

Article

Anaerobic Degradation of PLA Bioplastic: Effect of Pretreatment Methods on Biogas Production Efficiency and Biodegradability

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Abstract

In recent decades, bioplastics have gained increasing attention as an alternative material to conventional petroleum-based plastics due to their biodegradability, which contributes to reduced environmental pollution. After their use, bioplastics can be transformed into environmentally friendly products, such as compost, or energy carriers as biogas. This study investigates the short-term batch anaerobic biodegradability of polylactic acid (PLA) bioplastics accessible to household consumers, explores the impact of various pretreatment techniques on the anaerobic digestion process, and assesses the potential of PLA as a sustainable source of energy. Anaerobic digestion of thermally or biologically pretreated PLA, as well as of a PLA–ethyl acetate mixture, at 46.3 ± 0.5 °C resulted in a biogas yield ranging from 139.20 ± 22.14 to 510.26 ± 9.92 L/kg of raw material. However, PLA mass loss analysis indicated that a short-term 25-day digestion period was insufficient for complete biodegradation. Furthermore, considering the biogas production dynamics, it is unlikely that a longer digestion period would have significantly increased the biodegradation degree. Depending on the pretreatment method applied, the degree of PLA biodegradation after anaerobic processing ranged from 12.87% to 26.46%.

Keywords: bioplastic; polylactic acid; anaerobic digestion; pretreatment; biodegradability



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1. Introduction

The primary polymer used in everyday life is plastic derived from petroleum-based products. Due to its low cost, durability, and versatility, the demand for and production of plastic continues to grow annually, placing a significant burden on fossil fuel resources and petroleum consumption [1–3]. It is estimated that the annual production of conventional (petrochemical-based) plastics may exceed 754 million tonnes by 2050 [4]. However, only up to 10% of the plastic produced globally is recycled, while the vast majority is disposed of in landfills [2,5–7]. The extensive use of conventional plastics and their persistence in the environment pose serious global challenges, including environmental pollution and risks to human health [7–9]. For these reasons, it is essential to invest efforts in the integration and promotion of innovative, cost-effective, biodegradable, and environmentally friendly materials.

A sustainable alternative to petroleum-based plastics is bioplastics. Biodegradable bioplastics produced from biomass, such as polylactic acid (PLA), reduce the carbon footprint, offer improved waste management options, and decrease dependence on fossil fuels, thereby potentially limiting greenhouse gas emissions [3,10,11]. However, despite the growing interest in bioplastics as a sustainable substitute for conventional plastics, they also require appropriate end-of-life treatment [11,12]. Unfortunately, bioplastic degradation under natural conditions is usually too slow and sometimes incomplete, making it insufficient to prevent the accumulation of waste. This highlights the need for the exploration of biodegradation mechanisms, which can enable the development of more efficient techniques for waste disposal and utilization or upcycling [13,14].

Problems related to bioplastic waste can be addressed through appropriate end-of-life management strategies. Biodegradability enables bioplastics to return to the natural material cycle, resulting in minimal environmental impact [15]. Bioplastic waste can be managed through various methods, such as composting, which not only efficiently handles the waste stream but also generates added value. The concept of energy recovery through anaerobic digestion (AD) of bioplastics is highly promising within the framework of the circular bioeconomy. However, the complex chemical structure of various bioplastic polymers negatively influences their anaerobic conversion potential. The slow degradation rate of commercially available bioplastics under anaerobic conditions, particularly at mesophilic temperatures, highlights the necessity for pretreatment prior to processing in order to enhance their biodegradability and, consequently, increase biogas production [10,15]. Although numerous previous studies have demonstrated the effective biodegradation of bioplastics during biogas production, there is still a lack of knowledge regarding the biogas production potential and anaerobic treatment of commercially available bioplastics, including the role of pretreatment in enhancing anaerobic digestion performance [12,16,17].

Different strategies for plastic pretreatment have been suggested. Research studies proposed that microbial lipases (EC 3.1.1.3), carboxylesterases or true esterases (EC 3.1.1.1) and cutinases (EC 3.1.1.74), as well as highly related enzymes such as PETases (EC 3.1.1.101) and PHA depolymerases (EC 3.1.1.75 and EC 3.1.1.76), which, together, can be called polyesterases, can significantly improve biodegradation efficiency of polyester plastics, including PLA [3,13,18–21]. The structural homology of PLA with natural polymers permits its degradation by naturally occurring enzymes grouped as PLA depolymerases. According to primary substrate specificities, PLA depolymerases are classified as protease-type and lipase-type, the latter also including cutinases [3].

Chemical or thermal pretreatment of polyesters can also be used to improve PLA degradation. Although thermal decomposition of neat PLA is not very significant (1% per 20 min at 200 °C), thermal pretreatment has been successfully used by some researchers to enhance the following anaerobic digestion [22,23]. As a semicrystalline polymer, PLA consists of both crystalline and amorphous phases with a glass transition state of around 65 °C. In temperatures higher than the glass transition state, the amorphous regions of the polymer transition from a restricted to a mobile configuration [22,24]. Lower crystallinity of polyesters including PLA is associated with enhanced biodegradation. Even though glass transition is a reversible process, it has been shown that thermal history of PLA can strongly influence the properties of the polymer, including crystallinity [25]. Therefore, thermal pretreatment of PLA might have some lasting effect on the polymer's structure and enhance its biodegradation [24–27]. Chemical pretreatment of PLA can be used instead of or together with thermal pretreatment. For this purpose, alkaline substances are used since hydroxide ions catalyze polyester hydrolysis. The most studied pretreatment agent used is NaOH, but Ca(OH)₂ and KOH can also be used [28]. In some cases, untreated PLA did

not biodegrade under anaerobic conditions, while pretreatment was reported to influence hydrolysis rate and increase biogas production during anaerobic degradation [3,29,30].

More effective end-of-life management strategies for bioplastics can help reduce plastic pollution, while the simultaneous recovery of valuable products, such as biogas/biomethane, may increase the potential value of this process. It is essential that such research is conducted on commercially available PLA products rather than focusing exclusively on pure scientific-grade materials. Globally, a wide range of PLA blends containing various copolymers, dyes, and additives are produced, and these components may influence their biodegradation behavior and performance during anaerobic treatment. Therefore, this study aimed to analyze the anaerobic degradation process of PLA bioplastics available to household consumers, to investigate the effect of different PLA pretreatment methods on biogas production efficiency, evaluated based on cumulative biogas yield in batch assays, and to assess the potential of PLA bioplastics as a sustainable energy source.

2. Materials and Methods

2.1. Sample Preparation and Pretreatment

Home compostable PLA waste bags purchased from an online retailer in Lithuania were used in this study. The selected product is made primarily from corn starch and is certified with the TÜV Austria OK Compost HOME label, indicating its suitability for home composting conditions. The composition of the waste bags does not contain any hidden plastics, chemical additives that promote fragmentation into microplastics, or other potentially harmful components. PLA bags were shredded with scissors into pieces measuring 4–6 cm. All experiments were conducted in triplicate, using 5 g of the prepared sample for each trial. Three different pretreatment methods were applied: thermal, chemical, and biological.

2.1.1. Thermal Pretreatment

The prepared PLA sample was heated for 1 h in a CV-EL L GS autoclave (CertoClav Sterilizer GmbH, Leonding, Austria), maintaining a temperature of 140 ± 1 °C and a pressure of 4 bar. After autoclaving, the treated PLA melted into a single porous, rubber-like mass, which was then shredded with scissors into 1 ± 0.5 cm sized particles.

2.1.2. Preparation of PLA–Ethyl Acetate Mixtures

The pretreatment of PLA with ethyl acetate is a novel area of research aimed at improving the properties of the biomaterial for various applications, including biodegradability. Ethyl acetate is an organic solvent known for its ability to dissolve and modify polymers, influencing their physical and chemical properties. Moreover, ethyl acetate is rapidly biodegradable and less toxic to methanogens compared to other organic solvents such as ethanol, methanol, or acetone. For these reasons, ethyl acetate was chosen for the primary chemical treatment of PLA.

Each prepared sample was chemically treated using 5 g of ethyl acetate ($\geq 99\%$) (UAB Eurochemicals, Vilnius, Lithuania) in a ratio of 1:1. The chemical treatment lasted for 24 h at room temperature (20–22 °C). The entire mixture (PLA with ethyl acetate) was used in the biogas production potential experiments.

Initially, ethyl acetate was selected as a chemical pretreatment agent intended to enhance PLA degradation. However, based on the observed results, this approach was reconsidered, as ethyl acetate itself contributed significantly to biogas production. Therefore, for methodological accuracy, the system was further interpreted as the co-digestion of PLA and ethyl acetate rather than as a chemical pretreatment process. In this context, the synergistic index (SI) was used to evaluate the interaction between substrates and

was calculated as the ratio of the observed biogas yield (y_{exp}) to the theoretical biogas yield (y_{theo}). The theoretical biogas yield (y_{theo}) represents the expected biogas yield of the mixture calculated from the weighted contribution of individual substrates according to their proportions, assuming additive behavior, as described by Castro-Molano et al. [31]:

$$SI = \frac{y_{exp}}{y_{theo}} \quad (1)$$

2.1.3. Biological Pretreatment

Several lipolytic enzyme variants were tested on small-sized PLA samples: GD-95RM, GDEst-lip, and Cut+SP. These enzymes have been chosen based on previous works. GD-95RM lipase and GDEst-lip [32,33] possessed high activity, thermostability and the ability to degrade another polyester, polycaprolactone (PCL). Hydrolysis of PCL films higher than 50% was achieved using 0.05 mg of the enzyme at 50 °C, while at 30 °C, 20–30% degradation was observed [33]. *Streptomyces scabiei* 87.22 cutinase Cut+SP [20,34] shared 100% similarity with *S. scabiei* EF-35 (HER1481) Sub1 suberinase/cutinase [35], which demonstrated the ability to degrade PET. This lead to the hypothesis that Cut+SP, GD-95RM, and GDEst-lip can also be suitable for PLA degradation.

For primary PLA degradation at a low scale, three microbial lipolytic enzymes were tested: GD-95RM lipase [32,33], GDEst-lip [36] and *Streptomyces scabiei* 87.22 cutinase Cut+SP [20,34]. *E. coli* BL21 (DE3) cells harboring pET-21c(+) plasmids with inserted genes encoding either GD95-RM lipase, fused lipolytic enzyme GDEst-lip or Cut+SP were used as producers of lipolytic enzymes. The induction, expression, and purification of recombinant lipolytic enzymes GD-95RM, GDEst-lip, and Cut+SP were carried out according to previous studies [33].

PLA bag samples (20–30 mg) were fully submerged in 2 mL of the reaction mixture (Tris-HCl buffer solution (50 mM; pH 8) containing 0.05 mg/mL enzyme) and incubated at 35 °C for 72 h. Samples were weighed before and after degradation. The degradation efficiency was evaluated based on the residual film mass, calculated according to the following formula [37]:

$$w_l = \frac{w_i - w_d}{w_i} \times 100 [\%] \quad (2)$$

where w_l represents the weight loss (g); w_i —initial mass (g); and w_d —mass of dried sample after treatment (g).

For PLA bag pretreatment for the following anaerobic digestion, Cut+SP was chosen. Briefly, 5 g of PLA bag material was fully submerged in a 0.05 mg/mL protein solution (0.05 mg/mL enzyme in 60 mL of the reaction mixture (Tris-HCl buffer solution (50 mM; pH 8))). After a 72 h incubation at 35 °C, the samples were removed, rinsed with distilled water, and thoroughly dried. Samples were weighed before and after degradation, and residual film mass was evaluated. The obtained pretreated PLA bags were transferred for anaerobic digestion.

All necessary reagents for enzyme production, purification and PLA degradation analysis were purchased from Carl Roth GmbH & Co. Kg, Karlsruhe, Germany, unless otherwise specified. *Escherichia coli* strain BL21 (DE3) (Thermo Fisher Scientific, Waltham, MA, USA) was used for recombinant protein production.

2.2. Biogas Potential Testing

The biogas potential test was performed to assess the biogas generation potential from the tested feedstocks under anaerobic conditions. For biogas production, 1 L reactors were used, with an effective volume of 500 mL (495 g of inoculum and 5 g of bioplastic). The reactors were kept in a heated incubator, ensuring a semi-thermophilic regime by maintaining

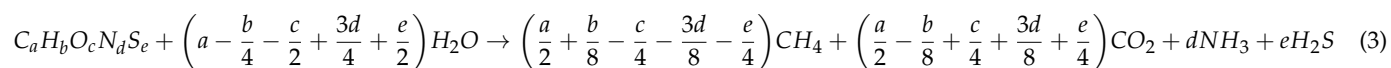
a temperature of 46.3 ± 0.5 °C. The reactors were connected to a BPC Instruments gas-flow measurement unit (BPC Instruments AB, Lund, Sweden). The gas produced in the unit was measured by water displacement using calibrated flow elements, which send a signal for every 2 mL of produced gas. The signal was recorded in a programmable controller. To prevent process-related mass loss of PLA, the pretreated samples were enclosed in 7×9 cm nylon mesh bags (pore size 90 μ m) prior to loading into the reactor. All biogas potential tests were conducted in triplicate.

The temperature regime of 46.3 ± 0.5 °C was selected based on studies reported in the scientific literature, where semi-thermophilic conditions demonstrated higher biogas production efficiency and improved process stability compared to both mesophilic and thermophilic regimes, particularly in the degradation of poorly biodegradable materials such as lignocellulose [38–40].

Inoculation was carried out using digestate obtained from an operating biogas plant in eastern Austria, where manure and grass biomass were used as feedstock, resulting in a microbial community adapted to the degradation of complex, slowly biodegradable substrates. The inoculum was kept for 5 days in a 19 L anaerobic reactor at a semi-thermophilic temperature (46.3 ± 0.5 °C) to ensure digestate deactivation and degassing. Blank reactors containing only the inoculum were used to quantify background gas production, which was subtracted from the total gas production in reactors containing the substrate to obtain net biogas yields for the tested feedstocks.

The experiment lasted for 25 days. During this investigation, the generated gases were collected using airtight 25 L sampling bags (RESTEK 22967, Bellefonte, PA, USA), which were connected to the outlet port of the gas flow meters. The collected gases were analyzed using an AwifLEX gas analyzer (Awite Bioenergie GmbH, Langenbach, Germany) with the following measurement ranges and accuracies: CH₄ 0–100 Vol.%, $\pm 0.2\%$; CO₂ 0–100 Vol.%, $\pm 0.2\%$; O₂ 0–25 Vol.%, $\pm 0.1\%$; H₂S 0–3000 ppm, $\pm 1.0\%$.

The theoretical biogas yield was calculated based on the modified Buswell and Hatfield equation [41]:



where a, b, c, d, e —molar numbers of atoms.

Methane yield was calculated using the following formula:

$$V_{CH_4} = B \times \left(\frac{C_{CH_4}}{100}\right) \quad (4)$$

where B —biogas yield from raw material (RM) (L/kg RM); C_{CH_4} —methane concentration in the biogas (%).

The theoretical methane concentration in biogas was calculated using the modified Buswell and Hatfield stoichiometric model (Equation 2) [41] based on the elemental composition of the substrate. According to this model, the molar amounts of methane and carbon dioxide were determined assuming that substrate carbon is distributed between these main gaseous products.

2.3. Assessment of Biodegradability

The assessment of biodegradability was performed using the weight loss method, where the weight of the samples was measured after anaerobic digestion. Prior to weighing, the samples were thoroughly washed and dried. For drying, the prepared samples were placed in a Memmert Model 100-800 drying oven (Mettler GmbH + Co. KG, Schwabach, Germany) and dried for 24 h at a temperature of 105 °C. The samples were weighed

using KERN EG4200-2NM analytical scales (max. 4200 g, min. 0.5 g) (Kern & Sohn GmbH, Balingen, Germany). The efficiency of PLA degradation was calculated using the following formula:

$$\text{Weight loss of PLA (\%)} = \frac{(W_i - W_p)}{W_i} \times 100 \quad (5)$$

where W_i —initial weight (g); W_p —weight after anaerobic digestion (g).

2.4. Chemical Composition Analyses

The chemical composition analyses of PLA were carried out by ALS Czech Republic, s. r. o (Prague, Czech Republic). Dry matter (DM) was determined according to method CZ_SOP_D06_01_045; ash content according to CZ_SOP_D06_07_47.C; carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and sulfur (S) according to CZ_SOP_D06_07_121.A; chlorine (Cl) according to CZ_SOP_D06_07_124.C; and phosphorus and sodium were determined using CZ_SOP_D06_07_006. The chemical composition of the PLA bags is presented in Table 1.

Table 1. Chemical composition of PLA.

Analysis	Value
Dry matter, %	96.4
Ash, %	1.38
Carbon, g C/g	0.554
Hydrogen, g H/g	0.064
Oxygen, g O/g	0.329
Nitrogen, g N/g	0.0015
Sulfur, g S/g	0.0014
Chlorine, g Cl/g	0.0004
Phosphorus, g P/g	0.000059
Sodium, g Na/g	0.00037

2.5. Scanning Electron Microscopy Analysis

The surface morphology of the PLA samples was investigated using a Schottky-type field-emission scanning electron microscope SU-70 (Hitachi, Tokyo, Japan). The surface images were obtained by recording secondary electrons using an accelerating voltage of 10 kV. The analysis was conducted in an ultra-high vacuum ($<10^{-7}$ Pa). In order to increase the electric conductivity and to avoid the charge accumulation, the specimens were coated with a 15 nm thick layer of silver via a Q150T ES sputter (Quorum, Lewes, UK) deposition prior to analysis.

2.6. Data Processing

Statistica 13 (StatSoft, Inc., Tulsa, OK, USA) and Microsoft Excel 2025 (Microsoft Corporation, Redmond, WA, USA) were used for data processing. The mean of the test results was compared using the t (t test) criterion. Significant differences were reported at a p value lower than 0.05.

3. Results

3.1. Anaerobic Digestion of a PLA–Ethyl Acetate Mixture

Cumulative biogas yield results (Figure 1) in the case of chemical pretreatment cannot be interpreted unambiguously. Unlike conventional chemical pretreatment methods, such as acid (e.g., HCl) or alkaline (e.g., NaOH) treatments, which involve intensive hydrolysis

of ester bonds, ethyl acetate acts as an organic solvent, inducing swelling and partial dissolution of the PLA structure, leading to the formation of a more porous matrix [42,43]. This effect increases substrate accessibility to microorganisms and enhances enzyme–substrate interactions; however, it is not associated with significant chemical depolymerization of the polymer [43,44]. Furthermore, as an organic compound, ethyl acetate can be biodegraded under anaerobic conditions and thus directly contributes to biogas production, as observed in this study. It was found that ethyl acetate serves as a suitable organic substrate for biogas generation, producing a high yield (963.55 ± 15.62 L/kg RM) (Table 2). As a result, the true effect of PLA pretreatment is not fully reflected, and interpretation of the results within the framework of conventional pretreatment concepts becomes problematic. Therefore, to more accurately interpret the results of chemical pretreatment, it is appropriate to evaluate biogas yield as a mixture of individual substrates (Figure 1) and to support these findings by assessing the synergistic index of the co-substrates.

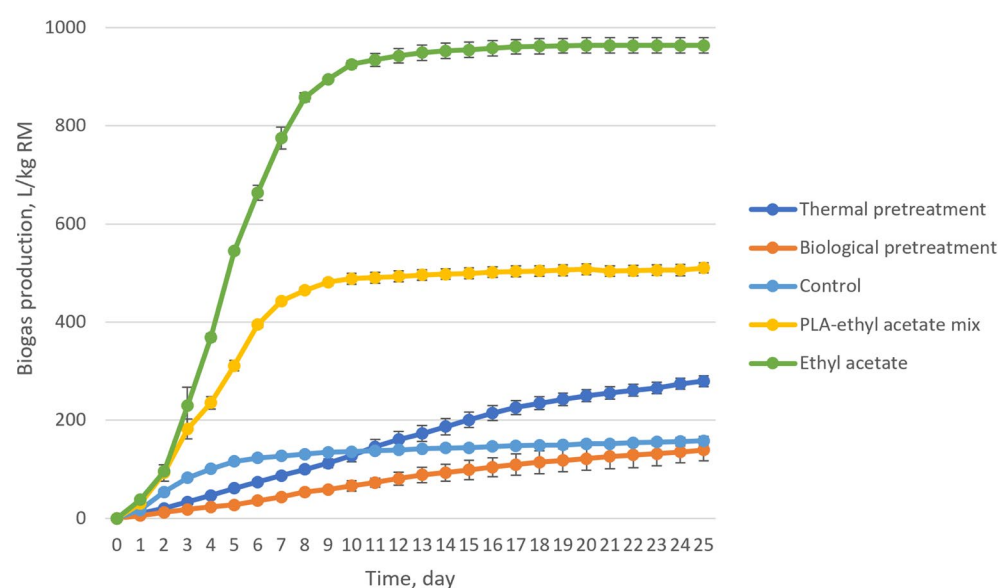


Figure 1. Cumulative biogas production. Values represent net cumulative biogas production after correction for biogas produced by the inoculum control. Error bars represent standard deviations of triplicate measurements ($n = 3$).

Table 2. Experimental and theoretical biogas and biomethane potential from substrate.

Pretreatment	Experimental Biogas Yield L/kg RM	Experimental Methane Yield L CH ₄ /kg RM	Theoretical Biogas Yield L/kg RM	Theoretical Methane Yield L CH ₄ /kg RM
Control	157.94 ± 9.37	38.00 ± 7.31		
Biological	139.20 ± 22.14	38.39 ± 3.97	996.53	557.56
Thermal	279.54 ± 11.29	109.83 ± 3.41		
Ethyl acetate	963.55 ± 15.62	513.09 ± 9.00	1017.60	636.00
PLA–ethyl acetate mix	510.26 ± 9.92	268.15 ± 12.06	1008.80	596.78

Based on calculations using experimentally measured and theoretically derived biogas yields (where theoretical values represent weighted mono-substrate biogas potentials), the synergistic index reached 1.8, indicating that the mixture of PLA and ethyl acetate exhibits a synergistic effect. However, both the experimental biogas yield and the experimental biomethane yield from the PLA–ethyl acetate mixture were significantly lower than the calculated theoretical yields (Table 2). Based on the analysis of biogas quality collected over a 25-day period, the methane yield potential for the PLA and ethyl acetate mixture was calculated to be 268.15 ± 12.06 L CH₄/kg RM, while for ethyl acetate alone, it was

513.09 ± 9.00 L CH₄/kg RM (Table 2). Although the calculated theoretical biogas yield from ethyl acetate is close to the experimentally measured biogas yield, significant differences were observed in methane yield.

According to theoretical calculations, the methane yield from ethyl acetate should reach 636.00 L CH₄/kg RM (Table 2). These discrepancies can be attributed to the significant differences between the measured and theoretical methane concentrations. Specifically, the experimental values for the PLA–ethyl acetate mixture and pure ethyl acetate were $52.53 \pm 1.36\%$ and $53.25 \pm 0.07\%$ CH₄, respectively, whereas the theoretical values based on the initial carbon (C) content were calculated at 59.21% and 62.50% CH₄. The reduced carbon conversion efficiency may be associated with substrate losses occurring before biological degradation, particularly due to the volatilization (stripping) of ethyl acetate. In addition, the accumulation of intermediate metabolites, such as volatile fatty acids, and the dissolution of CO₂ in the liquid phase may further contribute to the observed discrepancy between the theoretical and experimental methane yields, as not all carbon was converted into methane under the applied experimental conditions. Consequently, the theoretical methane yield is higher because it assumes complete conversion of all substrate carbon into methane, whereas, in practice, part of the carbon is lost or remains in intermediate products and dissolved phases. Nevertheless, in all cases, the obtained methane concentration, compared to values typically observed in standard biogas substrates such as maize (usually 50–60% CH₄), is not low and remains sufficient for efficient biogas utilization [45].

Unfortunately, there is a lack of information specifically regarding the use of ethyl acetate in biogas production. However, scientific literature does contain studies on other chemicals that contain an acetate group. For example, to enhance methane production and properly balance the carbon-to-nitrogen ratio of the feedstock, Soboh et al. [46] investigated the digestion of algae with sodium acetate. The results of this study revealed that sodium acetate is a highly effective and technologically promising substrate for methanogenic processes as it provides a rich and easily accessible carbon source [46]. While sodium acetate is a direct substrate for methanogenesis, ethyl acetate is an intermediate compound that can be hydrolyzed into products suitable for biogas production. Both studies revealed that acetate-based substances, when used as substrates, have significant potential for biogas production. Furthermore, both the actual biogas production results and the theoretical biogas production estimate (1017.60 L/kg RM) are closely aligned—the actual biogas yield from ethyl acetate (963.55 ± 15.62 L/kg RM) is only 54.05 L/kg RM lower than the theoretical value (Table 2).

Scanning electron microscopy (SEM) provides qualitative insights into surface morphology changes associated with biodegradation (Figure 2). SEM analysis was performed for an untreated PLA bag (A), the control sample after AD (B), the PLA sample after AD with ethyl acetate (C), and the thermally pretreated sample after AD (D). Typically, the surface of an untreated PLA sample is expected to be smooth, continuous, and free from morphological damage or defects. However, the surface shown in Figure 2A appears different—it is uneven and covered with numerous spherical formations (bumps or bubbles). Such a structure may have resulted from the migration and crystallization of additives on the surface or from specific characteristics of the bag-manufacturing process. In contrast, the surface of the PLA sample in Figure 2B shows clear signs of degradation, including irregular pores, holes, and cracks. These defects vary in size, with some cavities being larger than others, indicating an uneven biodegradation process across the PLA surface. However, SEM analysis alone does not provide information on crystallinity, molecular weight, or other structural parameters, and, therefore, additional analyses are required to confirm the proposed degradation-related interpretations and mechanisms.

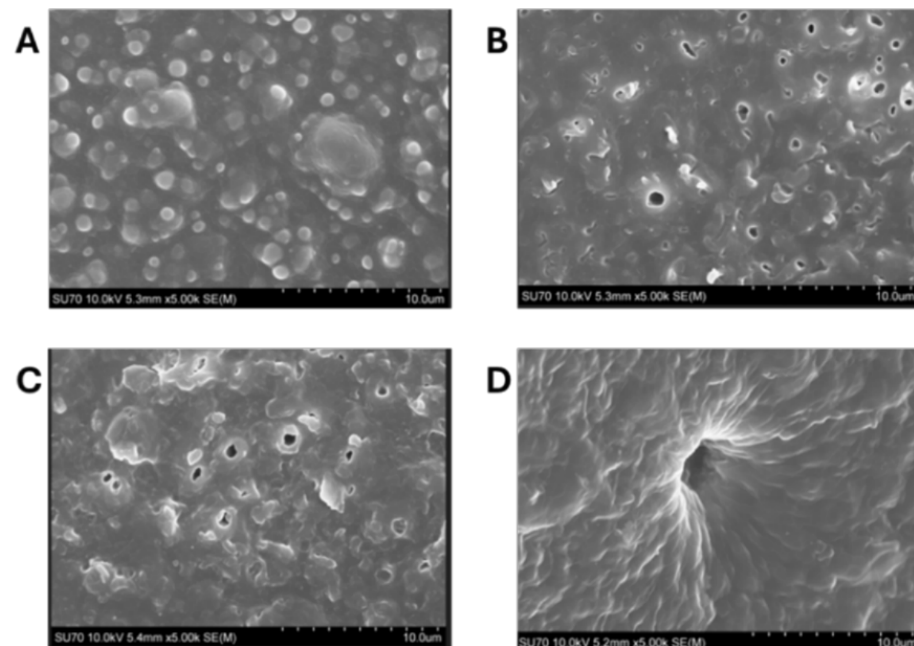


Figure 2. SEM micrographs of the primary PLA bag (A), control PLA bag after AD (B), PLA bag after PLA–ethyl acetate mix AD (C) and thermal pretreatment and AD (D).

After PLA–ethyl acetate mix AD, irregularly shaped pores, cavities, and flakes were observed on the PLA surface (Figure 2C). These surface irregularities may suggest a non-uniform crystallization of PLA and indicate that amorphous zones at the surface could remain susceptible to microbial attack. Furthermore, ethyl acetate may induce PLA swelling, particularly when the polymer is not fully crystallized. Although surface damage was evident, the greater mass loss observed in the control sample suggests that more extensive and efficient degradation occurred throughout the bulk of the material. This may be attributed to a higher amorphous content in the control sample, which provided more favorable conditions for anaerobic degradation, whereas the potentially increased crystallinity induced by chemical effects of ethyl acetate limited the depth of degradation, despite the visible surface deterioration [47–50].

3.2. Thermal Pretreatment

The conducted studies showed that the results of chemical PLA pretreatment cannot be evaluated unambiguously due to the high biogas yield from ethyl acetate (963.55 ± 11.05 L/kg RM). However, by considering other pretreatment methods, it is evident that thermal treatment at 140 °C had a significant impact on biogas production. Biogas generation from thermally treated PLA amounted to 279.54 ± 11.29 L/kg RM (Table 2), which is approximately two times more than the control group— 157.94 ± 9.37 L/kg RM (Table 2). The cumulative biogas production curves (Figure 1) indicate that biogas production was approaching the final stage of the digestion process but had not yet reached a stable phase. This observation is further supported by the protocol proposed by Holliger et al. [51], which indicated that after 25 days, the complete fermentation endpoint had not been reached, although calculations suggested it was close to completion. Daily biogas production during the final days of this study varied from 3.12% to 1.74% of the accumulated biogas volume. By calculating the theoretical biogas yield from the used PLA (996.53 L/kg RM), it was found that the actual biogas yield accounted for 28% of the calculated theoretical yield.

High-temperature treatment of PLA can affect the polymer structure, properties, and its ability to biodegrade. When PLA is treated at high temperatures, the polymer structure

becomes more amorphous, making it more prone to deformation and more accessible to enzymes and microorganisms [52–55]. Biodegradation can also be accelerated, as the heat effect can cause the formation of smaller molecules, such as monomers and oligomers, which are more readily available to microorganisms [56,57]. Therefore, thermal treatment of PLA can accelerate biodegradation by enhancing microorganism accessibility, as heat promotes the formation of smaller molecules and reduces the rigidity of PLA polymers. In the present study, the greatest mass loss after anaerobic digestion was also observed for thermally treated PLA, reaching $26.47 \pm 0.61\%$ (Table 3). However, as demonstrated by Zhang et al. [58], PLA mass loss under anaerobic conditions can exceed methane production because physical fragmentation occurs more rapidly than biological degradation. Therefore, weight loss alone cannot be considered a direct indicator of biodegradation, but rather reflects the extent of polymer fragmentation.

Table 3. Mass loss of PLA after anaerobic digestion.

Pretreatment	Initial Weight, g	Weight After Anaerobic Processing, g	Mass Change, g	Weight Loss, %
Control	5.00	3.88 ± 0.05	1.12 ± 0.05	22.40 ± 1.06
Biological	5.00	4.14 ± 0.07	0.86 ± 0.07	17.21 ± 1.30
Thermal	5.00	3.68 ± 0.03	1.32 ± 0.03	26.47 ± 0.61
PLA after PLA–ethyl acetate mix AD	5.00	4.36 ± 0.02	0.64 ± 0.02	12.87 ± 0.31

After analyzing the biogas quality, it was calculated that the methane yield potential after thermal pretreatment reached 109.83 ± 3.41 L CH₄/kg (Table 2). This calculated methane yield is more than five times lower than the theoretical yield of 557.56 L CH₄/kg at a methane concentration of 55.95% CH₄. Although identical yields are not expected, such large discrepancies may have been caused not only by the low degree of PLA degradation (Table 3) but also by the low methane concentration ($39.30 \pm 0.46\%$ CH₄). Unfortunately, due to the lack of specific information, it is challenging to compare the obtained biogas quality results with those reported by other researchers. For instance, although García-Depraet et al. [59] presented results on anaerobic biodegradation and carbon flux distribution analysis from commercial PLA powders, reporting carbon as CH₄ (pretreated PLA $47.4 \pm 0.2\%$ CH₄), the initial chemical composition of the PLA used remains unclear. Similarly, while Shrestha et al. [11] conducted biogas production studies using bioplastics made from cellulose and PLA, the biogas composition results were reported only for the bioplastic derived from cellulose.

Interestingly, although heat improves biodegradation, thermal treatment does not enhance the intensity of biogas production. After thermal treatment, biogas production was consistent and gradual throughout this study, unlike in the control group, where the most intense biogas production occurred during the first week of this study, after which the biogas yield flattened. Similar trends were observed in the study by Hobbs et al. [60], where untreated PLA and NaOH-treated PLA were anaerobically processed with food waste under mesophilic conditions. The minimal lag phase for untreated biochemical methane potential test (BMP) results was 2 days, and CH₄ production was rapid and peaked by day 12, after which the cumulative methane production curve flattened, continuing until day 30. While the lag phases between treated and untreated PLA were similar in the studies conducted, the treated PLA BMP test yielded the highest CH₄ production throughout the entire testing period (70 days), although CH₄ production from alkali-treated PLA was lower than from untreated PLA until day 40.

Figure 2D presents a markedly different microstructural appearance compared to the previously discussed SEM images. A prominent central cavity is visible, from which radiating, fibrous, and wavy structures extend, suggesting that the material underwent rupture or lateral dispersion as a result of thermal treatment. Although the treated samples fused into a homogeneous mass, the thermal pretreatment induced microstructural modifications and rearrangement of the amorphous phase. These changes contributed to a reduction in material density and an increase in porosity, which, in turn, facilitated higher biogas production (Table 2) and more efficient biodegradation (Table 3).

3.3. Biological Pretreatment

However, only the samples treated with the cutinase Cut+SP, derived from *Streptomyces scabiei* strain 87.22, exhibited a significant mass loss (approximately 16%). The other two enzymes tested, GD-95RM and GDEst-lip, did not possess significant PLA degradation ability, with mass loss in the enzyme samples not exceeding the mass loss of PCL films in negative control samples. After treatment with Cut+SP, the PLA samples appeared deformed, and large cracks were visible on the material surface. Based on these results, Cut+SP cutinase was selected for further investigation—specifically, for the pretreatment of PLA bags prior to biogas potential test. Images of the PLA bags before and after treatment with Cut+SP are presented in Figure 3.

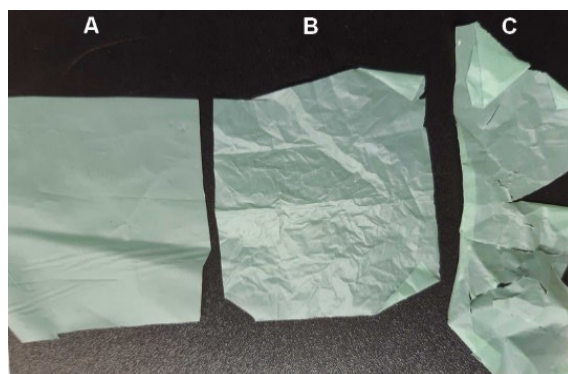


Figure 3. PLA bags before treatment with Cut+SP enzyme (A), control sample in Tris-HCl buffer (50 mM, pH 8) (B) and PLA bags after 72 h exposure at 35 °C exposure with Cut+SP enzyme (0.05 mg/mL) (C).

During biological degradation, microbial enzymes break down polymers into smaller fragments—metabolic monomers. As a result, the bioavailability of the material increases, allowing the degradation products to be readily assimilated across microbial membranes as a source of nutrients and energy, ultimately leading to the formation of CO₂, H₂O, and CH₄. The rate of polymer fragmentation depends on both the nature and the concentration of the enzymes involved. The main enzymes responsible for PLA depolymerization include carboxylesterases, cutinases, lipases, and serine proteases [54,61–64]. It was reported that the weight of PLA films was reduced by more than 5% after 12 h of incubation with triacylglycerol lipase from *Bacillus safensis*, although the degradation rate gradually declined over time. This reduction in PLA degradation efficiency was attributed to the accumulation of lactic acid and its oligomers, which inhibited the catalytic activity of the enzyme [65]. In our study, prolonged incubation of PLA with Cut+SP cutinase was also evaluated, but no further improvement in degradation was observed, likely due to the inhibitory effect of even low concentrations of free lactic acid on Cut+SP activity. In the biological pretreatment with Cut+SP, cutinase demonstrated the weakest performance in terms of biogas production. This result may be related to the low enzyme concentration used, as large-scale enzymatic treatment of plastics remains a significant challenge. Further

optimization is necessary to improve the effectiveness of Cut+SP cutinase as a biocatalyst for PLA degradation at larger scales.

Under these conditions, $8.94 \pm 1.65\%$ weight loss was measured. The negative control sample (Tris-HCl buffer (50 mM, pH 8) underwent $5.73\% \pm 3.04$ weight loss. Due to a large amount of replicates ($n = 12$), degradation catalyzed by Cut+SP can be considered statistically significant ($p = 0.016$), but the effect of the enzyme is insignificant. On average, the solutions containing Cut+SP were only 1.56 times more effective at degrading PLA samples compared to a buffer solution. The images acquired by SEM of treated films also support the results of the weight loss assay. A rough and porous surface was observed on both treated samples (Figure 4A,B). On the other hand, both treated and control (buffer solution) samples had a different surface structure compared to a completely untreated sample (Figure 2A).

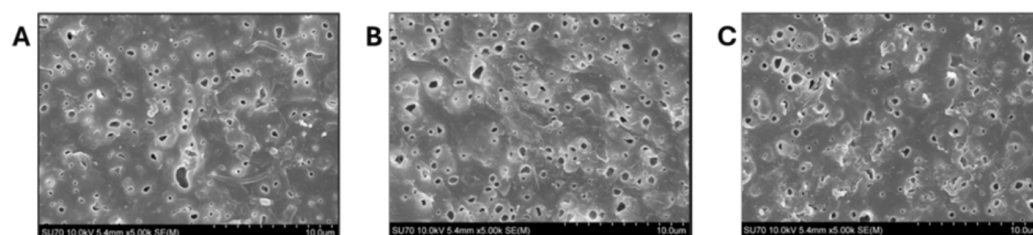


Figure 4. PLA bag treated with Tris-HCl buffer (50 mM, pH 8) (A), PLA bags treated with Cut+SP enzyme (B), and PLA bags after pretreatment with Cut+SP and AD (C).

Although scientific literature does provide information indicating that cutinase is one of the enzymes playing a key role in the breakdown of PLA, in this study, biological pretreatment of PLA with cutinase enzymes resulted in the poorest biogas production (139.20 ± 22.14) (Table 2). The biogas yield was more than 11% lower than that of the control group, and this difference in results was statistically significant at a 95% confidence level ($p < 0.05$). Using the protocol by Holliger et al. [51], it was determined that after 25 days, the complete fermentation endpoint had not been reached, although calculations indicated it was near completion. Daily biogas production during the final days of this study varied from 2.82% to 1.74% of the accumulated biogas volume (Figure 1). After analyzing the biogas quality, it was calculated that the methane yield potential after biological pretreatment reached 38.39 ± 3.97 L CH₄/kg (Table 2). This calculated methane yield is more than 14 times lower than the theoretical yield, which is 557.56 L CH₄/kg at a methane concentration of 56%. Again, although identical yields are not expected, such large discrepancies may have been caused not only by the low degree of PLA degradation (Table 3) but also by the low methane concentration.

The reduction in biogas yield and the low methane concentration may be explained by several mechanisms. The Cut+SP enzyme likely preferentially hydrolyzed the more accessible amorphous regions located at the PLA surface. The removal of these readily degradable regions could have increased the relative proportion of crystalline domains in the remaining material [66,67]. Since crystalline PLA is generally less susceptible to hydrolysis and microbial attack than amorphous PLA, an increase in crystallinity could have reduced substrate accessibility during anaerobic digestion. Another potential mechanism is the formation and accumulation of intermediate degradation products, such as lactic acid oligomers and short-chain hydrolysis products. Depending on their concentration, these compounds may have altered local environmental conditions or inhibited microbial activity, thereby reducing methane production and overall degradation efficiency. Furthermore, it cannot be excluded that enzyme adsorption on the PLA surface altered surface properties and influenced microbial colonization during anaerobic digestion.

The mass change in the biologically pretreated PLA samples after AD was $17.21 \pm 1.30\%$, while in the control sample, it reached $22.40 \pm 1.06\%$ (Table 3). Interestingly, comparison of the SEM images of the control sample after AD (Figure 2B) with those of the biologically pretreated sample after AD (Figure 4C) reveals more pronounced surface deformations in the latter. Specifically, the biologically pretreated sample shows a higher number and density of surface cavities, indicating more extensive surface disruption. However, SEM analysis of the PLA surface before (Figure 4B) and after AD (Figure 4C) revealed no significant morphological changes associated with Cut+SP pretreatment. These differences may be attributed to enzymatic pretreatment, which could have altered the surface and internal structure of PLA or removed the most readily degradable components, thereby inducing localized damage without substantially enhancing the overall biodegradability of the material.

4. Discussion

The rate of PLA degradation, both in terms of the biologically decomposed fraction and weight loss, varied across different studies ranging from no degradation at all [10,55,68] to nearly complete mineralization [10,69]. Although studies differ in various aspects, such as the nature of PLA, processing methods, the use of additives, and so on, a common trend can be observed that PLA is degraded more effectively and easily at higher temperatures. As temperature increases, so does the rate of hydrolysis reactions and microbial activity [70–73]. In the study by Yagi et al. [73], under mesophilic conditions (37 °C), PLA degradation reached only 7% after 90 days, whereas in a related study by Yagi et al. [69], a 1×1 cm PLA film at 55 °C was degraded by 25% in 20 days, 59% in 30 days, and 80–83% in 40 days. Research by Vargas et al. [68] revealed that untreated PLA degraded only under thermophilic conditions (58 °C), with weight loss under anaerobic mesophilic conditions being negligible. Similarly, the study by Vasmara and Marchetti [74] showed that no biodegradation of PLA occurred at 35 °C over a 90-day period, whereas at 55 °C, PLA BMP reached 282 NmL/g VS. Wang et al. [75] found that at particularly high temperatures (80 °C), PLA degraded even faster; within 3 days, 51.8% of 70% PLA film and 65.2% of 100% PLA film had decomposed.

Recently, an increasing number of studies have indicated that temperature ranges between mesophilic and thermophilic digestion (40–50 °C) may enhance biogas production from lignocellulosic biomass [38]. Hupfauf et al. [39] reported that biogas production at 45 °C was 12.8% and 9.6% higher compared to conventional mesophilic (37 °C) and thermophilic (55 °C) conditions, respectively, with system stability attributed to the coexistence of acetoclastic and hydrogenotrophic methanogens. Similarly, Tian et al. [40] investigated six batch reactors operated at 9, 15, 21, 35, 45, and 55 °C, observing the highest biogas yield at 45 °C. This temperature was identified as a transitional point in microbial community structure and metabolic pathways between mesophilic and thermophilic states. Collectively, these findings suggest that anaerobic digestion under semi-thermophilic conditions (40–50 °C) can yield favorable results. Nevertheless, considering the results obtained in the present study for PLA degradation, as well as findings from other researchers who have employed thermophilic conditions for PLA anaerobic digestion, it can be assumed that a temperature regime of 46.3 ± 0.5 °C may be insufficient for efficient biomass conversion. The observed mass reduction of $26.47 \pm 0.61\%$ (in the case of thermal pretreatment) is not adequate to ensure effective and sustainable management of such waste streams. However, this should be regarded as a preliminary assumption, and further research is required, particularly focusing on intermediate temperature ranges between mesophilic and thermophilic regimes.

Another important aspect that must be considered when aiming to recycle PLA in anaerobic systems is its co-digestion with other biodegradable feedstocks. Nachod et al. [76] found that the cumulative CH₄ production from food waste after 34 and 104 days was almost 8 and 2 times higher, respectively, compared to the co-digestion of starch-based PLA pellets with food waste. Maragkaki et al. [77] reported conflicting results, showing that the addition of PLA derived from food waste increased biogas production and had no apparent negative impact on the performance of reactors at both laboratory and pilot scales. In pilot-scale experiments, co-digestion of food waste with PLA increased biomethane production by 8%, and most importantly, PLA had no adverse effect on digester performance. Maragkaki et al. [77] found that mixing PLA with other high-biogas-yielding substrates resulted in a lower biogas yield than when PLA and these substrates were digested separately. In this study, when ethyl acetate was considered not as a chemical pretreatment method but as a separate substrate in a mixture with PLA, the calculated synergistic index (SI = 1.8) indicated a synergistic effect. Therefore, questions still remain regarding the necessity of mixing PLA with other biodegradable waste materials and the methodological considerations involved, such as determining the optimal mixing ratio of feedstocks to achieve comprehensive energy conversion efficiency.

The degradability of complex bioplastic products in an anaerobic reactor can be improved through various pretreatment strategies. Typically, different pretreatment options are explored to facilitate the degradation of biopolymers, but the majority of attention has been given to methods such as acid or alkaline treatment, photodegradation (UV), and cold plasma [16]. In this study, three different PLA pretreatment methods were tested. Considering that both the biogas yield and PLA mass loss in the case of biological treatment, compared to the control group, do not indicate a positive pretreatment effect, and that ethyl acetate should not be regarded as a pretreatment agent but rather as a co-substrate in the mixture due to its high intrinsic biogas yield, a positive pretreatment effect can only be observed in the case of thermal pretreatment. Although biogas production does not directly reflect PLA biodegradation, which was independently quantified in this study through mass loss analysis, it was found that the PLA mass reduction after anaerobic digestion (AD) following thermal pretreatment ($26.47 \pm 0.61\%$ over 25 days) remains insufficient to fully address the challenges associated with the management of bioplastics such as PLA. Therefore, there remains a need to find more effective ways to improve bioplastic degradation with the aim of generating added value, such as biogas. On the other hand, to improve the AD process and PLA degradation, future research could consider combining multiple pretreatment methods. For example, pretreating polyhydroxybutyrate (PHB) and PLA with thermal-alkaline methods has been shown to increase average BMP values by more than 100% [29]. Sourkouni et al. [3] found that, following initial physical treatment of PLA using ultrasound or UV, by composting it with an enzyme cocktail containing commercial alkaline proteases and lipases, degradation of PLA films reached up to a 90% mass reduction at 42 °C.

Although biological pretreatment was not very effective in this study, it has the biggest potential of further improvement. Due to protein-engineering methods, biocatalysts can be modified to become custom “tailored” for very specific processes. Modified mutant enzymes with enhanced polyester biodegradation capabilities are widely described in scientific literature [18,33,78]. In addition, chimeric enzymes with multiple catalytic sites can be synthesized and used in a similar way as the aforementioned enzymatic cocktails [18]. Enzymatic pretreatment of PLA can be optimized using different enzymes, chimeric enzymes, enzyme cocktails or by the modification of current enzymes via protein engineering. While complete degradation of solid PLA particles is still difficult to achieve, future research into and improvement in enzyme properties may provide an avenue for the development of

advanced procedures for PLA degradation and upcycling, utilizing its building blocks for further applications as envisaged by circular-economy principles. These developments are pivotal for the sustainable management of bioplastic waste and the advancement of circular-economy practices.

The BMP test, in this case adapted as a biogas potential test, is one of the most widely used analytical methods for assessing the ultimate biochemical methane potential of a substrate under anaerobic conditions, with a duration typically ranging from 20 to more than 100 days [79]. This study is based on batch biogas potential assays, which are intended to evaluate the intrinsic biodegradation potential of the substrate under controlled laboratory conditions, rather than to simulate full-scale or continuous anaerobic digestion systems. Accordingly, a 25-day digestion period was selected, which, as indicated by the obtained results, was not sufficient to achieve complete biodegradation of PLA under thermal and biological pretreatment conditions. However, the 25-day duration can be considered sufficient, as biogas production almost reached a plateau phase during this period, with the average yield over the last 5 days not exceeding 6 L/kg, indicating that further gas production was minimal. In addition, literature data suggest that after approximately 21 days, BMP results become sufficiently stable and informative, and further prediction is associated with an error of less than 10% [80]. Therefore, although these results can be considered sufficiently reliable for evaluating fermentation dynamics, longer-duration experiments would be more appropriate for a more detailed analysis of the process and a more accurate assessment of kinetic changes.

Undoubtedly, attention must also be given to the issue of further use or disposal of the digestate resulting from biogas production. The presence of bioplastic residues in the digestate can hinder its further application as fertilizer on fields, as this may allow bioplastics to enter and accumulate in the environment. An assessment conducted by Cucina et al. [12] revealed that PLA mixtures showed only a slight reduction in potential environmental release after AD processes due to the slow degradation of these materials under anaerobic conditions. However, the integration of anaerobic digestion with digestate composting emerged as a promising method for bioplastic degradation. This is a noteworthy finding, as combining AD and composting allows for both energy and nutrient recovery from organic waste, enabling improved environmental performance. Therefore, organic recycling coupled with energy recovery is a potentially effective approach to bioplastic waste management, provided that sustainability criteria are met [81].

5. Conclusions

As a sustainable alternative to conventional petroleum-based plastics, bioplastics are becoming increasingly accessible to household consumers, and their ability to biodegrade presents a potential solution to environmental pollution challenges. However, as with conventional plastics, bioplastics also require appropriate end-of-life management. Energy recovery through anaerobic digestion is a promising value-added option within the framework of a circular bioeconomy. Yet, the complex chemical structure of bioplastic polymers significantly affects their biodegradation potential.

Ethyl acetate, used in this study, should not be considered a pretreatment agent but rather a co-substrate, as it was identified as a high-yield feedstock for biogas production (963.55 ± 15.62 L/kg RM). The obtained biogas yields largely reflect the contribution of ethyl acetate rather than the actual effect of pretreatment on PLA. Therefore, although a positive synergistic effect was observed during co-digestion, the results should be interpreted with caution and should not be regarded as a recommendation for its application in anaerobic digestion systems aimed at PLA waste treatment.

Pretreatment of PLA can enhance biogas production efficiency. Therefore, selecting an appropriate pretreatment method is a critical factor in establishing effective end-of-life strategies for bioplastic waste. Among the tested methods, thermal pretreatment yielded the most favorable results in terms of biogas production (279.54 ± 11.29 L/kg RM). Although this method resulted in the highest PLA biodegradation degree ($26.47 \pm 0.61\%$) among all tested conditions, the overall degradation remained insufficient, indicating incomplete substrate conversion under the applied experimental conditions.

Overall, the results demonstrate that the selection of an appropriate pretreatment strategy is one of the key factors in improving PLA anaerobic biodegradation and biogas production. Thus, although PLA is often promoted as a sustainable alternative to conventional plastics, effective end-of-life management of PLA-based products remains an unresolved challenge. This highlights that achieving improved environmental performance requires not only the development of new biopolymers, but also the optimization of end-of-life treatment strategies for existing materials. Further research should therefore focus on developing more efficient pretreatment methods to enhance PLA degradation and support more effective waste management within the framework of a circular bioeconomy.

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