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Composting as a disposal route of PLA materials: kinetics of the aerobic biodegradation

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ABSTRACT

The current challenge for poly(lactic acid)-based materials (PLA) to be widely accepted in composting facilities at their end of life is their biodegradation rate. Aerobic biodegradation of PLA with compost as the inoculum (at the ratios of PLA:wet mass of compost of 1:40 (PLA1); 1:20 (PLA2); 1:10 (PLA3), all in w/w) was investigated (90 d, 58°C) concerning oxygen consumption (OC), rate of OC (r_{OC}) and changes of physical properties based on Fourier-transform infrared/photoacoustic infrared spectroscopy and macroscopic analysis. In 25–30 d, disintegration and biofragmentation of PLA took place. Then, PLA mineralization started. In PLA1 and PLA2, OC reached maximum ca. 1,050 mg O_2/g d.m. after 70 and 90 d, respectively. In PLA3, OC was ca. 680 mg O_2/g d.m. (65% of the final OC in PLA1 and PLA2) which means that 90 d was too short for complete mineralization. During mineralization two phases were distinguished: up to day 50 (r_{OC1}) and then from day 50 to 90 (r_{OC2}). In PLA1, r_{OC1} was ca. 15 mg O_2/g d.m. d, whereas r_{OC2} was 2-times higher. In PLA2, both r_{OC1} and r_{OC2} equaled ca. 15 mg O_2/g d.m. d. In PLA3 OC r_{OC1} was ca. 5 mg O_2/g d.m. d, although r_{OC2} was 3-times higher.

Keywords: Poly(lactic acid)-based materials; Aerobic biodegradation test; Oxygen consumption; Mineralization; Fourier-transform infrared/photoacoustic infrared spectroscopy analysis

1. Introduction

The basic raw material for the production of polymers is crude oil. Polymers produced from this feedstock are durable enough to last for many years, which causes waste to accumulate. Indeed, the amount of waste generated from packaging and disposable products made from conventional plastics is continually increasing. This has led to the establishment of European directives that prioritize the recovery of plastic waste as the first choice in the waste management hierarchy. Consequently, industries are paying increased attention to innovative materials that can be recycled or biodegraded at the end of their life-cycle. This has resulted in a search for new kinds of packaging and disposable products that are biodegradable. Thus, there has been an effort to introduce cost-effective methods for the production of biodegradable polymers derived from renewable sources, often called bio-based products/materials. Polymers that are susceptible to hydrolytic and/or biological degradation to small-molecule products that do not pose a threat to the environment are generally of natural origin and are derived from renewable raw materials. If producing polymers from these kinds of materials were economically competitive with the production

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of non-degradable plastics, then it would an attractive method of reducing the depletion of world oil resources. Moreover, biodegradable polymers is a promising way to reduce the amount of plastic waste disposed of in landfills and composting is considered as the preferred method for their disposal. Many biodegradable polymers that theoretically can be composted have been developed in the last two decades, for example, poly(lactic acid) (PLA), polyhydroxyalkanoate, starch plastics, cellulose esters, bio-polyethylene, bio-poly(ethylene terephthalate), bio-polyamide [1]. However, along with the development of these materials, evaluation of their biodegradation has become essential target for research.

Many studies have investigated the biodegradability of bioplastics in different environments, such as soil, compost, marine and other aquatic environments. Soil and compost have been most extensively investigated, due to their high microbial diversity [2]. It is known that the growth of microorganisms on or inside the surface of the polymers modifies the mechanical, chemical and physical properties of the polymer, and these changes are first observed at the macroscopic level as PLA rolling, breaking, fragmentation, etc. These changes are primarily the result of the shortening of the polymer chain, the length of which affects the properties of the polymers, such as tensile strength, bending or elongation at breakage. At the molecular level, the concentration of functional groups changes during degradation, for example, the amount of carbonyl groups (–(C=O)–). Next, the microorganisms convert polymers to oligomers and monomers. The microorganisms obtain the necessary carbon, energy and nutrients from the fragmentation of polymers and conversion of the polymer carbon to CO_2, water and biomass.

It is known the biodegradation efficiency depends on the composition and characteristics of the polymers. Lemmouchi et al. [3] reported that the disintegration of PLA in composting conditions was enhanced by the presence of plasticizers. Kalita et al. [4] evaluated the aerobic biodegradation of modified PLA. Samples of neat PLA, PLA/5% chitosan (modified) (PLA/chitosan), PLA/CNC (cellulose nanocrystal)-SO_4 (1%) (PLA/CNC), and PLA/5% Gum Arabic (modified) (PLA/Gum) were used to evaluate biodegradation under simulated composting conditions without adding any inoculum and utilizing the thermophilic microflora already present in the compost. According to those authors, biodegradation was confirmed by increased hydrophilicity, decreased transparency, the formation of hydroxyl and carboxyl groups, and increased crystallinity in all the degraded test samples. The percent crystallinity increased over time due to the assimilation of amorphous polymeric chains by microbes. Moreover, chain scission of the high molecular weight chains and converting them into intermediates of low molecular weight chains. The number average molecular weight decreased from 109 to 49 kDa, with the formation of low molecular weight chains of ca. 331 and ca. 82 Da observed on the 80th days of the biodegradation process. Similar behavior was recorded for all the test samples. The authors did not analyze, however, the mineralization process. Sedničková et al. [5] investigated changes in the physical properties of 3 biodegradable plastic materials during composting: pure poly(L-lactide) (PLA), PLA with the plasticizer triacetin (TAC), and a mixture of PLA/polyhydroxybutyrate (PHB) and TAC. The process was conducted at 58°C in compost and the degradation of PLA was determined on the basis of CO_2 evolved. Measurements indicated that the amorphous phase degrades substantially faster than the crystalline phase. Triacetin disappears rather quickly from the samples. In general, the samples maintained their shape for up to 8 d, after which the material disintegrated into a rough powder. However, the last step of biodegradation, in which microorganisms are supplied with the necessary carbon, energy and nutrients from the fragmentation of polymers and the conversion of the carbon in polymer to CO_2, water and biomass last much longer. In the mentioned work [5], the decomposition of the three materials lasted about 100 d and mineralization degree were close around 90% for PLA and PLA + TAC and more than 90% in the case of PLA/PHB + TAC. It means that to completely evaluate the biodegradation of bio-based materials, not only structural changes should be considered, but also mineralization.

During complete biodegradation, PLA carbon is transformed into carbon dioxide; thus, the most common method of monitoring the process is measuring the amount of carbon dioxide released in a closed system. To ensure accurate results, the system needs to maintain the necessary conditions for the proper functioning of the microorganisms (i.e., humidity, temperature, pH, lack of toxic substances). In addition, biodegradation can be monitored by measuring the consumption of oxygen (which is converted to carbon dioxide) in a closed system [6], which is the method that was used in the present study.

The appropriateness of treating of biodegradable bio-based products along with food and garden waste and the preparation of these wastes for organic recycling is debatable as, although, biodegradable plastics will degrade, they do not confirm the time frame in which commercial composting occurs. If the biodegradation rate of PLA and PLA-based products can be accelerated and/or tailored, it could greatly benefit their general use and acceptance in industrial composting facilities. Therefore, there is an urgent need to establish the biodegradation rate of bio-based products.

The aim of this study was to investigate the biodegradation of disposable vessels made of PLA under aerobic conditions using mature compost from sewage sludge and lignocellulosic waste as the inoculum. Three different doses of PLA were used to determine whether PLA content in compost affects the range and rate of its aerobic biodegradation. The following aspects of biodegradation were analyzed: morphological changes (based on microscopic analyses), structural (using Fourier-transform infrared/photoacoustic infrared spectroscopy, FTIR/PAS) and oxygen consumption over time. On the basis of the oxygen consumption data, the rate constant and rate of PLA mineralization were determined.

2. Material and methods

2.1. Inoculum and PLA

As inoculum, mature compost from a composting plant working at a technical scale was used. The feedstock for the compost was sewage sludge with lignocellulosic materials.

The basic characteristics of the compost were as follows: pH 7.95, moisture 66.2%, dry mass (d.m.) 33.8%, organic matter 62.0% d.m. and total nitrogen 2.89% d.m.

Cups (disposable vessels) made from PLA were used in the aerobic biodegradation tests. These cups were shredded to a particle size of 2 mm × 2 mm × 0.5 mm. These small pieces of PLA were mixed with compost.

2.2. Experiment organization

Four variants of the experiment were carried out (all in triplicate; three different doses of PLA in compost samples were used, one variant was a control sample):

- Control sample with compost only (30 g wet mass, ca. 11 g d.m.);
- PLA1 it was 0.75 g PLA and 30 g wet mass of compost, it means the ratio of PLA:wet mass of compost was 1:40 (w/w), PLA:dry mass of compost was ~1:15 (w/w);
- PLA2 it was 1.5 g PLA and 30 g wet mass of compost, it means the ratio of PLA:wet mass of compost was 1:20 (w/w), PLA:dry mass of compost was ~1:7 (w/w);
- PLA3 it was 3.0 g PLA and 30 g wet mass of compost, it means the ratio of PLA:wet mass of compost was 1:10 (w/w), PLA:dry mass of compost was ~1:3.5 (w/w).

The OxiTop® Control system (Germany) was used for the measurement of oxygen consumption during aerobic biodegradation of PLA at 58°C ± 0.5°C in a thermostatic incubator. The starting moisture content in all samples was established as ca. 60%. During the experiment, bioreactors with samples were aerated (passive aeration by unscrewing the head and removing the lid). Moisture was also controlled and corrected to ca. 60%, if necessary, by the addition of water.

Measurements were conducted for 90 d; however, changes in the structure of PLA pieces were observed during the first 50 d of biodegradation. Later, the PLA in the samples was crumbling, and as a result, the microscopic analysis could not be performed.

2.3. Analytical methods

Measurement of oxygen consumption was carried out in the OxiTop® Control system. In this system, pressure is measured in a sealed glass vessel (bioreactor) with a volume of 1 dm^3 equipped with a measuring head. The heads measured and recorded pressure changes in the bioreactors every 4 h. Pressure changes were converted to the amount of oxygen consumed, assuming that the pressure drop is inversely proportional to the biological activity of the compost sample.

During the aerobic biodegradation test moisture and dry mass (after drying at 105°C), pH, organic matter content, total nitrogen in compost were determined according to [7].

FTIR/PAS of the examined PLA samples were recorded by means of Bio-Rad Excalibur FTIR 3000 MX spectrometer (USA) over the 4,000–400 cm^{-1} range at room temperature, resolution 4 cm^{-1}, mirror velocity 2.5 kHz and maximum source aperture, using. MTEC Model 300 photoacoustic cell (USA). Dry helium was used to purge the photoacoustic cell before data collection. The spectra were normalized by computing the ratio of a sample spectrum to the spectrum of a MTEC carbon black standard. PLA sample was placed in a stainless steel cup (diameter 10 mm) and interferograms of 1,024 scans were averaged for the spectrum, providing good signal-to-noise (S/N) ratio. No smoothing functions were used. All spectral measurements were performed at least in triplicate.

In this work, FTIR/PAS spectroscopy has been used as it is a non-destructive technique that, due to its advantages, is increasingly used in the analysis of materials. The most commonly used attenuated total reflectance (ATR) technique suffers from a lack of good contact between the sample and refractive ATR crystal what can lead to non-accurate results (i.e., differences in peaks intensity, depending on the clamping force).

Moreover, changes occurring on the surface of PLA samples were also analyzed using a Leica M205C stereoscopic microscope (Switzerland) with a magnification of 1.25× and 4×, and a polarizing microscope Nikon Eclipse 50i with a magnification of 10× (Japan). Pictures were taken at 0, and also after 15, 25, 35, 45 and 50 d of the process (after this time PLA fragments could not be extracted).

3. Results and discussion

3.1. Changes in PLA structure – microscopic analysis

In the beginning, the PLA pieces were transparent, smooth and hard (Fig. 1). During the primary degradation phase of the aerobic biodegradation test, the PLA lost transparency and changes in its structure became more apparent. It is known that an autocatalytic process usually proceeds when a thick sample is subjected to degradation. It happens because the sample inside becomes more acidic as more carboxylic acid groups with the cleavage of ester bonds are generated and these acidic groups diffuse out of the material very slowly [8–11]. On the contrary, carboxylic acids that are generated on the surface can leave the surface immediately. The degradation behavior of PLA is affected by its semi-crystalline nature. Water that has diffused into the sample first cleaves the ester bonds in the amorphous regions. The crystalline regions start to be attacked by water just after cleavage of most of the ester bonds in the amorphous regions.

After 18 d, individual cracks and fissures had become visible on the surface of PLA pieces (Fig. 1). After 25 d of the process, the cracks had become deeper and more numerous. During the following 10 d, the PLA darkened and these changes in its structure became even more apparent. Between 40–50 d, there had been so much damage to the PLA structure that it had become delaminated. The material was fragile and crumbled when touched. Thus, after 50 d of aerobic biodegradation, the structure of PLA had substantially changed and was severely weakened (Fig. 1). In the next days, samples were to crumbling to analyze microscopically. Different results were obtained by Sedničková et al. [5] during the analysis of the biodegradation of PLA, PLA with the plasticizer TAC, and a mixture of PLA, PHB and TAC. Those authors showed that, during composting, the samples generally maintained their shape for up to 8 d, after which the material disintegrated into a rough powder.

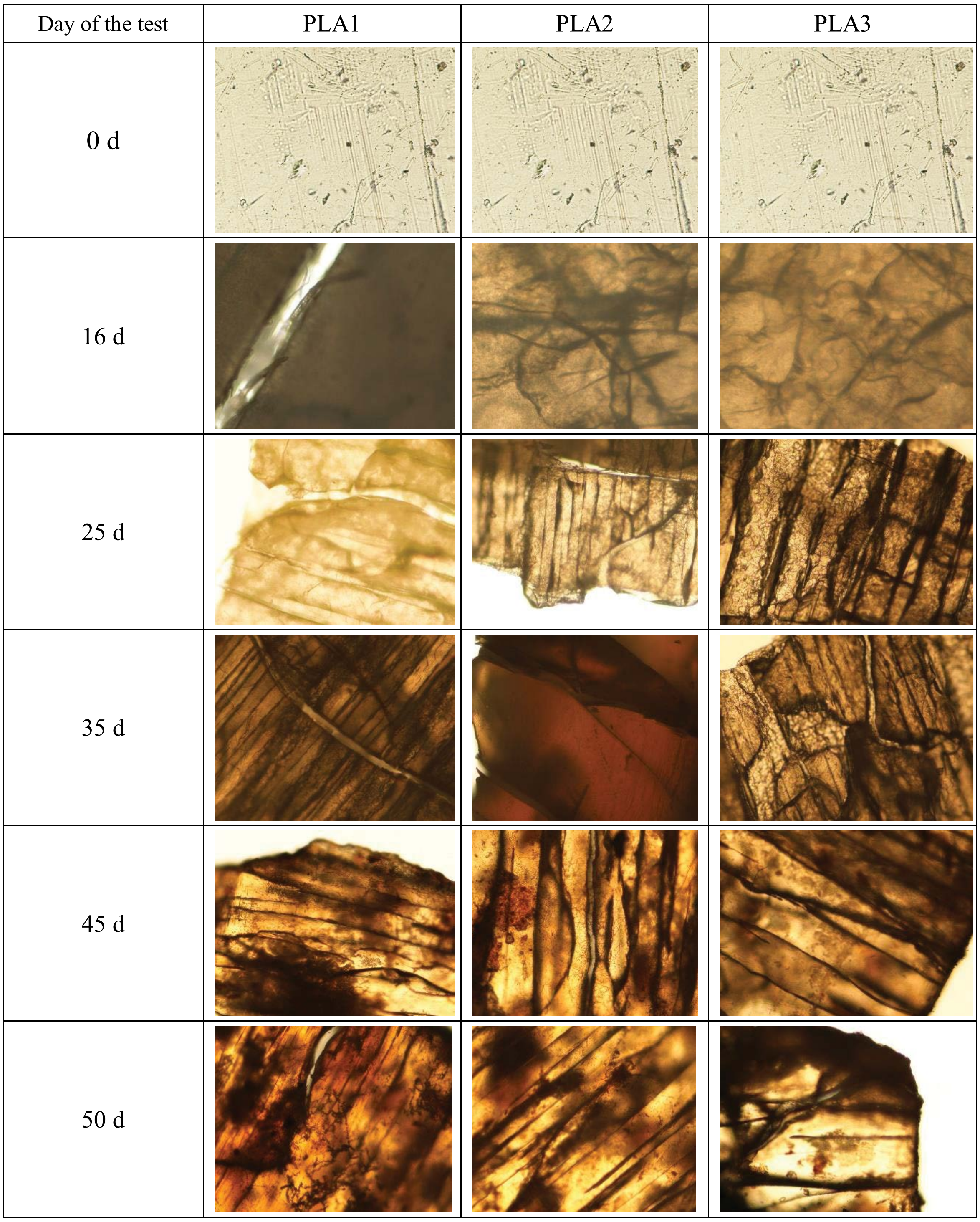

Fig. 1. Microscopic images of PLA structure during aerobic biodegradation (10× magnification).

Arrieta et al. [12] studied the disintegration, under composting conditions, of films based on PLA, PLA-PHB blends. Two different plasticizers, poly(ethylene glycol) (PEG) and acetyl-tri-*n*-butyl citrate (ATBC), were used to limit the inherent brittleness of both biopolymers. Those authors reported that all materials had increased opacity even at 7 d of the composting process. PHB noticeably slowed the rate of PLA disintegration, as formulations with PHB were still visible after 28 d, while those without PHB had completely disintegrated by that time. Disintegration began in the amorphous phase of the polymers, which was attacked by microorganisms mostly in the initial stage of the process. This led to a noticeable loss of transparency in films after treatment. The increase in crystallinity in the materials decreased their degradation rate since the ordered structure in the crystalline fractions slowed the action of the microorganisms. Thus, the addition of a large amount of PHB, which is mostly crystalline, slowed down the disintegration of the PLA matrix. On the other hand, the addition of plasticizers resulted in a clearly increased rate of disintegration.

3.2. Changes in PLA structure - FTIR/PAS analysis

In the FTIR/PAS spectra of the PLA taken for composting ($PLA1_{0d}$, $PLA2_{0d}$ and $PLA3_{0d}$, Figs. 2 and 3), bands characteristic of PLA are visible.

For the purpose of better visibility, all spectra discussed further in this work were divided into two ranges: 3,600–2,400 cm^{-1} and 2,000–600 cm^{-1}. The band at 3,503 cm^{-1} (Fig. 2) indicates the presence of hydrogen-bonded (intra- and intermolecular) –OH groups in PLA. The bands at 2,998 and 2,946 cm^{-1} correspond to the asymmetric and symmetric vibrations of the $–CH_3$ groups, respectively, while the bands at 2,918 and 2,881 cm^{-1} correspond to the asymmetric and symmetric vibrations of $–CH_2–$ groups, respectively.

Asymmetric deformation and scissor vibrations of $–CH_2–$ groups are also visible at 1,455 cm^{-1}, while asymmetric and symmetric deformations of $–CH_3$ groups are visible at 1,384 and 1,366 cm^{-1}, respectively (Fig. 3). The band with a maximum at 1,770 cm^{-1} is typical of asymmetric stretching of the carbonyl C=O group in the amorphous phase of PLA. The weak-intensity band at ~1,720 cm^{-1} could correspond to C=O stretching in the crystalline phase of PLA [13]. The bands at 1,265; 1,212; 1,187; 1,095; 1,046 cm^{-1} are the result of stretching vibrations of the C–O–C and –CH–O– groups. In addition, the spectrum shows bands at 871 cm^{-1} ($O–CH–CH_3$, esters and/or C–C) and at 756 cm^{-1} (rocking vibrations of $–CH_3$ groups). Siracusa et al. [14] and Arrieta et al. [12] assign these bands to the amorphous and crystalline phases of PLA, respectively. The bands at 956 and 921 cm^{-1} are the results of the contributions of both $–CH_3$ rocking and deformation of the carboxylic $–OH \cdots H$ groups [15].

According to Raghuvanshi et al. [16], PLA polymers undergo a three-phase mechanism of degradation. The first stage is random chain scission, in which the molecular weight of the polymer decreases significantly, without appreciable mass loss. The second stage is a decrease in molecular weight accompanied by a rapid loss of mass. In the final stage, soluble monomer products are formed from soluble oligomeric fragments. An increase in the number of –OH groups, therefore, corresponds to the first stage of degradation. Subsequently, as the polymer degrades, it enters into the second stage, where mass loss is rapid. Once a mass loss occurs, a decrease in the number of C=O groups can be observed. This would indicate the start of the second stage of degradation.

During the first 25 d of PLA composting in our study, there are no significant changes in the area corresponding to –OH vibrations (3,500–3,100 cm^{-1}, Fig. 2). However, in the fingerprint region (2,000–600 cm^{-1}), slight changes in the intensity of the C–O–C bands at 1,265–1,095 cm^{-1} appear (cleavage of the ester bonds) in the $PLA1_{25d}$, $PLA2_{25d}$ and $PLA3_{25d}$ spectra. The decrease in the intensity of these bands may be the first indicator of the PLA hydrolytic degradation process.

Significant changes are observed after 35 d in the 2,000–600 cm^{-1} region ($PLA1_{35d}$, $PLA2_{35d}$ and $PLA3_{35d}$ spectra, in the area of C–O and C=O groups vibrations). Such changes in the spectra within the range corresponding to –OH groups (3,500–3,100 cm^{-1}) appear only after 45 d of composting ($PLA1_{45d}$, $PLA2_{45d}$ and $PLA3_{45d}$, Fig. 2), and in spectra from the $PLA1_{50d}$, $PLA2_{50d}$ and $PLA3_{50d}$ samples, those changes are substantial.

These results are in contrast with those of Sedničková et al. [5], who investigated changes in the physical properties of pure PLA, PLA with TAC, and a mixture of PLA, PHB and TAC. They observed that, in spite of the large decrease in molecular weight, the chemical composition did not change substantially. Changes in the PLA spectra appeared after 16 d in the range corresponding to hydroxyl –OH bands (appearance of 3,340 cm^{-1} bands), while other bands from 2,000 to 400 cm^{-1} did not change during the entire time of composting. In their spectra, the most significant change during disintegration was the appearance of a broadband in the 3,300–3,100 cm^{-1} region, which indicated the formation of a biofilm on the sample surfaces. This band appeared in the spectra from all three materials, but it was much more pronounced with samples that contained TAC.

Arrieta et al. [12] analyzed the disintegration of PLA, PLA-PHB blends and mixtures of these polymers with plasticizers (PEG, ATBC). In contrast to Sedničková et al. [5], they observed a band around 1,600 cm^{-1}, corresponding to the formation of carboxylate ions (COO^-) by the action of microorganisms that consume lactic acid and PLA oligomers on the film surface and leave carboxylate ions at the chain ends. This band was particularly noticeable in PLA-ATBC films after 21 d of composting. The band appeared after 7 d in PLA and plasticized PLA samples, and after 14 d in plasticized PLA/PHB films, indicating that PHB helps to slow down the PLA disintegration rate under composting conditions. In our study, the band corresponding to the formation of carboxylate ions appears at ~1,608 cm^{-1} after 35 d of composting and is present in all spectra from PLA samples ($PLA1_{35d}$, $PLA2_{35d}$ and $PLA3_{35d}$). At the same time, the intensity of the band at 1,770 cm^{-1} (C=O in the amorphous phase of PLA) noticeably decreased, while the intensity of the weak shoulder at ~1,720 cm^{-1} (C=O in the crystalline phase) remained practically the same. The next indicator of the PLA degradation process in our study is the appearance of carboxylates and carboxylic acids replacing the ester groups in the main polymeric chain of PLA. In addition, some bands corresponding to methyl groups ($–CH_3$) and/or C–O linkages (1,455; 1,384; 1,366; 1,265 cm^{-1}) are less intense in degraded samples than in non-degraded ones. Moreover, these results indicate that degradation occurred mainly in the amorphous phase of PLA. The appearance of hydroxyl groups (broad bands at 3,300 and 1,640 cm^{-1}) in the spectra after 45 d of composting indicates further degradation and a significant change in the hydrophilicity of PLA. The strong bands at ~3,279 and 1,546 cm^{-1} and the weak band at 3,082 cm^{-1} indicate the appearance of amide II [15], which may indicate the presence of microorganisms on the PLA surface. This band has the highest intensity in the $PLA1_{50d}$,

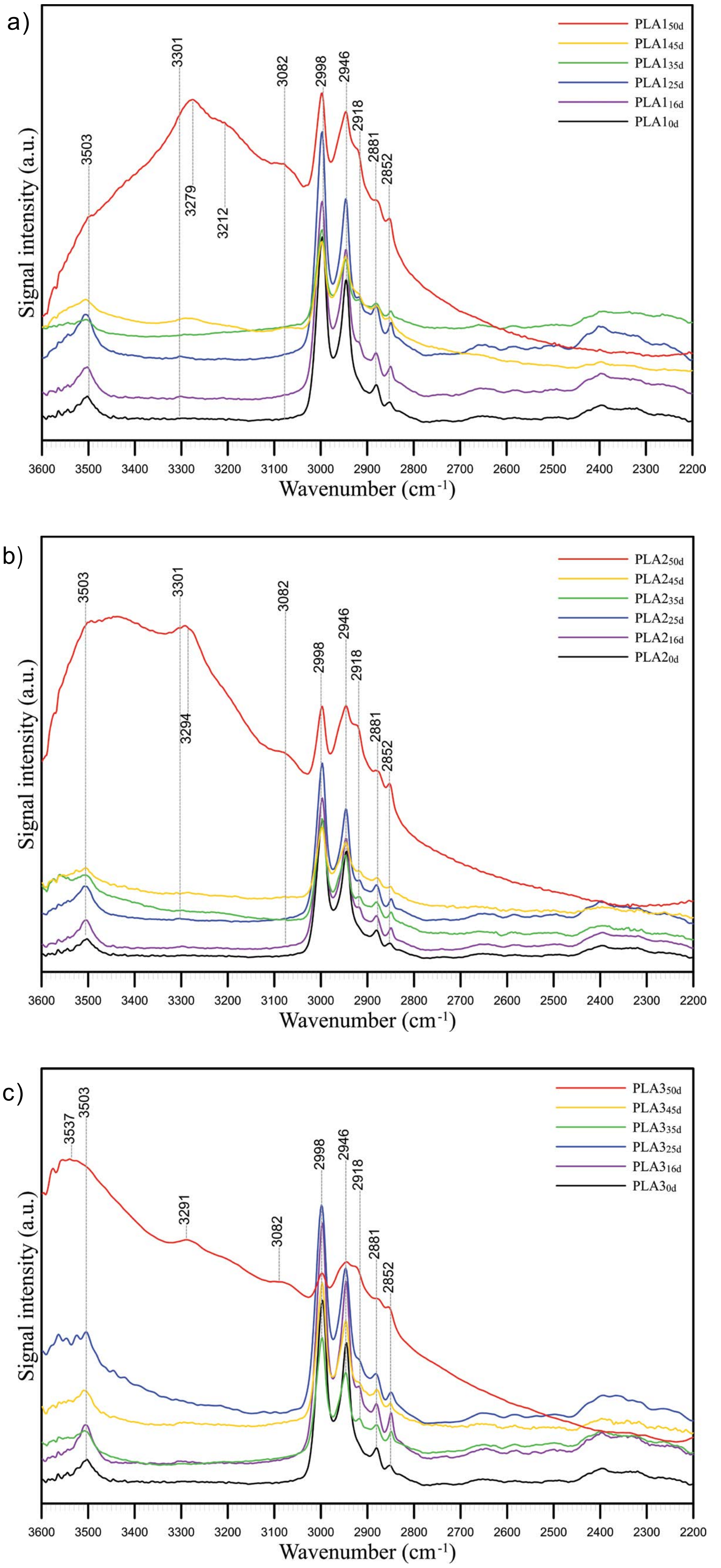

Fig. 2. FTIR/PAS spectra of PLA samples in the ranges of 3,600–2,200 cm^{-1} (a) PLA1, (b) PLA2 and (c) PLA3.

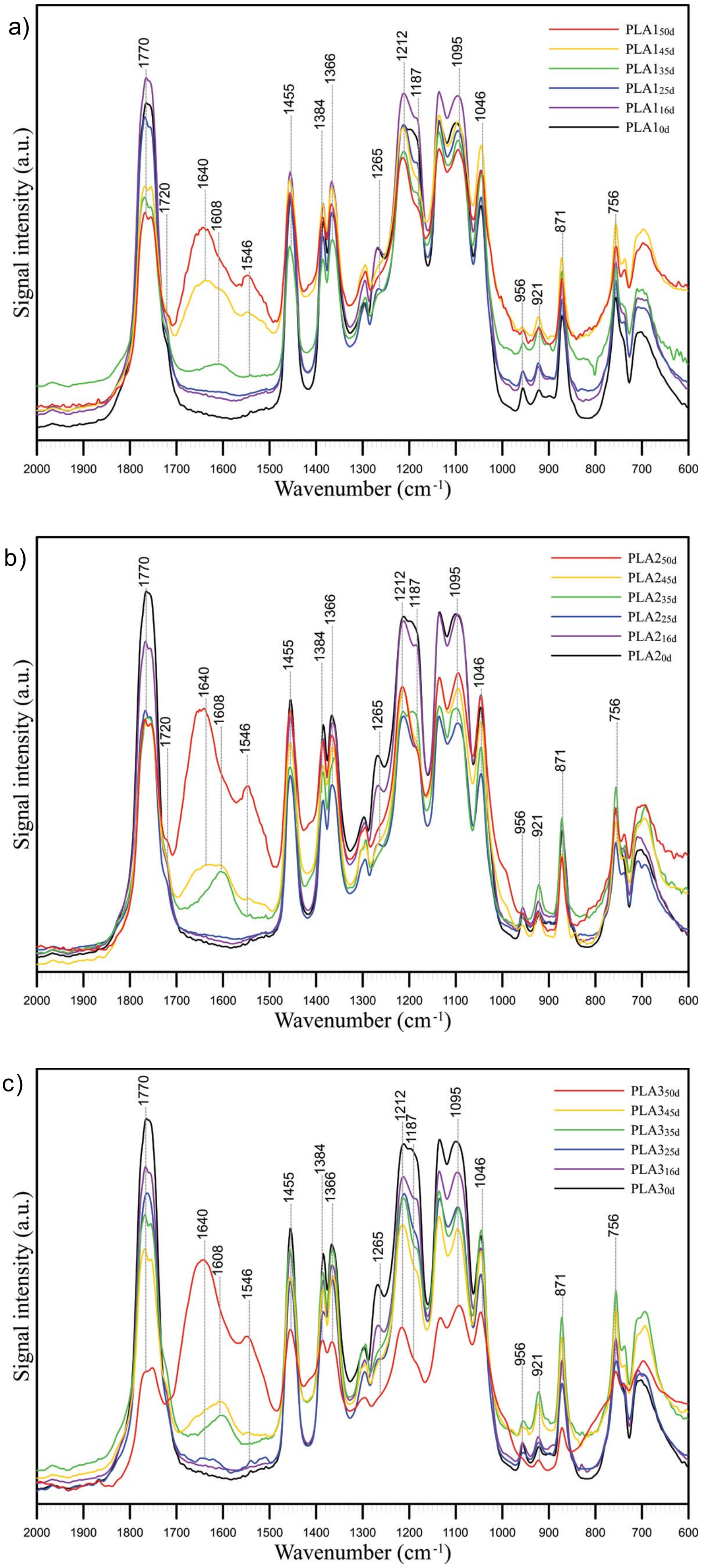

Fig. 3. FTIR/PAS spectra of PLA1 samples in the ranges of 2,000–600 cm^{-1} (a) PLA1, (b) PLA2 and (c) PLA3.

$PLA2_{50d}$ and $PLA3_{50d}$ spectra. Recording PLA spectra after more than 50 d of composting were impossible because PLA particles could not be visually detected in all samples.

3.3. Oxygen consumption

During the primary phase of the aerobic biodegradation test (from 0 to 25–28 d), oxygen consumption (OC) in samples PLA1, PLA2 and PLA3 remained the same level as in the compost alone (control sample) (as shown by the raw results expressed as pressure changes; then these results were converted into OC) (Figs. 4 and 5). This primary phase was slightly shorter for PLA1, which had the lowest content of PLA in compost, and was somewhat longer when the content of PLA in compost was increased. At the end of the primary phase, oxygen consumption in the compost (control

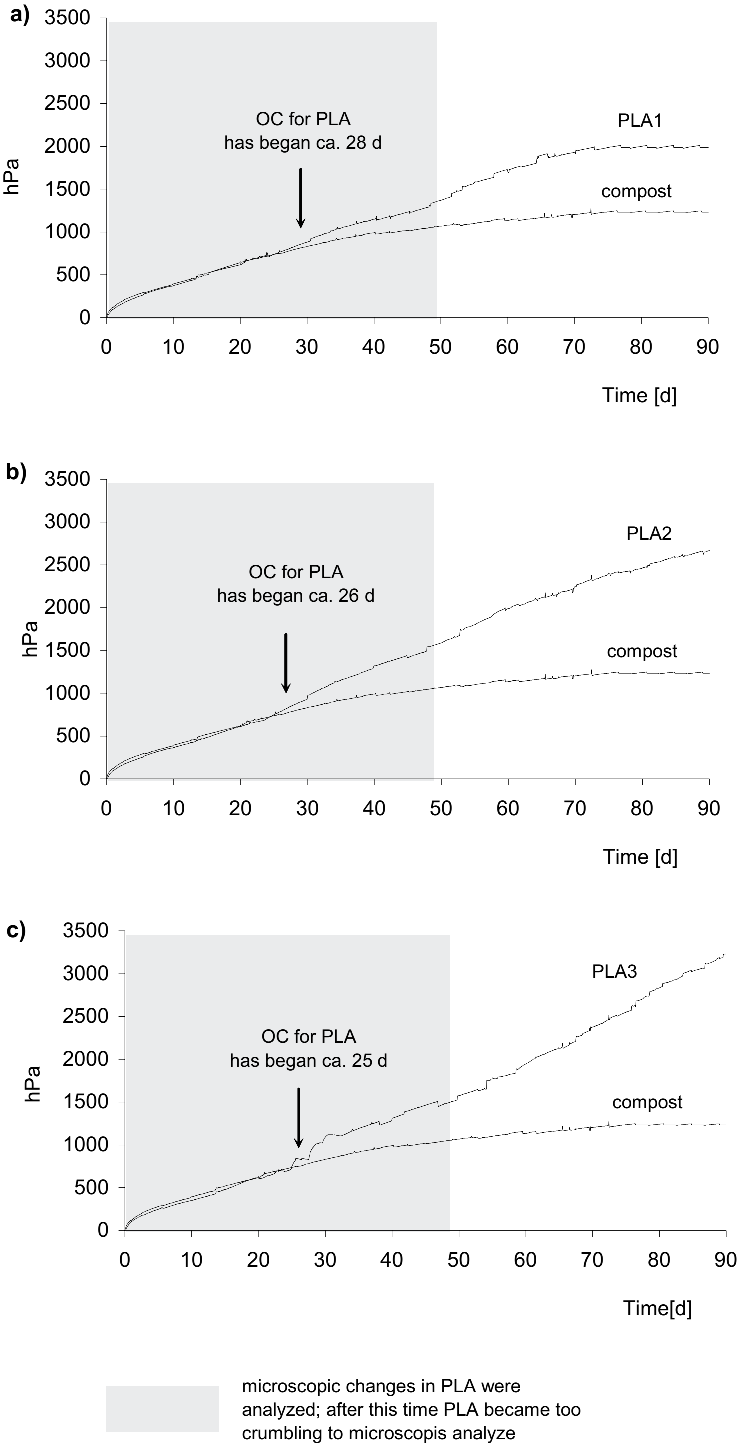

Fig. 4. Pressure changes in PLA samples resulting from oxygen consumption (a) PLA1, (b) PLA2 and (c) PLA3.

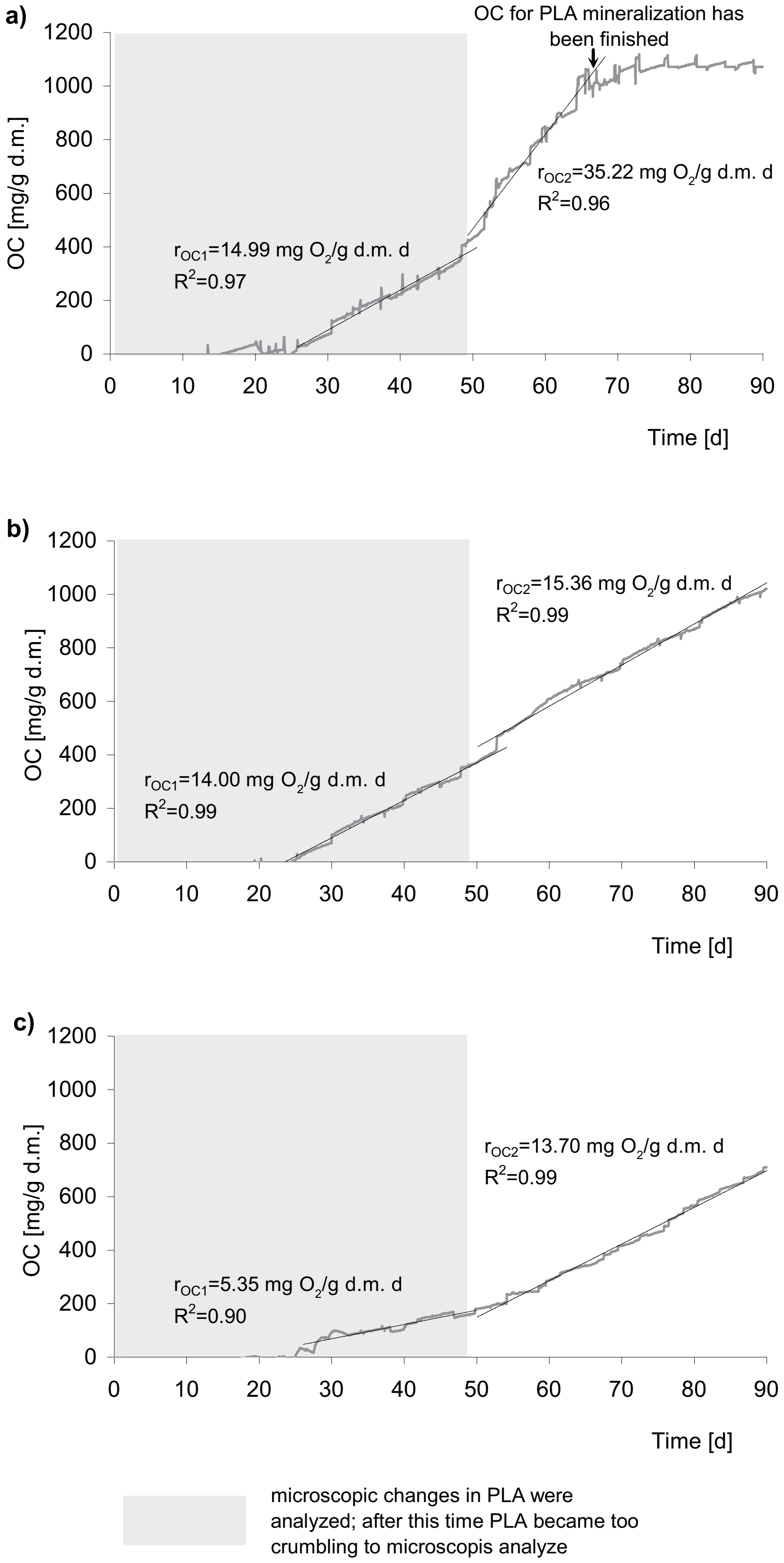

Fig. 5. Oxygen consumption in PLA samples during mineralization (a) PLA1, (b) PLA2 and (c) PLA3.

samples) was 31 ± 1.4 mg O_2/g d.m. and was the same for all PLA samples (that was inoculated with compost). It means that there was no oxygen consumption for PLA.

At the same time, based on microscopic analyses (Fig. 1), changes in the PLA surface were found and FTIR analyses showed slight changes in the content of functional groups. This means that, during the primary phase of the aerobic biodegradation test, PLA underwent chemical hydrolysis, which is both temperature- and humidity-dependent and probably does not involve the action of any microorganisms. H_2O attacks C(C=O), forming carboxyl groups and hydroxy groups. This hydrolytic degradation process paves the way for the degradation of PLA by the formation of intermediates of lower molecular weight. These intermediates can be assimilated by microorganisms for further biodegradation and conversion into CO_2, water and energy [17]. Other authors have also stated that surface erosion of samples occurs due to hydrolytic degradation prior to biodegradation, leading to the formation of intermediates for assimilation by microorganisms and subsequent conversion into CO_2 and water [4].

Taken together, the same OC in control samples and in the PLA samples, the macroscopic changes in PLA samples, and the results of the FTIR analysis clearly indicate that, in the first days of the aerobic biodegradation test, only disintegration and biofragmentation took place.

After the primary phase, that is, from day 30 to day 90 of biodegradation measurements, the pressure in samples PLA1, PLA2 and PLA3 increased faster than that in the control samples (compost). During this time, oxygen consumption in the control samples increased slowly to 90 ± 2.6 mg O_2/g d.m. (Fig. 5).

The values of pressure in the control samples between 30 and 90 d of the biodegradation test were subtracted from the total pressure in the PLA samples. The extra pressure in the PLA samples was converted into OC (mg O_2), and then divided by the content of d.m. of PLA. The increase in OC in the PLA samples after the primary phase indicated that PLA mineralization began on about the 30th day of the experiment.

As mentioned, between days 40–50 of the aerobic biodegradation test, the PLA structure was damaged, and the PLA pieces in the compost in PLA1–PLA3 were crumbling and almost invisible to the naked eye (Fig. 5). After this time, in PLA1, OC was 420 ± 11.5 mg O_2/g d.m. and then OC intensified. Interestingly, in PLA1, which had the lowest content of PLA (PLA:wet mass of compost 1:40, w/w), OC reached its maximum value between 60 to 70 d and then remained at the same level until the end of the experiment (1,060 ± 11.5 mg O_2/g d.m.).

Kinetic constants (the rate constant of oxygen consumption, k_{OC}, and the rate of oxygen consumption, r_{OC}) and of oxygen consumption was calculated from the zero-order kinetic equation. In zero-order kinetic k_{OC} is equal r_{OC}.

On the basis of the rate of OC, two phases of mineralization were distinguished: up to day 50, and then from day 50 to 90, when PLA was not visible under macroscopic analysis. In all PLA samples, the rate of oxygen consumption (r_{OC}) in these two phases was determined. In PLA1, the first phase, r_{OC1} equal ca. 15 mg O_2/g d.m. d, whereas in the second phase, r_{OC2} was 2-times higher, ca. 35 mg O_2/g d.m. d.

In contrast, OC did not plateau in PLA2 and PLA3, in PLA:wet mass of compost were 1:20 and 1:10 (w/w), respectively. Instead, OC increased constantly until the end of the test. However, similarly to PLA1, two phases of OC were also distinguished in PLA2 and PLA3 on the basis of the rate of OC.

In the first phase in PLA2, r_{OC1} (ca. 14 mg O_2/g d.m. d) was similar to r_{OC1} in PLA1. In the second phase in PLA2, r_{OC2} (ca. 15 mg O_2/g d.m. d) was almost the same as r_{OC1} in this variant. After the first phase, OC in PLA2 (418 ± 7.5 mg O_2/g d.m.) was similar to that in PLA1, after which, OC continued to increase, reaching 1,043 ± 7.5 mg O_2/g d.m. after 90 d of biodegradation. This value was only slightly lower than the maximum value in PLA1.

Oxygen consumption proceeded differently in PLA3 than in the other two variants. In the first phase, r_{OC1} was only ca. 5 mg O_2/g d.m. d, although in the second phase, it was almost 3-times higher, ca. 14 mg O_2/g d.m. d. As a result of the slow rate of oxygen consumption in the first phase, after 50 d OC (ca. 200 mg O_2/g d.m.) was more than 2-times lower in PLA3 than in PLA and PLA. After 90 d of biodegradation, OC in PLA3 reached 679 ± 8.2 mg O_2/g d.m., which was ca. 65% of the final OC in PLA1 and PLA2 (Fig. 5).

As mentioned above, in the first days of the experiment only disintegration and biofragmentation of PLA took place. PLA mineralization began on about the 30th day of the experiment. In PLA1, OC reached a maximum between 60 and 70 d, indicating that mineralization was complete. In PLA2, the same maximal OC (expressed as mg O_2/g d.m.) was achieved after 90 d. In PLA3, however, OC after 90 d had reached only 65% of the value that it reached in the other two variants, which indicates that 90 d was not long enough for complete mineralization to take place in PLA3. Thus, increased PLA content lengthened the time needed for complete mineralization. Moreover, 3-times lower r_{OC1} in the first phase in PLA3 in comparison to r_{OC1} in PLA1 and PLA2 could be caused by inhibition at the beginning of the mineralization of PLA. For example, Ghorpade et al. [18] reported inhibition during composting of extruded PLA with yard waste (mixtures containing 0%, 10%, or 30% PLA; dry weight basis). Those authors stated that, after the first week, significantly greater amounts of CO_2 were generated in vessels with 10% or 30% PLA than in the control vessel (0% PLA). At the end of the process, however, CO_2 emission did not differ significantly between 10% and 30% PLA mixtures. According to the authors, the most likely explanation is that, in the 30% PLA mixture, substantial chemical hydrolysis and lactic acid generation lowered the compost pH. The lowered pH likely suppressed microbial activity, thus explaining the lack of difference in carbon dioxide emissions between 10% and 30% PLA mixtures. They concluded that PLA can be efficiently composted when added in small amounts.

Other authors have also reported that for PLA degradation a long time is necessary. For example, Sedničková et al. [5] 100 d. The time required for PLA degradation means that one of the main obstacles to PLA-based materials becoming widely accepted in composting facilities at their end-of-life is that PLA usually degrades more slowly than other organic wastes commonly used in composting. For this reason, some authors have investigated methods of accelerating the biodegradation of compostable plastics. Increasing the biodegradation rate of PLA should facilitate its disposal via composting since PLA based products and organic materials could biodegrade in a similar period of time. For example, Castro-Aguirre et al. [19] evaluated the effect of bioaugmentation with *Geobacillus* on the biodegradation of PLA and PLA bio-nanocomposites (that is, PLA with 5% organo-modified montmorillonite (OMMT) (PLA-OMMT5), and PLA with 0.4% organo-modifier, methyl, tallow, bis(2-hydroxyethyl), quaternary ammonium (QAC) (PLA- QAC0.4) films) in simulated composting conditions.

They showed that the compost alone and the compost with *Geobacillus* did not produce significantly different amounts of CO_2. However, when an additional source of carbon was introduced (PLA), there was a significantly higher production of CO_2 in the presence of *Geobacillus* during the early part of the test (<25 d). Those authors attributed this behavior to the synergistic effect of the actions of *Geobacillus* along with those of the other microbial strains present in the compost. The lag time was slightly reduced when *Geobacillus* was present, meaning that the biodegradation phase started earlier than in the samples without *Geobacillus*. Moreover, the lag time was shorter with PLA-OMMT5 film than with PLA, suggesting that polymer modification may facilitate colonization and the resulting degradation of polymers. Stloukal et al. [20] also observed a lag phase when composting high-molecular-weight PLA in nanocomposite films containing various commercially available native or OMMT. Moreover, the authors reported that the lag phase was evident with pure PLA and its mixture with native OMMT. Stloukal et al. [20] and Husárová et al. [21] suggested that the limiting step in the biodegradation process is abiotic hydrolysis. For example, Husárová et al. [21] analyzed the biodegradation of four PLA samples with molecular weights (MW) ranging from approximately 34–160 kg mol^{-1}. As the MW of the polymer increased, the biodegradation rate decreased, and the initial retardation was discernible. The addition of a limited amount of low MW PLA did not accelerate the biodegradation of high MW PLA, suggesting that the process is not limited to the number of specific degraders and/or the induction of specific enzymes. Moreover, the courses of abiotic hydrolysis were quasi–identical during biodegradation of all four PLA samples. This suggests that abiotic hydrolysis is a rate-limiting step in the biodegradation process and the organisms present are not able to significantly accelerate depolymerization by the action of their enzymes.

4. Conclusions

The results of this study indicate that, in the first 25–30 d of PLA biodegradation, only disintegration and biofragmentation took place. PLA mineralization occurred in the latter part of the experiment. The time needed for PLA mineralization depended on PLA content in the compost. It required 70–90 d for PLA mineralization in PLA:wet mass of compost of 1:40 and 1:20 (w/w), respectively. However, in PLA:wet mass of compost of 1:10 (w/w), 90 d was not enough time for complete mineralization of PLA. It must be emphasized that, during the entire time of the experiment, the temperature was 58°C, which is a standard temperature in tests of PLA biodegradation. Thus, intensive composting at a technical scale, which usually lasts 2–4 weeks, may not be long enough for PLA mineralization. However, taking into account that, during technical composting, the temperature changes from mesophilic to thermophilic (reaching temperatures above even 70°C) and back to mesophilic, biodegradation of PLA should be tested and conducted with typical temperature profiles for composting.

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