Effect of enzymatic-bacterial bioproduct on PLA biodegradation under industrial composting conditions*)

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Abstract: The effect of the new bioproduct in the form of granulate and liquid concentrate on PLA biodegradation during industrial composting was investigated. The analysis included compost parameters, PLA mass loss, structural changes (SEM) and chemical properties (FTIR-ATR, O/C ratio). The effectiveness of the bioproduct, especially in liquid form, in accelerating PLA degradation and improving compost properties was confirmed what matters for sustainable waste management.

Keywords: biodegradation, PLA, industrial composting, bioproduct.

Wpływ bioproduktu enzymatyczno-bakteryjnego na biodegradację PLA w warunkach kompostowania przemysłowego

Streszczenie: Zbadano wpływ nowego bioproduktu w postaci granulatu i płynnego koncentratu na biodegradację PLA podczas przemysłowego kompostowania. Analiza obejmowała parametry kompostu, utratę masy PLA, zmiany strukturalne (SEM) i właściwości chemiczne (FTIR-ATR, stosunek O/C). Potwierdzono skuteczność bioproduktu, szczególnie w postaci płynnej, w przyspieszaniu degradacji PLA i poprawianiu właściwości kompostu, co ma znaczenie dla zrównoważonego zarządzania odpadami.

Słowa kluczowe: biodegradacja, PLA, przemysłowe kompostowanie, bioprodukt.

Polymeric materials, one of the cornerstones of modern industry, simultaneously represent one of the greatest environmental challenges of the 21st century. Currently, the annual global production of polymer granulates for processing amounts to approximately 348 million tons [1]. Each year, around 5 trillion plastic bags are consumed, with approximately 40% of total plastic production used for packaging [2]. Such products rapidly become waste. Over just seven years (2015–2022), approximately 6 300 Mt of plastic waste was generated, of which about 9% was recycled, 12% was incinerated, and 79% accumulated in landfills or the natural environment [2]. Despite growing environmental awareness and technological advancements, polymer waste management still requires new, more sustainable solutions [3–5]. Materials classified as biodegradable, such as polylactide (PLA), the most widely used commercial material of this type — require further research and innovative methods for utilization [6, 7]. PLA, while considered biodegradable, effectively degrades only under specific industrial composting conditions [8], where high temperatures (above 50°C) and the activity of thermophilic microorganisms facilitate degradation processes. Under mesophilic conditions, PLA degradation is limited, resulting in prolonged processes and waste accumulation [9–12].

Although industrial composting has significant potential, it faces numerous challenges. The presence of contaminants in biowaste, such as glass, metals, or fragments of polymeric materials, significantly disrupts the efficiency of this process. Particularly problematic are thin film waste, which can cause the mechanical equipment used for turning and processing compost piles to halt or even become damaged. Consequently, such contaminants must often be removed manually, increasing costs and processing time while exposing workers to biological hazards associated with contact with organic waste [13–15].

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The scientific literature focuses on laboratory studies of PLA degradation under controlled conditions [16–18]. Field studies conducted under industrial composting conditions are far less common, and even less attention is paid to unprocessed biowaste [19, 20]. In practice, industrial composting comprises several key stages: i) segregation and preliminary sorting of biowaste; ii) shredding and homogenization of materials; iii) formation of compost piles; iv) intensive composting phase (mesophilic and thermophilic); and v) compost maturation phase [21–25].

During the first stage, contaminants such as glass, metals, and polymeric materials are removed. Shredding and homogenization facilitate uniform aeration and initiate biological processes. After forming compost piles, they undergo aeration and periodic temperature measurements. In the intensive composting phase (mesophilic and thermophilic), active microorganisms play a crucial role in organic matter degradation. It has been shown that enzymatic hydrolysis may contribute more significantly than chemical processes during this stage [26]. The final phase, compost maturation, results in a stable fertilizer product [27–30].

Most field studies focus on post-preprocessing stages of biowaste, where materials are already shredded and formed into piles [31-33]. In contrast, this study was conducted directly on unsorted biowaste, enabling the assessment of the real impact of the introduced bioproduct during the initial stages of composting. This methodology captures the practical challenges of industrial composting and addresses a research gap concerning bioproduct application in unprocessed biowaste.

This publication is a continuation of earlier research [34], where a starch-based granulate bioproduct enriched with selected bacterial strains and hydrolytic enzymes was developed. The previous studies focused on optimizing the composition and dosage of the bioproduct. In this work, the research scope was expanded to industrial composting conditions. Additionally, for the first time, two application forms of the bioproduct — granulate and its water-based concentrate — were compared to evaluate their practicality and effectiveness at different process stages. The results provide valuable practical insights into industrial implementation.

The bioproduct used in the study contains bacterial strains of *Pseudomonas alloputida* and *Pseudomonas taiwanensis*, isolated from anthropogenically degraded environments, such as landfills [35]. The hydrolytic enzymes (papain, proteinase K, nattokinase) support polymer breakdown, while the granulate composition (starch, glycerin, linseed cakes, coffee grounds, urea) promotes the proliferation of autochthonous microflora [36, 37]. The study evaluated structural changes in PLA (mass loss, carbonyl index, O/C ratio) and the impact of the bioproduct on key compost parameters such as pH, redox potential, and microbial abundance [38–40].

The main objective of the study was to assess the effectiveness of the bioproduct in accelerating the biodegradation of polylactide under industrial composting conditions. Emphasis was placed on comparing two forms of bioproduct application – granulate and water concentrate – and their effect on the intensity of PLA biodegradation processes, as well as on the properties of compost. This publication is an extension of earlier works and focuses on the results obtained under industrial conditions. The results provide valuable practical data that can optimize industrial biowaste composting processes. They also serve as a foundation for further research into the biodegradation of other polymeric materials under industrial conditions, contributing to the development of a more sustainable waste management system.

EXPERIMENTAL PART

Materials

The study applied a bioproduct composed of starch-based granulate, selected bacterial strains, and hydrolytic enzymes. The composition of the granulate, developed based on previous research [34], is presented in Table 1.

The granulate was produced using a laboratory-scale production line equipped with a co-rotating twin-screw extruder BTSK 20/40D (Bühler, Germany). The resulting granulate was mixed with a bacterial-enzymatic lyophilizate, the composition of which is presented in Table 2.

PLA film was prepared from Ingeo™ 2003D granulate (NatureWorks LLC, USA). The extrusion process was carried out using a Plasti-Corder PLV 151 single-screw extruder (Brabender, Germany) with a screw diameter of 19 mm and a plasticizing system length of 25 D, a compression ratio of 3:1, a slot die with a working width of 100 mm and a gap of 0.1 mm. The extrusion temperature was 190°C, and the screw rotation speed was 29 min^{-1}. PLA was dried for 8 h at 80°C before forming a 0.09 mm thick film, from which samples of dimensions 100×15 mm (± 1 mm) was cut.

Samples preparation

The study was conducted at an industrial composting facility in Niedźwiedź, Poland. Compost piles with a capacity of 500 L were prepared using biowaste from households. The study included two variants of bioproduct application: i) granulate – added directly at a dose of 0.5 g/L of compost; ii) water-based concentrate – prepared by dissolving the granulate in water (0.5 g/L) and leaving it at room temperature for 24 hours. The concentrate was then diluted at a 1:10 ratio and applied to the pile using a manual garden sprayer (Kwazar Orion, Poland) with a capacity of 12 L and a long lance to ensure even distribution of the solution. Approximately 5 L of solution was used per 1 m^2 of pile surface during each application.

PLA film samples (45 g per cage) were placed in metal cages with dimensions of 40×16×16 cm, designed to allow easy retrieval without disrupting the compost structure. The samples were arranged in a single layer of strips measuring 100×15 mm (± 1 mm) with a thickness

T a b l e 1. Composition of the starch-based granulate bioproduct

Component	Producer	Amount wt%
Potato starch	Cargill, Poland	40
Chalk ≤ 45 μm	NB Minerals, Poland	15
Linseed cakes	HorseLen, Poland	15
Glycerin	Chempur, Poland	10
Urea	Azoty Tarnów, Poland	9
Palm oil	Master Martini, Poland	8
Coffee grounds (Robusta)	Gimoka, Italy	3

T a b l e 2. Composition of the bacterial-enzymatic lyophilizate

	Component	Amount g/100 g of granulate
Bacteria	*Bacillus subtilis* (PCM 2021, Poland)	0.01
	Bacillus cereus (PCM 2018, Poland)	0.01
	Pseudomonas frederiksbergensis (KJ-D-3-1, environmental – own collection)	0.01
	Pseudomonas taiwanensis (KJ-LIN-3-1, environmental – own collection)	0.01
	Pseudomonas alloputida (KJ-OS-5-3, environmental – own collection)	0.01
Enzymes	Proteinase K (Sigma-Aldrich, USA)	0.03
	Bromelain (GymBeam, Germany)	0.02
	Nattokinase (GymBeam, Germany)	0.02
	Papain (GymBeam, Germany)	0.03

of 0.09 mm. This configuration ensured uniform exposure to composting conditions and facilitated subsequent analysis. The cages were evenly distributed within the compost piles to minimize variability caused by spatial differences in microbial activity and temperature.

Methods

The experiment lasted 12 weeks (June–August) and included periodic analyses at 4-week intervals. The results obtained from different bioproduct application variants enabled a comprehensive assessment of its effectiveness and its impact on key compost parameters.

Compost analysis

The following methods were used to assess the properties of the compost: pile temperature, pH and redox potential, total microbial count, and macronutrient content.

The pile temperature was measured using a thermometer probe (Hanna Instruments, Poland) inserted approximately 50 cm into the pile.

pH and redox potential measurements were performed using a laboratory pH meter (WTW, Germany) after diluting the compost samples tenfold with distilled water.

The total microbial count was determined using the serial dilution method and plating on PCA agar (Plate Count Agar, VWR). The mesophilic and thermophilic microorganisms were quantified in homogenized 10 g compost samples and recalculated per gram. To determine the mesophilic microbial count, the samples were incubated at 26°C for 24 hours. To determine the thermophilic microbial count, the samples were incubated at 58°C for the same duration.

The macronutrient content (N, P, K, Ca, Mg) was analyzed using aquarium test kits (Aqualab, Poland) after diluting the samples fivefold.

PLA film analysis

The analysis of PLA film included mass loss, carbonyl index, O/C ratio, and surface structure.

Mass loss was determined by measuring the difference in sample weight before and after composting using an analytical balance (Radwag, Poland) with an accuracy of 0.001 g.

The carbonyl index was calculated by Fourier transform attenuated total reflectance infrared spectroscopy (FT-IR-ATR) using a Cary 630 FT-IR spectrometer (Agilent Technologies, USA). The calculation was based on the ratio of the absorbance of carbonyl groups (1746 cm^{-1}) to CH_3 groups (1450 cm^{-1}) according to the previously established methodology [41].

The O/C ratio was analyzed using scanning electron microscopy (SEM) with energy dispersive X-ray spectroscopy (EDX). The analysis was performed using a Hitachi SU8010 microscope (Japan) with a Thermo Scientific Ultra Dry EDX detector (USA), following the methodology described by Janczak *et al*. [42].

The surface structure was visually assessed using SEM at 100× magnification.

RESULTS AND DISCUSSION

Compost analysis

A detailed analysis of the substrate and PLA film was conducted. The tests were performed on samples collected before the start of the experiment and subsequently after 4, 8, and 12 weeks of composting. The analyzed compost parameters included: temperature, pH, redox potential, total number of mesophilic (*TNM*) and thermophilic (*TNT*) microorganisms and macronutrient content. To facilitate the interpretation of the results, the following experimental variants were defined: i) Ctr – control, without PLA; ii) Ctr + PLA – control, with PLA; iii) G – bioproduct in granulate form, without PLA; iv) G + PLA – bioproduct in granulate form, with PLA; v) C – bioproduct in concentrate form, without PLA; vi) C + PLA – bioproduct in concentrate form, with PLA (Fig. 1).

The analysis of temperature changes in the compost piles allowed for assessing the dynamics of biological pro-

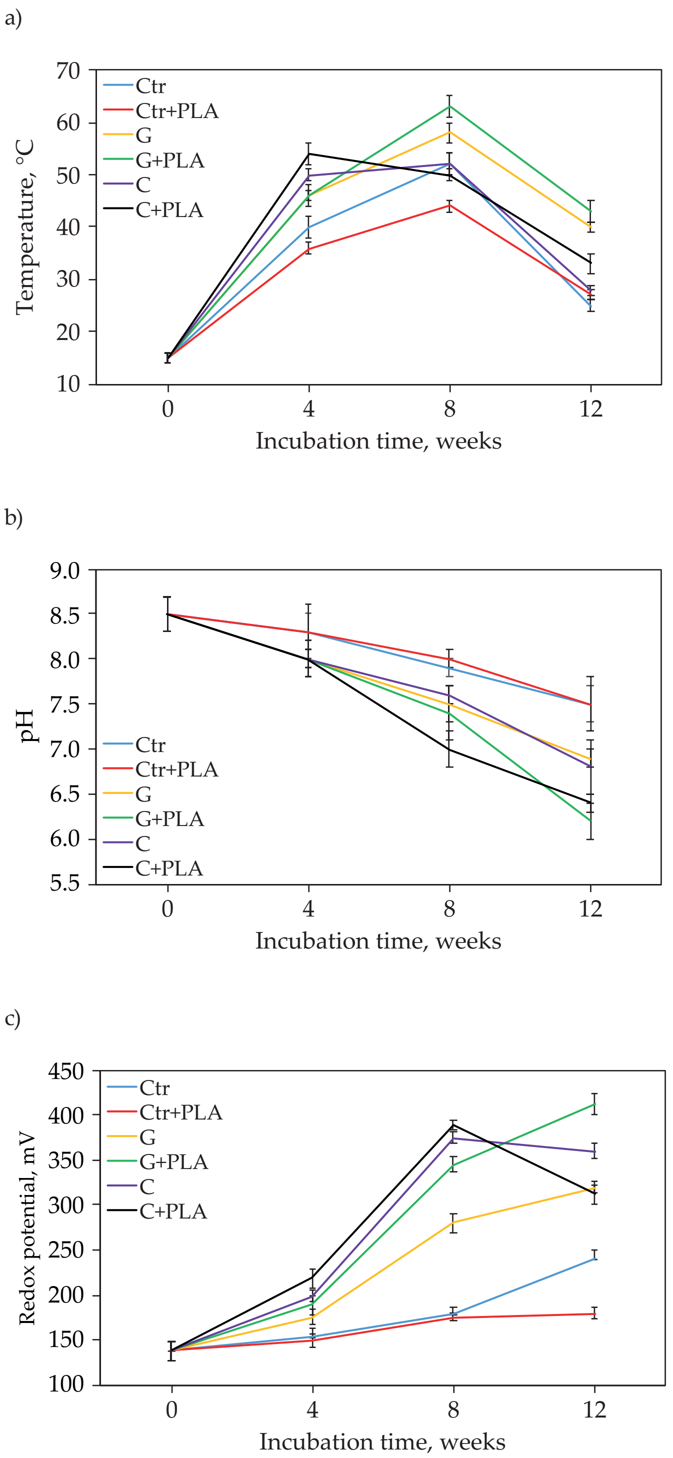

Fig. 1. Changes in compost pile parameters during the 12-week composting process: a) temperature changes in compost piles, b) compost pH, c) compost redox potential

cesses in different variants. At the start of the process, the temperature in all piles was 15°C, corresponding to the initial composting conditions and ambient temperature. In the following weeks, a temperature increase was observed, a characteristic indicator of microbial activity responsible for the intensification of organic matter degradation processes. After 4 weeks, the highest temperature increase was noted in the variants with the addition of the bioproduct in concentrate form. In the presence of PLA (C + PLA), the temperature reached 54°C. Piles with granulate (G and G + PLA) achieved similar values at around 46°C. The control variants exhibited lower values, reaching 40°C (Ctr) and 36°C (Ctr + PLA). The lower temperature in the control piles suggests less intense biological processes in these variants, highlighting the influence of the bioproduct on microbial activity (Fig. 1a).

After 8 weeks, the highest temperature, 63°C, was recorded in the pile with granulate and PLA (G + PLA). Variants with concentrate stabilized at 50–52°C, while the control piles achieved lower values of 52°C (Ctr) and 44°C (Ctr + PLA). These results indicate that bioproducts, especially in granulate form, significantly enhanced the efficiency of thermophilic processes, intensifying the decomposition of organic matter.

After 12 weeks, a temperature decrease was observed across all variants, characteristic of the final composting phase associated with compost maturation. The highest temperatures were maintained in the granulate variants, regardless of the presence of PLA, at 40°C (G) and 43°C (G + PLA). In contrast, control piles and those with concentrate containing PLA exhibited significantly lower temperatures of 27°C (Ctr + PLA) and 33°C (C + PLA). These results suggest more stable biological conditions in the bioproduct variants, particularly in granulate form, potentially due to more effective enzymatic and microbial activity in this application form.

The pH values for the experimental variants are presented in Fig. 1b. The initial pH value across all piles was 8.5, indicating the alkaline nature of biowaste at the start of the process. As composting progressed, a gradual pH decrease was observed, varying across experimental variants, driven by biological and chemical processes in the piles. In the control piles (Ctr and Ctr + PLA), pH decreased to 7.5 after 12 weeks. These results suggest that the presence of PLA film (Ctr + PLA) had no significant impact on the dynamics of pH changes compared to the control without PLA (Ctr).

Variants with granulate application (G and G + PLA) showed a more intense pH decrease compared to controls. After 12 weeks, pH values were 6.9 for the granulate without PLA (G) and 6.2 for the granulate with PLA (G + PLA). The significant difference between granulate variants may result from substrate acidification caused by PLA degradation, a polymer derived from lactic acid. This process may release organic acids, intensifying compost acidification.

The redox potential changes for the experimental variants are presented in Fig. 1c. The redox potential (mV) changes in compost piles across experimental variants are shown in Fig. 1c. Initial redox potential values were identical in all variants, measuring 138 mV, corresponding to moderately oxidative conditions typical of biowaste at the beginning of the composting process.

T a b l e 3. Changes in mesophilic (*TNM*) and thermophilic (*TNT*) microorganism counts in compost piles; values are presented as means calculated from three replicates

		Incubation time, weeks		
		4	8	12
TNM, cfu/g	Ctr	5.1×10^4	4.3×10^3	5.8×10^5
	Ctr + PLA	4.9×10^4	2.2×10^4	3.8×10^5
	G	7.2×10^6	3.9×10^4	7.8×10^6
	G + PLA	6.5×10^6	4.3×10^4	3.7×10^7
	C	8.4×10^7	5.3×10^4	4.1×10^5
	C + PLA	6.7×10^7	8.2×10^4	6.6×10^6
TNT, cfu/g	Ctr	2.3×10^2	4.2×10^3	5.4×10^1
	Ctr + PLA	1.1×10^2	8.7×10^3	6.2×10^1
	G	4.7×10^3	8.7×10^3	7.2×10^2
	G + PLA	6.5×10^4	5.8×10^4	9.8×10^3
	C	7.5×10^4	3.6×10^3	7.3×10^3
	C + PLA	8.4×10^4	9.9×10^4	5.2×10^3

TNM (total number of mesophiles) – total count of mesophilic microorganisms (cfu/g), *TNT* (total number of thermophiles) – total count of thermophilic microorganisms (cfu/g).

In the control variants (Ctr and Ctr + PLA), a moderate increase in redox potential was observed during incubation. After 12 weeks, the values were 240 mV for control without PLA (Ctr) and 180 mV for the control with PLA (Ctr + PLA). The presence of PLA in the control piles (Ctr + PLA) reduced the dynamics of redox potential increase, potentially due to PLA's limited impact on improving aerobic conditions and possible local anaerobic processes caused by PLA's slow degradation.

Granulate variants (G and G + PLA) exhibited a significant redox potential increase during incubation, indicating improved aerobic conditions. After 12 weeks, the redox potential was 320 mV for granulate without PLA (G) and 412 mV for granulate with PLA (G + PLA). Higher values in the G + PLA variant suggest that PLA degradation might have further stimulated microbial metabolic activity, enhancing oxidative processes.

Piles with concentrate (C and C + PLA) reached the highest redox potential values among all variants. After 12 weeks, the redox potential was 360 mV for concentration without PLA (C) and 312 mV for concentration with PLA (C + PLA). Higher redox potential values in the concentrate variants suggest that the water-based bioproduct form might more effectively distribute enzymes and microorganisms throughout the piles, supporting oxygen diffusion and intensifying oxidative processes.

Slightly lower values in the C + PLA variant compared to C might be due to microbial competition for PLA degradation products or local zones with lower oxygen potential. The highest redox potential values were achieved in the granulate with PLA (G + PLA) and concentrate with PLA (C + PLA) variants, indicating a possible synergistic effect of bioproduct application and PLA presence. This may enhance aerobic conditions in the piles through intensified oxidative processes associated with microbial activity and PLA degradation, which releases organic acids.

The microbial counts for the experimental variants are presented in Table 3. Mesophilic (*TNM*) and thermophilic (*TNT*) microorganism counts in the compost piles were monitored at the start of incubation and after 4, 8, and 12 weeks.

At the start of the process, *TNM* values were similar across all experimental variants. As the process progressed, a significant *TNM* increase was observed in bioproduct variants (granulate and concentrate) compared to controls. The highest *TNM* values were recorded in concentrate variants (C and C + PLA), indicating the stimulating effect of this bioproduct form on mesophilic microorganism development. After 12 weeks, *TNM* in the C + PLA variant was about 10 times higher than in control with PLA (Ctr + PLA).

Thermophilic microorganism counts (*TNT*) increased as composting advanced, peaking at 8 weeks. Bioproduct variants, particularly concentrate forms, exhibited the highest *TNT* values. The C + PLA variant reached the peak thermophilic count at 8 weeks, suggesting a particularly favorable effect of the bioproduct on the development of this microbial group in the later composting stages.

Bioproduct application, in both granulate and concentrate forms, significantly influenced the increase in mesophilic and thermophilic microorganism counts in the compost piles. Higher *TNM* and *TNT* values in concentrate variants may result from better bioproduct distribution in its water-based form and faster release of active substances. These results unequivocally demonstrate that the bioproduct supports key microbial groups responsible for intensifying biodegradation processes.

The analysis of macronutrient content in the composted substrate after 12 weeks of the experiment revealed significant differences between the experimental variants, particularly regarding nitrogen (N), phosphorus (P), calcium (Ca), and magnesium (Mg). Detailed data are presented in Table 4. The potassium content in all variants exceeded the measurement limit of the method (>100 mg/L), which is attributed to the naturally high potassium content in biowaste.

The highest nitrogen content was recorded in the granulate variant without PLA film (G), amounting to 40.0±1.0 mg/L. This result may be associated with the presence of nitrogen in linseed cakes and urea, which were components of the granulate. The concentrate variants (C and C + PLA) exhibited lower nitrogen values compared to the granulate, due to differences in the release rates of active ingredients depending on the bioproduct form. The control variants (Ctr and Ctr + PLA) showed the lowest nitrogen content, suggesting limited mineralization of organic matter in these piles.

All bioproduct variants (G, G + PLA, C, C + PLA) exhibited higher phosphorus content compared to the controls. The highest phosphorus content was observed in the granulate variant without PLA film (G), amounting to 1.2±0.2 mg/L. The phosphorus present in the granulate composition, derived in part from linseed cakes, contributed significantly to this result. The presence of PLA film did not have a significant effect on phosphorus content in the piles.

Calcium content was notably higher in the bioproduct variants compared to the controls. The highest value was recorded in the granulate variant with PLA film (G + PLA), reaching 28.0±2.5 mg/L. The elevated calcium levels in the granulate variants may be linked to the presence of chalk as a granulate component. These findings suggest that bioproducts, particularly in granulate form, promote calcium release from biowaste.

A similar trend was observed for magnesium. Bioproduct variants exhibited higher magnesium content compared to the controls. The highest magnesium content was found in the concentrate variant with PLA film (C + PLA), amounting to 11.52±0.06 mg/L. The higher magnesium content in the concentrate variants may result from better organic matter decomposition and potential interactions between PLA degradation products and compost microorganisms.

These results indicate that the bioproduct, in both granulate and concentrate forms, significantly increases the nitrogen, phosphorus, calcium, and magnesium content in the compost substrate, enhancing its fertilizing properties. The presence of components such as urea, linseed cakes, and chalk in the granulate contributed to higher nitrogen and calcium values compared to the concentrate. On the other hand, the water-based concentrate form favored higher magnesium content due to more uniform bioproduct distribution and intensified microbial activity.

The presence of PLA may have further stimulated the release of certain elements through the degradation of lactic acid contained in the film.

PLA film degradation analysis

In the studies on PLA film biodegradation, a comprehensive assessment of changes in its properties during the 12-week composting process was conducted. The analysis included the determination of mass loss, changes in the carbonyl index, and the oxygen: carbon content ratio (O/C) in the film structure. Detailed microscopic observations of the film surface were also performed using scanning electron microscopy (SEM), allowing for visualization of structural signs of biodegradation. The results were compared across three variants: control (Ctr), with the addition of bioproduct in granulate form (G), and in water-based concentrate form (C). The aim of the study was to evaluate the effectiveness of the bioproduct in accelerating PLA degradation under industrial composting conditions and to examine the impact of the bioproduct application form on the rate and nature of the degradation processes.

The changes in PLA film mass during the composting process are presented in Fig. 2a. The results indicate significant differences in the degradation rate between the experimental variants. The control variant exhibited minimal mass loss of the PLA film, indicating limited activity of autochthonous microflora in the absence of the bioproduct. After 4 weeks, no changes in film mass were observed (0.0%), while after 8 weeks, the mass loss was 1.7±0.58%. After 12 weeks, the mass loss reached 4.0±1.73%, confirming the low intensity of biodegradation processes in this variant.

The granulate variant showed a significantly higher rate of PLA film degradation compared to the control. After 4 weeks, the mass loss was 13.7±1.15%. After 8 weeks,

T a b l e 4. Macronutrient content (N, P, K, Ca, Mg) in compost piles after 12 weeks of incubation

	Macronutrient content, mg/L				
	N	P	K*	Ca	Mg
Ctr	2.5±0.5	0.8±0.1	>100	6.0±0.2	0.72±0.04
Ctr + PLA	3.0±0.5	0.6±0.1	>100	10.4±0.4	0.96±0.06
G	40.0±1.0	1.2±0.2	>100	22.0±2.0	7.20±0.02
G + PLA	35.0±1.5	1.0±0.1	>100	28.0±2.5	9.60±0.06
C	15.0±1.0	1.0±0.1	>100	11.2±1.0	10.32±0.06
C + PLA	10.0±0.5	0.9±0.1	>100	14.4±1.2	11.52±0.06

*Measurement limit for potassium (K): values exceeding 100 mg/L are marked as >100 mg/L.

the degradation of the film increased to 59.3±4.04%, and after 12 weeks reached 93.7±2.52%. These results indicate the high effectiveness of the granulate in stimulating degradation processes, which can be associated with the presence of hydrolytic enzymes and active microorganisms in the bioproduct.

The highest mass loss value of the PLA film was observed in the water-based concentrate variant. After 4 weeks, the degradation reached 26.0±2.00%, and after 8 weeks, it increased to 84.3±2.52%. After 12 weeks, complete degradation was achieved (99.3±1.15%). The higher effectiveness of the concentrate may result from better distribution of the bioproduct in its water-based form and faster release of enzymes and microorganisms.

The most significant influence on the degradation rate of PLA film was observed in the concentrate variant, indicating the superiority of this application form under industrial composting conditions. The granulate variant also demonstrated high effectiveness, although slightly lower than the concentrate, which can be attributed to the slower release of active substances from the solid form. The minimal degradation rate in the control variant (Ctr) underscores the need for biotechnological additives to accelerate the biodegradation processes of PLA film.

The carbonyl index of PLA immediately after extrusion was 6.55±0.236. During composting, a gradual decrease in this value was observed across all experimental variants, indicating the progressive degradation of the polymer structure. The reduction in the carbonyl index resulted from a decrease in the carbonyl group content in the material, reflecting the intensity of biodegradation processes involving enzymatic hydrolysis of ester bonds between lactic acid monomers, followed by the release and assimilation of carbon from carbonyl groups. The analysis was conducted exclusively on PLA film fragments that had not completely disintegrated during composting. However, the mass loss results suggest that most samples, particularly in variants with the bioproduct, were significantly degraded, limiting the scope of analysis at later stages of the study.

The average values of the carbonyl index and their standard deviations are presented in Fig. 2b. Control variants (Ctr) exhibited the smallest decrease in the carbonyl index, with values of 5.89±0.564, 5.63±0.046, and 5.48±0.137 after 4, 8, and 12 weeks, respectively. Variants with granulate (G) showed a more pronounced decrease, with values of 5.07±0.055, 4.75±0.056, and 4.53±0.084 at the same time intervals. Concentrate variants (C) followed a similar trend, with values of 4.98±0.025, 4.70±0.026, and 4.53±0.103 after 4, 8, and 12 weeks, respectively. The changes shown in Fig. 2b highlight differences in the intensity of degradation between the experimental variants. The fastest decrease in the carbonyl index was observed in the bioproduct variants, particularly in granulate and concentrate forms, confirming their effectiveness in intensifying PLA polymer structure degradation. The control variant exhibited the lowest intensity of these changes,

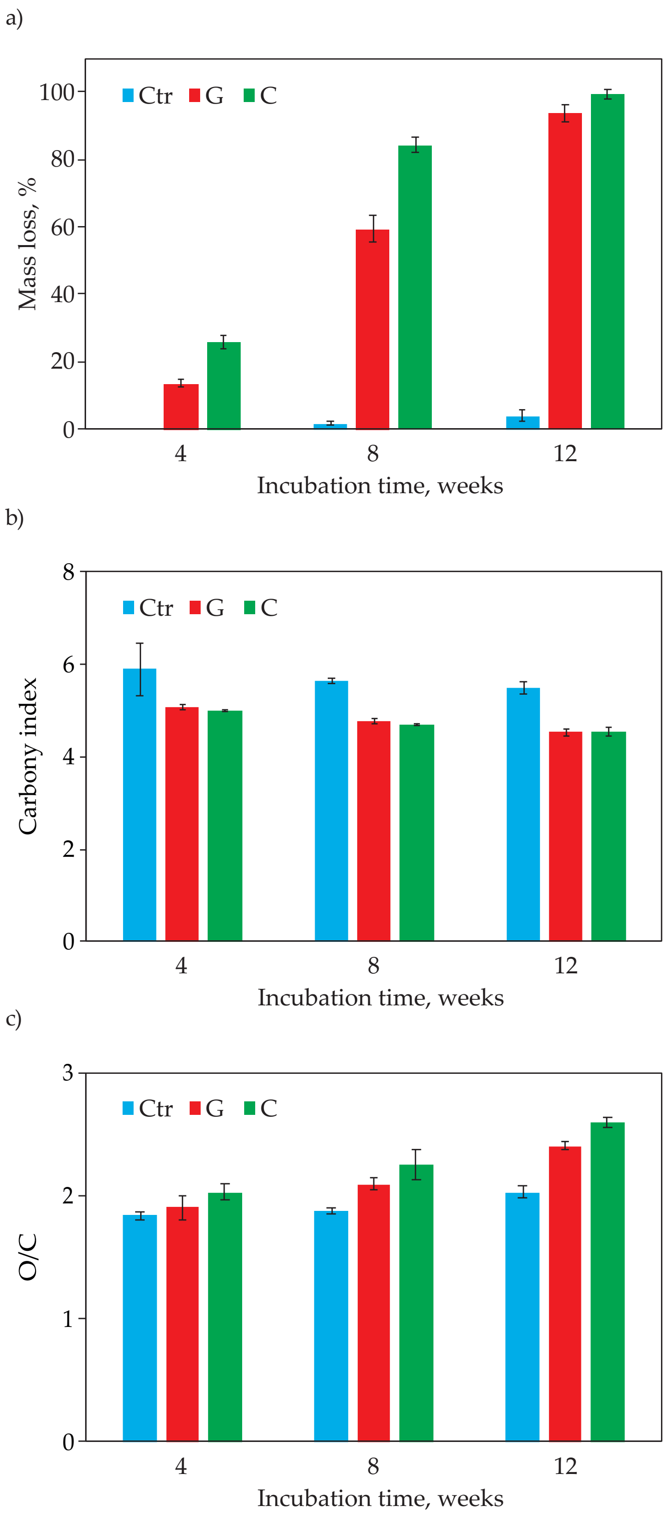

Fig. 2. Analysis of PLA films during composting: a) mass loss, b) carbonyl index, c) O/C ratio (Ctr – control, G – granulate, C – concentrate)

indicating limited activity of autochthonous microflora in the absence of the bioproduct.

The oxygen: carbon (O/C) mass ratio for the initial PLA film was 1.52. During the composting process, a systematic increase in this parameter was observed across all experimental variants, reflecting the microbial utiliza-

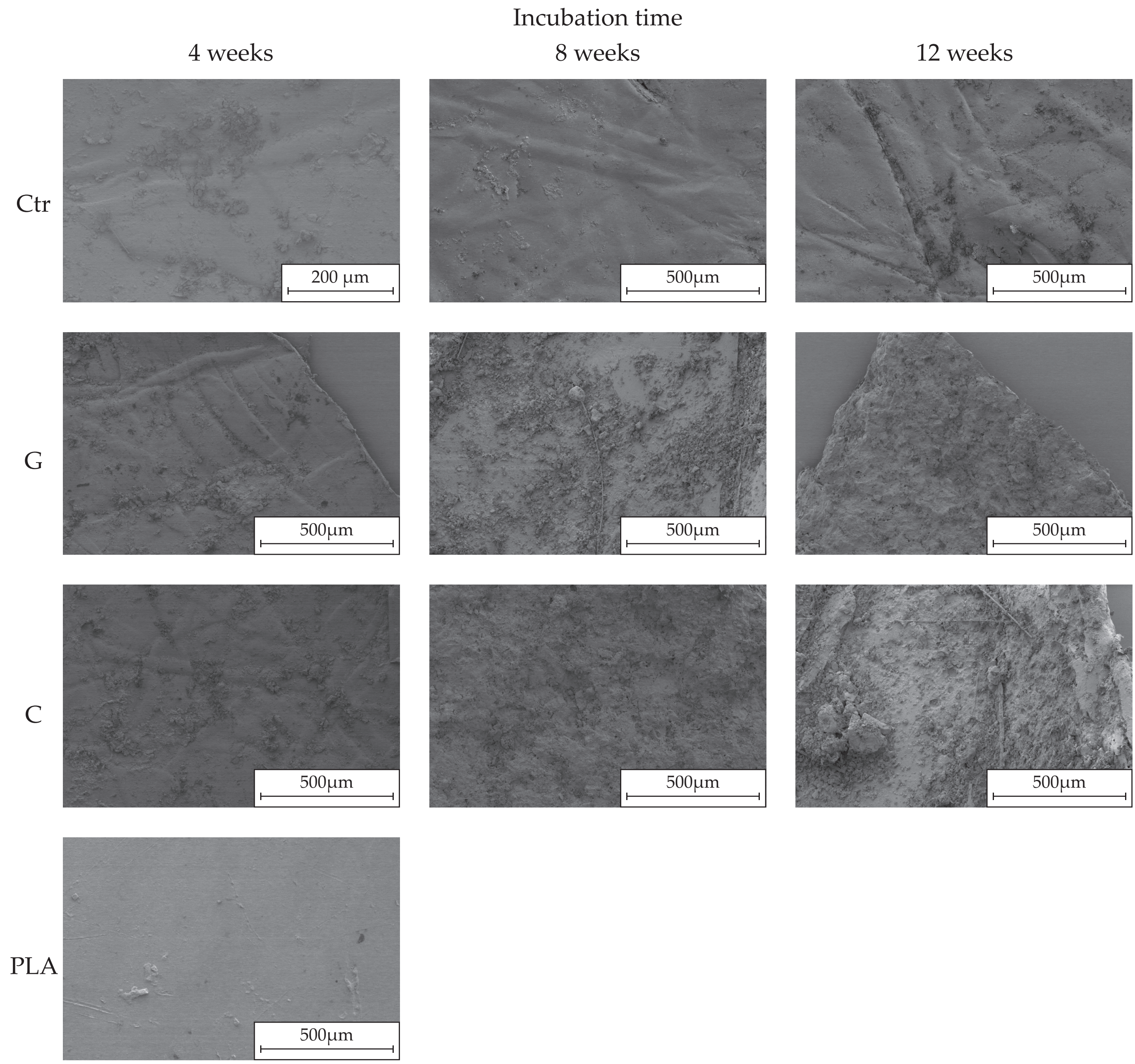

Fig. 3. SEM images of morphological changes on the surface of PLA film during composting depending on the experimental variant (Ctr + PLA, G + PLA, C + PLA) and incubation time (4, 8 and 12 weeks); PLA – film not subjected to incubation

tion of carbon from the polymer material [42]. The O/C ratio changes during composting are shown in Fig. 2c. In the control samples (Ctr), the increase was moderate, reaching 1.83±0.03 after 4 weeks and 2.03±0.05 after 12 weeks. Variants with the bioproduct, in both granulate (G) and concentrate (C) forms, exhibited a more dynamic increase in the O/C ratio compared to the control. After 12 weeks, the values were 2.40±0.03 for granulate (G) and 2.59±0.05 for concentrate (C). The highest O/C ratio was observed in the concentrate variant, confirming the effectiveness of this form of the bioproduct in accelerating PLA degradation processes. Granulate variants also showed a significant increase, indicating their efficiency in intensifying degradation, although to a lesser extent than the concentrate. The differences between the control and bioproduct variants clearly highlight the impact of additives on the intensity of metabolic transformations in the compost piles. The increase in the O/C ratio in PLA variants may also be linked to the release of polymer degradation products, which promote increased oxygen availability within the film structure.

Observations made using scanning electron microscopy (SEM) enabled a detailed evaluation of structural changes on the surface of PLA films during composting. Morphological changes depending on incubation time and experimental variants are presented in Fig. 3. Before incubation (PLA), the PLA film was characterized by a smooth and uniform surface, reflecting the state of the material after extrusion. Control variants (without bioproduct, Ctr) exhibited only surface wrinkling, which increased over time and can be associated with high temperature in compost piles. However, no damage such as clusters or delamination, typical for bioproduct variants, was observed.

Granulate variants (G + PLA) showed more intense morphological changes. After 4 weeks, initial signs of wrinkling and uneven material distribution were observed. After 8 and 12 weeks, the film surface was covered with numerous clusters and delamination's, indicating advanced degradation processes. The most pronounced structural changes were observed in the concentrate variant (C + PLA). After 4 weeks, the film surface was clearly delaminated, and after 8 and 12 weeks, irregular structures and large clusters were visible, indicating intense material degradation.

The analysis in all variants was hindered by the presence of silicon-based contaminants from the soil, which could not be completely removed despite sample cleaning. The recorded differences in morphology clearly demonstrate the significant impact of the bioproduct and incubation time on the intensity of PLA film degradation processes.

The synergistic effect of the bioproduct and the presence of PLA film indicates the potential for significant intensification of biodegradation processes under industrial composting conditions. The acidification of the substrate resulting from PLA degradation may have further stimulated microbial metabolic processes, particularly in the concentrate variants, as clearly reflected in parameters such as redox potential, the increase in thermophilic microorganism counts, and morphological changes in the PLA film.

The observed synergistic effect arises from a combination of factors. The degradation of PLA releases lactic acid, which acidifies the compost substrate, creating conditions favorable for microbial growth and activity. This acidification enhances the performance of hydrolytic enzymes present in the bioproduct, such as papain and proteinase K, which accelerate the breakdown of polymer chains. Furthermore, the starch-based components and organic additives in the granulate, including linseed cakes and coffee grounds, provide additional carbon sources that stimulate microbial proliferation. This interaction between the bioproduct components and PLA degradation products intensifies microbial metabolic activity, as evidenced by increased mesophilic and thermophilic microorganism counts and enhanced redox potential. These findings highlight the importance of combining biodegradable polymers like PLA with tailored bioproducts to optimize composting processes.

The obtained results suggest that using bioproducts in concentration form may be more effective under industrial composting conditions, especially in processes aimed at the rapid decomposition of polymer materials such as PLA. Granulate, while slightly less effective, could be applied in scenarios requiring longer substrate stability or in the environment with high initial moisture content.

CONCLUSIONS

The conducted studies have shown that the use of bioproduct in the form of granulate and concentrate significantly increases the biodegradation processes of the PLA film and modifies the properties of the compost substrate. Analysis of parameters such as temperature, pH, redox potential, the number of mesophilic and thermophilic microorganisms, indicated that the bioproduct in both forms supports microbiological activity in compost piles. The concentrate variant proved to be the most effective, as evidenced by the higher rate of PLA degradation and more visible changes in the morphology of the film surface, confirmed by SEM analysis.

Acidification of the substrate associated with PLA degradation played a key role in stimulating microbial metabolic processes, especially in the concentrate variants. The higher O/C ratio and the reduction of the carbonyl index in these samples indicate advanced degradation processes in the PLA polymer structure. The granulate variant also contributed to PLA film degradation, although to a lesser extent, making it a potential solution for applications requiring longer substrate stability.

The results suggest that the synergistic use of bioproduct and PLA film waste may be an effective approach to accelerate biodegradation processes in industrial composting conditions. The bio-based product in concentrated form is promising for applications focused on the rapid degradation of polymeric materials such as PLA, while the granulate may be more suitable for scenarios requiring gradual nutrient release and extended process duration.

This study highlights the importance of biotechnological solutions in the context of the circular economy, offering practical insights for the optimization of industrial composting processes involving bio-based products and biodegradable materials. The results can contribute to the design of more sustainable bio-waste management systems, supporting the development of environmentally friendly technologies.

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Authors contribution

K.J. – conceptualization, methodology, validation, formal analysis, writing-original draft, writing-review and editing, visualization, project administration, funding acquisition; D.L. – methodology, validation, formal analysis, investigation, writing-review and editing, project administration; A.M. – methodology, validation, formal analysis, investigation, writing-review and editing, visualization; A.Ż. – formal analysis, investigation; O.K. – resources; L.S. – resources.

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Conflict of interest
The authors declare no conflict of interest.

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