Investigation. **Andrea Armirotti:** Methodology, Data curation. **Despina Fragouli:** Writing – review & editing, Visualization, Supervision, Methodology, Conceptualization. **Athanassia Athanassiou:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Acknowledgements

We thank the Materials Characterization Facility at the Fondazione Istituto Italiano di Tecnologia for help with X-ray Diffraction (XRD) and Profilometer analysis. This work is part of the "Technologies for Sustainability" Flagship program of the Italian Institute of Technology (IIT). Also, part of this research has been funded under Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU. Project code CN.00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP J33C22001200001, Project title "National Biodiversity Future Center – NBFC".

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biortech.2025.132807>.

Data availability

Data will be made available on request.

References

- Abu Hajleh, M.N., Al-Samydai, A., Al-Dujaili, E.A.S., 2020. Nano, micro particulate and cosmetic delivery systems of poly(lactic acid): a mini review. *J. Cosmet. Dermatol.* <https://doi.org/10.1111/jocd.13696>.
- Araújo, A., Oliveira, M., Oliveira, R., Botelho, G., Machado, A.V., 2014. Biodegradation assessment of PLA and its nanocomposites. *Environ. Sci. Pollut. Res.* 21, 9477–9486. <https://doi.org/10.1007/s11356-013-2256-y>.
- Athanassiou, A., Varda, M., Mele, E., Lygeraki, M.I., Pisignano, D., Farsari, M., Fotakis, C., Cingolani, R., Anastasiadis, S.H., 2006. Combination of microstructuring and laser-light irradiation for the reversible wettability of photosensitized polymer surfaces. *Appl. Phys. A Mater. Sci. Process.* 83, 351–356. <https://doi.org/10.1007/s00339-006-3561-9>.
- Babichuk, I.S., Lin, C., Qiu, Y., Zhu, H., Ye, T.T., Gao, Z., Yang, J., 2022. Raman mapping of piezoelectric poly(l-lactic acid) films for force sensors. *RSC Adv.* 12, 27687–27697. <https://doi.org/10.1039/d2ra04241j>.
- Bell, E.L., Smithson, R., Kilbride, S., Foster, J., Hardy, F.J., Ramachandran, S., Tedstone, A.A., Haigh, S.J., Garforth, A.A., Day, P.J.R., Levy, C., Shaver, M.P., Green, A.P., 2022. Directed evolution of an efficient and thermostable PET depolymerase. *Nat. Catal.* 5, 673–681. <https://doi.org/10.1038/s41929-022-00821-3>.
- Beltrán, F.R., de la Orden, M.U., Lorenzo, V., Pérez, E., Cerrada, M.L., Martínez Urreaga, J., 2016. Water-induced structural changes in poly(lactic acid) and PLLA-clay nanocomposites. *Polymer (guildf)* 107, 211–222. <https://doi.org/10.1016/j.polymer.2016.11.031>.
- Bradford, M.M., 1976. A novel method for protein estimation assay using brilliant blue G. *Anal. Biochem.* 72, 248–254.
- Carniel, A., Valoni, É., Nicomedes, J., da Gomes, A., C., Castro, A.M. de., 2017. Lipase from *Candida antarctica* (CALB) and cutinase from *Humicola insolens* act synergistically for PET hydrolysis to terephthalic acid. *Process Biochem.* 59, 84–90. <https://doi.org/10.1016/j.procbio.2016.07.023>.
- Carrasco, F., Pagès, P., Gámez-Pérez, J., Santana, O.O., Maspoch, M.L., 2010. Processing of poly(lactic acid): Characterization of chemical structure, thermal stability and mechanical properties. *Polym. Degrad. Stab.* 95, 116–125. <https://doi.org/10.1016/j.polydegradstab.2009.11.045>.
- Casalini, T., Rossi, F., Castrovinci, A., Perale, G., 2019. A Perspective on Poly(lactic Acid)-based Polymers Use for Nanoparticles Synthesis and applications. *Front. Bioeng. Biotechnol.* <https://doi.org/10.3389/fbioe.2019.00259>.
- Chandra, P., Enespa, S., R., Arora, P.K., 2020. Microbial lipases and their industrial applications: a comprehensive review. *Microb. Cell Fact.* <https://doi.org/10.1186/s12934-020-01428-8>.
- Cui, L., Wang, X., Szarka, G., Hegyesi, N., Wang, Y., Sui, X., Pukánszky, B., 2022. Quantitative analysis of factors determining the enzymatic degradation of poly(lactic acid). *Int. J. Biol. Macromol.* 209, 1703–1709. <https://doi.org/10.1016/j.ijbiomac.2022.04.121>.
- Daba, G.M., Mostafa, F.A., Elkhateeb, W.A., 2021. The ancient koji mold (*Aspergillus oryzae*) as a modern biotechnological tool. *Bioresour. Bioprocess.* <https://doi.org/10.1186/s40643-021-00408-z>.
- de Castro, A.M., Carniel, A., Nicomedes Junior, J., da Conceição Gomes, A., Valoni, É., 2017. Screening of commercial enzymes for poly(ethylene terephthalate) (PET) hydrolysis and synergy studies on different substrate sources. *J. Ind. Microbiol. Biotechnol.* 44, 835–844. <https://doi.org/10.1007/s10295-017-1942-z>.
- De Jong, S.J., Arias, E.R., Rijkers, D.T.S., Van Nostrum, C.F., Kettenes-Van Den Bosch, J.J., Hennink, W.E., 2001. New insights into the hydrolytic degradation of poly(lactic acid): participation of the alcohol terminus. *Polymer (guildf)* 42, 2795–2802.
- Ellis, L.D., Rorrer, N.A., Sullivan, K.P., Otto, M., McGeehan, J.E., Román-Leshkov, Y., Wierckx, N., Beckham, G.T., 2021. Chemical and biological catalysis for plastics recycling and upcycling. *Nat. Catal.* <https://doi.org/10.1038/s41929-021-00648-4>.
- European Bioplastics, Global production capacities of bioplastics 2028 [WWW Document] https://www.european-bioplastics.org/news/multimedia-pictures-ideos/#lightbox/gallery_image_11/2 2023 accessed 10.23.24.
- Gao, A., Shen, H., Zhang, H., Feng, G., Xie, K., 2017. Hydrophilic modification of polyester fabric by synergetic effect of biological enzymolysis and non-ionic surfactant, and applications in cleaner production. *J. Clean. Prod.* 164, 277–287. <https://doi.org/10.1016/j.jclepro.2017.06.214>.
- Gilani, I.E., Sayadi, S., Zouari, N., Al-Ghouti, M.A., 2023. Plastic waste impact and biotechnology: Exploring polymer degradation, microbial role, and sustainable development implications. *Bioresour. Technol. Rep.* <https://doi.org/10.1016/j.biteb.2023.101606>.
- Giraldo-Narcizo, S., Guenani, N., Sánchez-Pérez, A.M., Guerrero, A., 2023. Accelerated polyethylene terephthalate (PET) enzymatic degradation by room temperature alkali pre-treatment for reduced polymer crystallinity. *Chembiochem* 24. <https://doi.org/10.1002/cbic.202200503>.
- Hajjghasemi, M., Nocek, B.P., Tchigvintsev, A., Brown, G., Flick, R., Xu, X., Cui, H., Hai, T., Joachimiak, A., Golyshin, P.N., Savchenko, A., Edwards, E.A., Yakunin, A.F., 2016. Biochemical and Structural Insights into Enzymatic Depolymerization of Poly(lactic acid) and Other Polyesters by Microbial Carboxylesterases. *Biomacromolecules* 17, 2027–2039. <https://doi.org/10.1021/acs.biomac.6b00223>.
- Hino, S., Kawasaki, N., Yamano, N., Nakamura, T., Nakayama, A., 2023. Effects of particle size on marine biodegradation of poly(L-lactic acid) and poly(ϵ -caprolactone). *Mater. Chem. Phys.* 303. <https://doi.org/10.1016/j.matchemphys.2023.127813>.
- Jung, E., Yim, D., Kim, H., Peterson, G.I., Choi, T.L., 2023. Depolymerization of poly(α -methyl styrene) with ball-mill grinding. *J. Polym. Sci.* 61, 553–560. <https://doi.org/10.1002/pol.20220578>.
- Jung, J.H., Ree, M., Kim, H., 2006. Acid- and base-catalyzed hydrolyses of aliphatic polycarbonates and polyesters. *Catal. Today* 115, 283–287. <https://doi.org/10.1016/j.cattod.2006.02.060>.
- Karamanlioglu, M., Houlden, A., Robson, G.D., 2014. Isolation and characterisation of fungal communities associated with degradation and growth on the surface of poly(lactic acid) (PLA) in soil and compost. *Int. Biodeter. Biodegr.* 95, 301–310. <https://doi.org/10.1016/j.ibiod.2014.09.006>.
- Karimi-Avargani, M., Bazooyar, F., Biriá, D., Zamani, A., Skrifvars, M., 2020. The special effect of the *Aspergillus flavus* and its enzymes on biological degradation of the intact poly(lactic acid) (PLA) and PLA-Jute composite. *Polym. Degrad. Stab.* 179. <https://doi.org/10.1016/j.polydegradstab.2020.109295>.
- Khan, S., Nadir, S., Shah, Z.U., Shah, A.A., Karunarathna, S.C., Xu, J., Khan, A., Munir, S., Hasan, F., 2017. Biodegradation of polyester polyurethane by *Aspergillus tubingensis*. *Environ. Pollut.* 225, 469–480. <https://doi.org/10.1016/j.envpol.2017.03.012>.
- Kister, G., Cassanas, G., Vert, M., 1998. Effects of morphology, conformation and configuration on the IR and Raman spectra of various poly(lactic acid)s.
- Krikorian, V., Pochan, D.J., 2005. Crystallization behavior of poly(L-lactic acid) nanocomposites: Nucleation and growth probed by infrared spectroscopy. *Macromolecules* 38, 6520–6527. <https://doi.org/10.1021/ma050739z>.
- Kulikowska, D., Bernat, K., Wojnowska-Baryła, I., Pasieczna-Patkowska, S., Jabłoński, R., 2020. Composting as a disposal route of pla materials: Kinetics of the aerobic biodegradation. *Desalination Water Treat* 206, 153–164. <https://doi.org/10.5004/dwt.2020.26355>.
- Kumar, A., Mukhia, S., Kumar, N., Acharya, V., Kumar, S., Kumar, R., 2020. A Broad Temperature active Lipase Purified from a Psychrotrophic Bacterium of Sikkim Himalaya with potential Application in Detergent Formulation. *Front. Bioeng. Biotechnol.* 8. <https://doi.org/10.3389/fbioe.2020.00642>.

- Lee, S.H., Kim, I.Y., Song, W.S., 2014. Biodegradation of polylactic acid (PLA) fibers using different enzymes. *Macromol. Res.* 22, 657–663. <https://doi.org/10.1007/s13233-014-2107-9>.
- Li, S., McCarthy, S., 1999. Further investigations on the hydrolytic degradation of poly (DL-lactide). *Biomaterials*.
- Lin, C.C., Anseth, K.S., 2013. The Biodegradation of Biodegradable Polymeric Biomaterials, in: *Biomaterials Science: An Introduction to Materials*: Third Edition. Elsevier Inc., pp. 716–728. doi: 10.1016/B978-0-08-087780-8.00061-9.
- Lipsa, R., Tudorachi, N., Darie-Nita, R.N., Oprică, L., Vasile, C., Chiriac, A., 2016. Biodegradation of poly(lactic acid) and some of its based systems with *Trichoderma viride*. *Int. J. Biol. Macromol.* 88, 515–526. <https://doi.org/10.1016/j.ijbiomac.2016.04.017>.
- Luo, W., Yang, Y., Weng, Y., Huang, Z., Zhang, C., 2024. Epoxidized cardanol oleate (ECD-OA) as an effective biobased chain extender of polylactic acid (PLA). *Int. J. Biol. Macromol.* 282, 136903. <https://doi.org/10.1016/j.ijbiomac.2024.136903>.
- Meaurio, E., Martinez De Arenaza, I., Lizundia, E., Sarasua, J.R., 2009. Analysis of the C=O stretching band of the α -crystal of poly(L-lactide). *Macromolecules* 42, 5717–5727. <https://doi.org/10.1021/ma9008109>.
- Mehta, A., Bodh, U., Gupta, R., 2017. Fungal lipases: a review. *J. Biotech Res* 8, 58–77.
- Meza Huaman, S.M., Nicholson, J.H., Brogan, A.P.S., 2024. A general route to retooling hydrolytic enzymes toward plastic degradation. *Cell Rep. Phys. Sci.* 5. <https://doi.org/10.1016/j.xcrp.2024.101783>.
- Mo, A., Zhang, Y., Gao, W., Jiang, J., He, D., 2023. Environmental fate and impacts of biodegradable plastics in agricultural soil ecosystems. *Appl. Soil Ecol.* 181. <https://doi.org/10.1016/j.apsoil.2022.104667>.
- Monsoor, M.A., Kalapathy, U., Proctor, A., 2001. Improved method for determination of pectin degree of esterification by diffuse reflectance Fourier transform infrared spectroscopy. *J. Agric. Food Chem.* 49, 2756–2760. <https://doi.org/10.1021/jf0009448>.
- Nakajima-Kambe, T., Edwinoliver, N.G., Maeda, H., Thirunavukarasu, K., Gowthaman, M.K., Masaki, K., Mahalingam, S., Kamini, N.R., 2012. Purification, cloning and expression of an *Aspergillus niger* lipase for degradation of poly(lactic acid) and poly(ϵ -caprolactone). *Polym. Degrad. Stab.* 97, 139–144. <https://doi.org/10.1016/j.polymdegradstab.2011.11.009>.
- Noor, H., Satti, S.M., Din, S., ud, Farman, M., Hasan, F., Khan, S., Badshah, M., Shah, A. A., 2020. Insight on esterase from *Pseudomonas aeruginosa* strain S3 that depolymerize poly(lactic acid) (PLA) at ambient temperature. *Polym. Degrad. Stab.* 174. <https://doi.org/10.1016/j.polymdegradstab.2020.109096>.
- Oliveira, M., Santos, E., Araújo, A., Fechine, G.J.M., Machado, A.V., Botelho, G., 2016. The role of shear and stabilizer on PLA degradation. *Polym. Test.* 51, 109–116. <https://doi.org/10.1016/j.polymertesting.2016.03.005>.
- Painter, P.C., Starsinic, M., Squires, E., Davis, A.A., 1983. Short Communications concerning the 1600 cm⁻¹ region in the i.r. spectrum of coal. *Fuel* 62, 742–744.
- Panyachanakul, T., Kitpreechavanich, V., Lorliam, W., Krajangsang, S., 2024. Improvement of thermo-stability and solvent tolerant property of *Streptomyces* sp. A3301 lipase by immobilization techniques with application in poly (lactic acid) polymerization by using biological process. *Trends. Sciences* 21, 8377. <https://doi.org/10.48048/tis.2024.8377>.
- Park, B., Peterson, G.I., 2023. Comparing molecular weight models for polymer degradation with ball-mill grinding. *Polym. Degrad. Stab.* 218. <https://doi.org/10.1016/j.polymdegradstab.2023.110549>.
- Penkhruw, W., Khanongnuch, C., Masaki, K., Pathom-aree, W., Punyodom, W., Lumyong, S., 2015. Isolation and screening of biopolymer-degrading microorganisms from northern Thailand. *World J. Microbiol. Biotechnol.* 31, 1431–1442. <https://doi.org/10.1007/s11274-015-1895-1>.
- Qi, X., Ren, Y., Wang, X., 2017. New advances in the biodegradation of Poly(lactic acid). *Int. Biodeter. Biodegr.* <https://doi.org/10.1016/j.ibiod.2017.01.010>.
- Rahmayetty, W., Y., Sukirno, Rahman, S.F., Suyono, E.A., Yohda, M., Gozan, M., 2018. Use of *Candida rugosa* lipase as a biocatalyst for L-lactide ring-opening polymerization and polylactic acid production. *Biocatal. Agric. Biotechnol.* 16, 683–691. <https://doi.org/10.1016/j.cbac.2018.09.015>.
- Rezvani Ghomi, E., Khosravi, F., Saedi Ardahaiei, A., Dai, Y., Neisiany, R.E., Foroughi, F., Wu, M., Das, O., Ramakrishna, S., 2021. The life cycle assessment for polylactic acid (PLA) to make it a low-carbon material. *Polymers (basel)*. <https://doi.org/10.3390/polym13111854>.
- Rodriguez, E.J., Marcos, B., Huneault, M.A., 2016. Hydrolysis of polylactide in aqueous media. *J. Appl. Polym. Sci.* 133. <https://doi.org/10.1002/app.44152>.
- Safdar, A., Ismail, F., Imran, M., 2024. Biodegradation of synthetic plastics by the extracellular lipase of *Aspergillus niger*. *Environ. Adv.* 17, 100563. <https://doi.org/10.1016/j.envadv.2024.100563>.
- Satti, S.M., Shah, A.A., Marsh, T.L., Auras, R., 2018. Biodegradation of Poly(lactic acid) in Soil Microcosms at Ambient Temperature: Evaluation of Natural Attenuation, Bio-augmentation and Bio-stimulation. *J. Polym. Environ.* 26, 3848–3857. <https://doi.org/10.1007/s10924-018-1264-x>.
- Silva Almeida, C., Simão Neto, F., da Silva Sousa, P., da Silva Aires, F.I., de Matos Filho, J.R., Gama Cavalcante, A.L., de Sousa Junior, P.G., Melo, R.L.F., Dos Santos, J. C.S., 2024. Enhancing lipase immobilization via physical adsorption: Advancements in stability, reusability, and industrial applications for sustainable biotechnological processes. *ACS Omega* 9, 46698–46732. <https://doi.org/10.1021/acsomega.4c07088>.
- Spier, M.R., Peron-Schlösser, B., Paludo, L.C., Gallo-García, L.A., Zanette, C.M., 2020. Microalgae as enzymes biofactories, in: *Handbook of Microalgae-Based Processes and Products: Fundamentals and Advances in Energy, Food, Feed, Fertilizer, and Bioactive Compounds*. Elsevier, pp. 687–706. doi: 10.1016/B978-0-12-818536-0.00025-7.
- Srikanth, M., Sandeep, T.S.R.S., Sucharitha, K., Godi, S., 2022. Biodegradation of plastic polymers by fungi: a brief review. *Bioresour. Bioprocess.* <https://doi.org/10.1186/s40643-022-00532-4>.
- Teixeira, S., Eblagon, K.M., Miranda, F., Pereira, R., M.F., Figueiredo, J.L., 2021. Towards Controlled Degradation of Poly(lactic acid) in Technical applications. *C (basel)* 7, 42. <https://doi.org/10.3390/c7020042>.
- Tsuji, H., Ikada, Y., 2000. Properties and morphology of poly(l-lactide) 4. Effects of structural parameters on long-term hydrolysis of poly(l-lactide) in phosphate-buffered solution. *Polym. Degrad. Stab.* 67, 179–189.
- Urayama, H., Moon, S.I., Kimura, Y., 2003. Microstructure and thermal properties of polylactides with different L- and D-unit sequences: Importance of the helical nature of the L-sequenced segments. *Macromol. Mater. Eng.* 288, 137–143. <https://doi.org/10.1002/mame.200390006>.
- Velghe, I., Buffel, B., Vandeginste, V., Thielemans, W., Desplentere, F., 2023. Review on the Degradation of Poly(lactic acid) during Melt Processing. *Polymers (basel)*. <https://doi.org/10.3390/polym15092047>.
- Von Burkersroda, F., Schedl, L., Opferich, A.G., 2002. Why degradable polymers undergo surface erosion or bulk erosion. *Biomaterials*.
- Wang, D.K., Varanasi, S., Fredericks, P.M., Hill, D.J.T., Symons, A.L., Whittaker, A.K., Rasoul, F., 2013. FT-IR characterization and hydrolysis of PLA-PEG-PLA based copolyester hydrogels with short PLA segments and a cytocompatibility study. *J. Polym. Sci. A Polym. Chem.* 51, 5163–5176. <https://doi.org/10.1002/pola.26930>.
- Wang, Z., Wang, Y., Guo, Z., Li, F., Chen, S., 2011. Purification and characterization of poly(L-lactic acid) depolymerase from *Pseudomonas* sp. strain DS04-T. *Polym. Eng. Sci.* 51, 454–459. <https://doi.org/10.1002/pen.21857>.
- Wenzel, R.N., 1936. Resistance of solid surfaces to wetting by water. *Ind. Eng. Chem.* 28, 988–994.
- Xu, B., Chen, Y., He, J., Cao, S., Liu, J., Xue, R., Qian, X., Zhou, J., Dong, W., Jiang, M., 2022. New insights into the biodegradation of polylactic acid: from 2 degradation to upcycling. *Environ. Rev.* 30, 30–38.
- Yu, S., Chen, J., Gomard, G., Hölscher, H., Lemmer, U., 2023. Recent Progress in Light-Scattering Porous Polymers and their applications. *Adv. Opt. Mater.* <https://doi.org/10.1002/adom.202203134>.
- Zaaba, N.F., Jaafar, M., 2020. A review on degradation mechanisms of polylactic acid: Hydrolytic, photodegradative, microbial, and enzymatic degradation. *Polym. Eng. Sci.* <https://doi.org/10.1002/pen.25511>.
- Zhang, J., Duan, Y., Sato, H., Noda, I., Yan, S., Ozaki, Y., 2005. Crystal modifications and thermal behavior of poly(L-lactic acid) revealed by infrared spectroscopy. *Macromolecules* 38, 8012–8021. <https://doi.org/10.1021/ma051232r>.
- Zhu, Z., Bian, Y., Zhang, X., Zeng, R., Yang, B., 2022. Study of Crystallinity and Conformation of Poly(lactic acid) by Terahertz Spectroscopy. *Anal. Chem.* 94, 11104–11111. <https://doi.org/10.1021/acs.analchem.2c02652>.