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Anaerobic biodegradation of disposable PLA-based products: Assessing the correlation with physical, chemical and microstructural properties

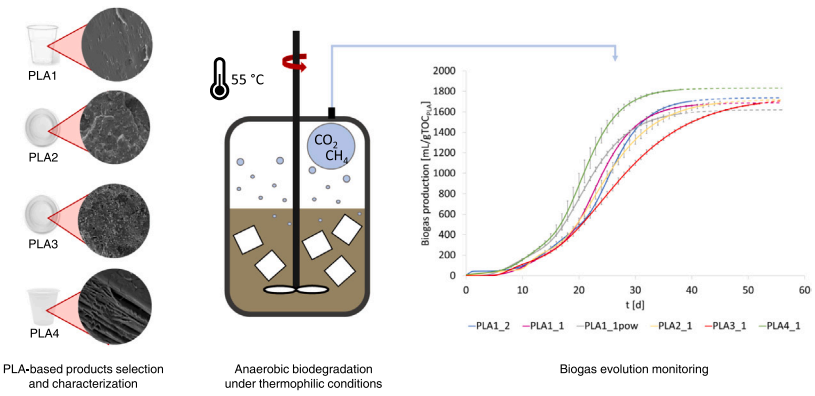
Maria Paola Bracciale [a], Giorgia De Gioannis [b], Marica Falzarano [c,*], Aldo Muntoni [b], Alessandra Polettini [c], Raffaella Pomi [c], Andreina Rossi [c], Fabrizio Sarasini [a], Jacopo Tirillò [a], Tatiana Zonfa [c]

[a] DICMA Department, Sapienza University of Rome, Via Eudossiana 18, 00184 Roma, Italy
[b] DICAAR Department, University of Cagliari, Piazza D'Armi 1, 09123 Cagliari, Italy
[c] DICEA Department, Sapienza University of Rome, Via Eudossiana 18, 00184 Roma, Italy

HIGHLIGHTS

- Thermophilic anaerobic biodegradation of commercial bioplastic products was studied.
- Similar degrees of biodegradation (86–100%) were attained in all tests.
- Product type, material size and digestion conditions influence the biodegradation rate.
- Prolonged durations (31–52 d) were required to attain 95% of maximum biogas production.
- Correlations among degradation parameters and products composition were derived.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study commercial Polylactic Acid-based disposable cups and plates were selected for lab scale anaerobic degradability tests. The experiments were carried out under thermophilic conditions at different inoculum to substrate ratios and test material sizes, and the specific biogas production and associated kinetics were evaluated. Maximum biogas production was comparable for almost all the experimental runs (1620 and 1830 NmL/gTOC$_{PLA}$) and a biodegradation degree in the range 86–100% was attained. Moreover, physical, chemical and microscopical analyses were used to characterize the tested materials before and after the degradation. The products composition was assessed and the presence of some additives (mainly Ca-based) was detected. Potential correlations among the process parameters and product composition were derived and a delay in process kinetics with increasing amount of additives embedded in the polymeric matrix was observed, confirming the relevant influence of the chemical blend on the biodegradation process.

* Corresponding author.
E-mail address: marica.falzarano@uniroma1.it (M. Falzarano).

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1. Introduction

Conventional fossil-based plastics are currently under scrutiny due to their acknowledged adverse environmental impacts, and in particular single-use items have been targeted for reduction as they are litter-prone products. At the European level, some measures such as banning of plastic cups and containers by 2028 have been implemented [1], which indirectly resulted in the research of a substitutive material for these applications.

Biodegradable polymers have received considerable attention over the past two decades, being regarded as a sustainable alternative to conventional plastics, since once discarded they can be managed through aerobic/anaerobic biological processes as a means of materials and energy recovery, or as a stabilization treatment before final disposal [2]. These materials belong to the wider family of biopolymers, which includes biodegradable polymers that are both bio-based (e.g. starch, polylactic acid (PLA)) and fossil-based (e.g. polycaprolactone (PCL), polybutylene succinate (PBS)), as well as bio-based and non biodegradable polymers (bio-ethylene, bio-polyethylene (bio-PE), bio-propylene (bio-PP), and bio-polyethylene terephthalate (bio-PET)). The biopolymer market has been constantly growing over the last decades, with a global production of 2.22 Mt in 2022 [3].

The industrial biodegradable plastics sector has developed a variety of biopolymer formulations with different chemical structures, technical properties, and potential applications. Among these, polylactic acid (PLA) is one of the most popular and widespread biopolymers that is commonly derived from direct polycondensation of lactic acid or via ring opening polymerization of lactide [4], and belongs to the category of bio-based and biodegradable plastics. PLA is already being employed for many different applications, and in particular represents a good alternative to plastic packaging and disposable items, such as tableware [5]. Given its brittle behaviour, PLA is often blended with either fossil- or bio-based polymers [6] and additives of different nature (e.g. natural and synthetic fibers [wood/plant fibers, glass fibers, cellulose etc.], inorganic fillers [TiO_2, talc, gypsum, $CaCO_3$, clay minerals etc.]) [7] to improve its mechanical and physical properties. In particular, additives can be used for nucleation purposes to improve the crystallization of the polymer [8], which plays an important role in improving the material performance but may also affect the biodegradation behaviour, since the degradation of crystalline PLA is usually slower than for the amorphous form [9]. Depending on the nature and amount of additives used, the final PLA blends may have very different chemical compositions and physical properties that could in turn influence the degradation mechanisms. Moreover, it has been widely demonstrated that the specific environmental conditions the material is subjected to (e.g., aerobic/anaerobic, natural soils, surface water, marine water) are also concurring in dictating its actual biodegradation profile [10,11], with even notably different degradation rates depending on both specific abiotic and biotic factors. The correlation among the material composition, the environmental conditions as well as the kinetics and yield of the biodegradation process, together with the assessment of the final products of the biodegradation pathways, are some of the aspects that need deeper understanding, along with the environmental implications of the extensive use of biodegradable plastics [12]. Currently, biodegradable plastics are mostly designed to be degraded under aerobic conditions, which explains the fact that the scientific literature on this topic has mainly focused on evaluating their aerobic degradability. As far as PLA is concerned, most of the studies available in the literature have also considered pure PLA or ad-hoc synthesized PLA blends [13].

The investigation of the anaerobic biodegradation of commercial bioplastic products, including PLA, has moved its first steps only very recently and is therefore still far away from having attained conclusive and indisputable outcomes. In general, it has been shown that mesophilic anaerobic digestion is considerably less efficient compared to the thermophilic process in degrading various types of biodegradable plastics. In particular, thermophilic conditions are recognized to be able to enhance the kinetics of the biodegradation process, since high temperatures can promote the hydrolysis phase [14] and can also affect the physical properties of the polymer thereby making it more susceptible to microbial attack. Some PLA-based (PLA ≈ 80%) laboratory blends were tested by Narancic and colleagues both under mesophilic (35 °C) and thermophilic (55 °C) conditions and biodegradation (84 – 104%) was observed only in the second case [15]. Disposable PLA-based cups were tested by Vasmara and colleagues [16], who observed no degradation at 35 °C and 56% degradation at 55 °C after 90 days; on the other hand, PLA sheets tested both at 38 °C and 58 °C reached 75% degradation in 500 and 100 days, respectively [17]. However, PLA cups tested by other authors reached 66% conversion at 37 °C in 280 days [18] and 96% conversion at 55 °C in 100 days [19]; other thermophilic (55 °C) experiments on PLA-based cutlery and dishes showed 58.4% and 78.3% in 90 days [20], while no degradation was observed at 55 °C for some coffee capsules and packaging in another study [21].

The data reported above suggest that multiple factors concur in dictating the biodegradation process of bioplastic products. Factors that have been overlooked in the scientific literature so far include the specific composition of the bioplastic blend, the specific polymer characteristics as well as the shape and size of the material tested, the effect of which may be even more prominent than the anaerobic degradation temperature. To this regard, only a few studies have focused on PLA products tested in different forms. In particular, Zhang and co-workers [22] tested PLA items in the form of both film and pellets at 37 °C, reaching 18.8% and 2.6% biodegradation (evaluated in terms of substrate conversion into methane), respectively, in 65 days. The crystallinity grade of the polymer was taken into account in another study [23] that investigated semi-crystalline and amorphous PLA in the form of pellets subjected to mesophilic (35 °C) anaerobic digestion for 170 days. Biogas production resulting from PLA biodegradation was only detected for the amorphous PLA product, which reached a biodegradation yield of 40%.

Efforts to enhance the degree of biodegradation and reduce the required treatment duration have been made by a number of researchers, who investigated the effects of mechanical, thermal or thermochemical pre-treatments. For example, material pulverization was reported to be effective in enhancing the kinetics of the process, reducing by 50% the time required to reach 50% of biodegradation when PLA pellets were grounded to 0.05–0.3 mm [24]. The exposure of the test material at 120 °C for 3 h was found to have an effect on the elimination of the lag phase [25], while in another research maintaining the material at 150 °C for 6 h allowed to obtain 75% degradation compared to 3% of untreated material [24]. Lag phase reduction and enhancement of methane production were also obtained with thermochemical pre-treatments. Benn and Zitomer tested a PLA cup after applying a thermo-alkaline treatment (48 h at 90 °C and pH = 12) and reached a methane production of 86 L/kgThOD, while the untreated material only produced 1 L/kgThOD [26]. Cazaudehore and colleagues enhanced PLA biodegradation from 4% to 65–73% in 30 days by applying a pretreatment of 48 h at 90 °C and 70 °C with different concentrations of $Ca(OH)_2$ (2.5 – 5%). The use of a chemical pre-treatment (48 h at pH = 2 and pH = 12) on PLA commercial items was reported to be ineffective [27].

Investigating the correlations between the product chemical characteristics and the degradation yield and kinetics is a key to understanding how to handle bioplastic waste correctly. To this regard, only few studies have used advanced chemical characterization techniques to describe the changes in the physical properties and chemical structure of biodegradable plastics during the anaerobic biodegradation process. Some of the analyses used to track the progress of degradation on the polymers are Fourier-transform infrared (FT-IR) spectroscopy [18], Scanning Electron Microscopy (SEM) [28,29] and Differential Scanning Calorimetry (DSC) [19], but a methodological approach in this way is still lacking.

While the number of studies on the anaerobic degradability of

biopolymers has increased considerably over the past two years, a systematic description of the biodegradation profile of different commercial bioplastic products and the associated by-products is still missing. In the present study, we aimed to contribute to filling in the existing knowledge gaps related to the anaerobic degradability of commercial disposable PLA-based items by combining different physical, chemical and microscopical testing methods to provide a thorough assessment of the anaerobic biodegradation of the materials of concern and identify potential correlations among the main process parameters. The findings of this study are deemed useful with a view to identifying the appropriate operating conditions for the anaerobic processing of PLA-based bioplastics and assessing the potential of such a process for materials and energy recovery from bioplastic-based residues.

2. Materials and methods

2.1. Feedstock materials

Disposable PLA tableware items were selected among commercially available products compliant with the EN 13432 standard [30]. Four different materials (two cups and two plates) from different industrial manufactures were selected for the study. The samples were manually cut into 1.5-cm side squares, leaving out the edges and the bottom of cups to ensure test material homogeneity. In addition, PLA1 was powdered prior to the degradation tests to a final particle size below 1 mm using MF 10 basic Microfine grinder drive coupled with MF 10.1 cutting-grinding head (IKA). Pulverization was obtained through mechanical grinding using dry ice to prevent material softening and particle sticking effects. PLA2 and PLA3 were the same type of plate produced by the same manufacturer but were found to have different chemical structures and blend compositions and were therefore dealt with as different samples.

Anaerobic sludge was used as the inoculum for the anaerobic degradation tests. The sludge was collected at a full-scale anaerobic digestion plant treating a mixture of organic residues from food industries and operating under mesophilic conditions. The main characteristics of the sludge are shown in Table 1. The sludge was sieved at 0.84 mm to remove the coarser fraction (mainly composed of fibers and inorganic particles) and stored at − 15 °C until use. It was then defrosted at room conditions before the experimental trials and used directly without any preliminary acclimation phase.

Calcium lactate (LACT) was used as a positive control material to assess the validity of the experiment (for the sake of reference, according to ISO 14853 [31] a test is valid if the reference material biodegradation exceeds 70% within 60 days). The main characteristics of the feedstock materials are reported in Table 1.

2.2. Analytical methods

The products thickness was measured prior to the degradation process using an object micrometer.

Table 1
Main characterization parameters of substrates and inoculum in terms of total solids (TS), volatile solids (VS), total organic carbon (TOC) and thickness.

Material	Type of item	TS [% ww]	VS [% ww]	TOC [gC/kg]	Thickness [μm]
Inoculum	—	6.4	4.2	29.1± 2.4	—
PLA1	Cup	99.6	99.6	530 ± 1.4	205
PLA2	Plate	99.6	97	484.4 ± 0.2	257
PLA3	Plate	99.7	83.6	434.5 ± 0.1	257
PLA4	Cup	99.6	99.4	494.1	133
Calcium lactate (LACT)	—	77.5	42.3	317.9	—

The morphological analysis of bioplastic items was performed by SEM (Mira3, Tescan) equipped with energy dispersive spectroscopy (EDS) (Octane Elect, EDAX).

DSC analysis was carried out with a Netzsch DSC 214 Polyma apparatus to measure the glass transition (T_g), melting (T_m) and crystallization (T_c) temperatures. The analysis was performed under a 40 mL/min N_2 gas flow in cycle ranges from − 40–210 °C at a heating/cooling rate of 10 °C/min. The associated enthalpy changes were used to calculate the crystallization degree of the PLA matrix (χ), using Eq. (1):

$$\chi(\%) = \left[\frac{\Delta H_m - \Delta H_{cc}}{\Delta H_0}\right] * \frac{100}{w} \quad (1)$$

where ΔH_m is the melting enthalpy, ΔH_{cc} is the cold crystallization enthalpy, $\Delta H_0$is the theoretical melting enthalpy of 100% crystalline PLA (93 J/g [32]), and w is the weight fraction of PLA in the blend, which was identified as the mass percentage lost during thermogravimetric (TG) analysis.

The thermal stability of the selected items was evaluated using a SetSys Evolution TGA/DSC (Setaram Instrumentation) thermogravimetric analyser with a temperature range up to 800 °C and a heating rate of 10 °C/min in an N_2 atmosphere.

Chemical composition was derived from FT-IR analyses carried out with a Vertex 70 spectrometer (Bruker Optik GmbH) equipped with a single reflection Diamond ATR cell. The spectra were recorded with 256 scans in the mid infrared range (400–4000 cm^{-1}) at a resolution of 3 cm^{-1}. SEM, TG and FT-IR analyses were also performed on the final digestates at the end of the degradation process.

The process performance was evaluated by measuring the concentrations of total solids (TS), volatile solids (VS), total organic carbon (TOC), soluble organic carbon (DOC), soluble carbohydrates and volatile fatty acids (VFAs), both at the beginning and at the end of the experiments. TS and VS measurements were performed according to the Standard Methods for the Examination of Water and Wastewater [33]. DOC, VFAs and soluble carboydrates were analyzed in the liquid phase after sample centrifugation at 5500 rpm for 15 min and subsequent filtration through a glass microfiber filter (1.2 μm pore size). Carbohydrates were determined with spectrophotometric analyses using the colorimetric phenol-sulphuric acid method using glucose as the standard [34]. The concentrations of VFAs (acetic [HAc], propionic [HPr], butyric + iso-butyric [HBu], valeric + iso-valeric [HVal], caproic + iso-caproic [HCa], heptanoic [HHp]) and ethanol (EtOH) were determined using a gas chromatograph (Model 3600 CX, VARIAN) equipped with a flame ionization detector and a 30 m capillary column (TRBWAX) with an inner diameter of 0.53 mm. The temperatures of the detector and the injector were 270 and 250 °C, respectively. The oven temperature was initially set at 60 °C, held for 3 min at this value, subsequently increased to 180 °C at a rate of 10 °C/min and finally increased to 220 °C at a rate of 30 °C/min and held for 2 min. The TOC and DOC concentration was measured using a Shimadzu TOC analyser equipped with modules for the analysis of both liquid and solid samples. The biogas produced during the anaerobic degradation tests was periodically sampled from the gasbags used for gas storage and its composition was determined by a gas chromatograph (Model 3600 CX, VARIAN) equipped with a thermal conductivity detector and a 2-m stainless-steel packed column (ShinCarbon ST) with an inner diameter of 1 mm. The operating temperatures of the injector and detector were 100 and 130 °C, respectively, with He as the carrier gas. The oven temperature was initially set at 80 °C and subsequently increased to 100 °C at 2 °C/min. All the analytical determinations were performed in triplicate.

2.3. Thermophilic anaerobic biodegradation tests

The biodegradation tests were carried out in 500-mL reactors equipped with overhead mechanical stirring using the Gas Endeavour®

apparatus by Bioprocess Control. The bottles were filled with 400 g of inoculum plus substrate mixture and then flushed with N_2 gas to ensure the onset of anaerobic conditions. Temperature was set at 55 °C and maintained constant with a thermostatic bath. The evolved biogas was conveyed from each reactor to the coupled flow cell unit integrated with a data acquisition system and stored in 5-L gasbags afterwards. The instrument was also equipped with sensors for room temperature and pressure measurement that were used to convert the evolved gas volume to standard temperature and pressure conditions (T = 273.15 K, p = 101 kPa). Thus, in the following, the reported biogas volumes are always expressed at standard conditions. The biogas collected in the gasbags was periodically analyzed for its main constituents (H_2, CO_2, CH_4, N_2). Blank tests were conducted to evaluate biogas production by the inoculum, while abiotic tests with no inoculum addition were also performed to quantify the extent of abiotic degradation of PLA. All tests were carried out in duplicate at inoculum-to-substrate (I/S) ratios of 1 and 2 g $VS_{inoculum}$/ g $VS_{substrate}$. The details of the experimental runs are reported in Table 2.

The incubation period was considered to be complete when the daily methane production during three consecutive days was below 1% of the cumulated volume of methane produced [35]. In addition, runs PLA1_2, PLA1_1 and PLA3_1 were also carried out at intermediate sets of time to investigate the process evolution at different stages of progress. Three additional reactors for each test were run in parallel and stopped respectively at day 18 (t_1), 24 (t_2) and 32 (t_3). These intervals correspond to the time needed for the biogas production rate to increase to ~50% of the maximum value (R_m) (t_1), to reach R_m (t_2) and to get back down to ~50% of R_m (t_3).

The results were modelled using the Gompertz equation (Eq. 2):

$$P(t) = P_m * \exp\left\{ -\exp\left[\frac{R_m * e}{P_m}(\lambda - t) + 1\right]\right\} \quad (2)$$

where $P(t)$ is the cumulative biogas production at time t, P_m is the maximum biogas production and λ indicates the lag phase duration. The parameters of the Gompertz curve were determined by fitting of the experimental data through mathematical regression using the software TableCurve2D®. In all cases the degree of fitting of the experimental data by the theoretical curve was found to be highly accurate, with values for the correlation coefficient (R^2) higher than 0.99. Considering the influence of multiple kinetic parameters on biogas evolution over time, the biodegradation rate was evaluated in overall terms through the time, $t_{x\%}$, required to reach a predetermined percentage, x%, of the maximum conversion yield into biogas (with x % = 10, 25, 50, 75 or 95%).

The degree of biodegradation was calculated using Eq. (3) [36]:

$$Biodegradation(t) = \frac{P_{net}(t) + P_{IC}(t)}{P_{th}} \quad (3)$$

where $P_{net}(t)$ is the actual cumulative biogas production (net of blank) at time t, $P_{IC}(t)$ is the dissolved inorganic carbon (IC) (net of blank), and P_{th} is the theoretical biogas production calculated according to the Buswell equation [37] from the elemental composition of the investigated materials. The IC content was indirectly calculated from the digestate pH and the CO_2 partial pressure in the reactor's headspace assuming thermodynamic equilibrium between the liquid and gaseous phases. To this aim, Visual Minteq [38] was used for the theoretical calculation of the equilibrium carbon concentration in the liquid phase.

Bioplastics disintegration was evaluated at the end of each test. According to the EN 13432 standard [30], disintegration is considered to be complete if a maximum of 10 wt% of the initial material is retained by a 2 mm sieve. However, in this study disintegration was evaluated using a 0.84 mm sieve, which was previously used for inoculum pretreatment, thus adopting a stricter condition. The plastic fragments retained were carefully rinsed with deionized water and then dried at 35 °C until constant weight.

Table 2
Experimental design. Test codes refer to the substrate, the I/S ratio and material size (pow = powder) used; t_1, t_2 or t_3 at the end of the test code indicate whether the experimental run was ended at day 18, 24 or 32; the acronym ABIO is used to indicate abiotic tests.

Run	Material size [cm]	I/S [g $VS_{inoculum}$/ g $VS_{substrate}$]
PLA1_2_t_1	1.5 × 1.5	2
PLA1_2_t_2	1.5 × 1.5	2
PLA1_2_t_3	1.5 × 1.5	2
PLA1_2	1.5 × 1.5	2
PLA1_1_t_1	1.5 × 1.5	1
PLA1_1_t_2	1.5 × 1.5	1
PLA1_1_t_3	1.5 × 1.5	1
PLA1_1	1.5 × 1.5	1
PLA1_1pow	< 0.1	1
PLA2_1	1.5 × 1.5	1
PLA3_1_t_1	1.5 × 1.5	1
PLA3_1_t_2	1.5 × 1.5	1
PLA3_1_t_3	1.5 × 1.5	1
PLA3_1	1.5 × 1.5	1
PLA4_1	1.5 × 1.5	1
LACT_1	—	1
PLA1_ABIO	1.5 × 1.5	—
PLA1_pow_ABIO	< 0.1	—
PLA2_ABIO	1.5 × 1.5	—

3. Results and discussion

3.1. Bioplastics characterization

The results of the morphological characterization of the samples by SEM are shown in Fig. 1. PLA1 and PLA4 were distinguished by surface uniformity and brittle behaviour. On the other hand, PLA2 and PLA3 showed irregular particles embedded in a homogeneous polymeric matrix; a larger number of inclusions was also observed for PLA3, suggesting a higher additive content. EDS analyses conducted at selected locations on the sample surface showed that carbon and oxygen were the major elements in the PLA blends. For both PLA2 and PLA3, Ca was detected at significant concentrations along with Ti, Mg and Si. Some Ca-containing particles were found in PLA4 (Fig. 1d). This might suggest that for all products tested, with the exception of PLA1, fillers were used, which likely included CaO (commonly used to absorb moisture during extrusion), $CaCO_3$ (used to improve thermal and mechanical properties [39]), TiO_2 (used as a filler and colorant and to improve product stability) and $Mg_3Si_4O_{10}(OH)_2$ (talc filler used to improve mechanical properties).

The results of DSC analyses, as obtained from the first heating cycle, are summarized in Table 3. The glass transition temperature for all samples was in agreement with the typical range for PLA, which is around 60 °C. The different values of ΔH_m calculated for the samples appear to be associated to differences in the crystallization degree of the polymer. According to several studies [40–42], additives such as TiO_2, $CaCO_3$ or talc usually act as nucleating agents, therefore are able to increase matrix crystallization. On cooling from the melt, only PLA3 among the four PLA samples showed a clear crystallization exotherm. This may be due to a faster crystallization rate induced by the nucleating efficiency of the different fillers, which also resulted in a significantly lower cold crystallization temperature of the amorphous regions during the heating ramp (around 95.9 °C). During the first heating, which is more representative of the as-received material than the second heating, sample PLA1 featured the presence of a weak and broad cold crystallization exotherm, thus explaining the high degree of crystallinity inherited from the manufacturing stage. PLA3 was found to show a cold crystallization peak during the first heating, which almost disappeared during the second heating scan, thus confirming its ability to crystallize during controlled cooling. It is worth mentioning that polylactides can be either amorphous or semicrystalline at room temperature, depending

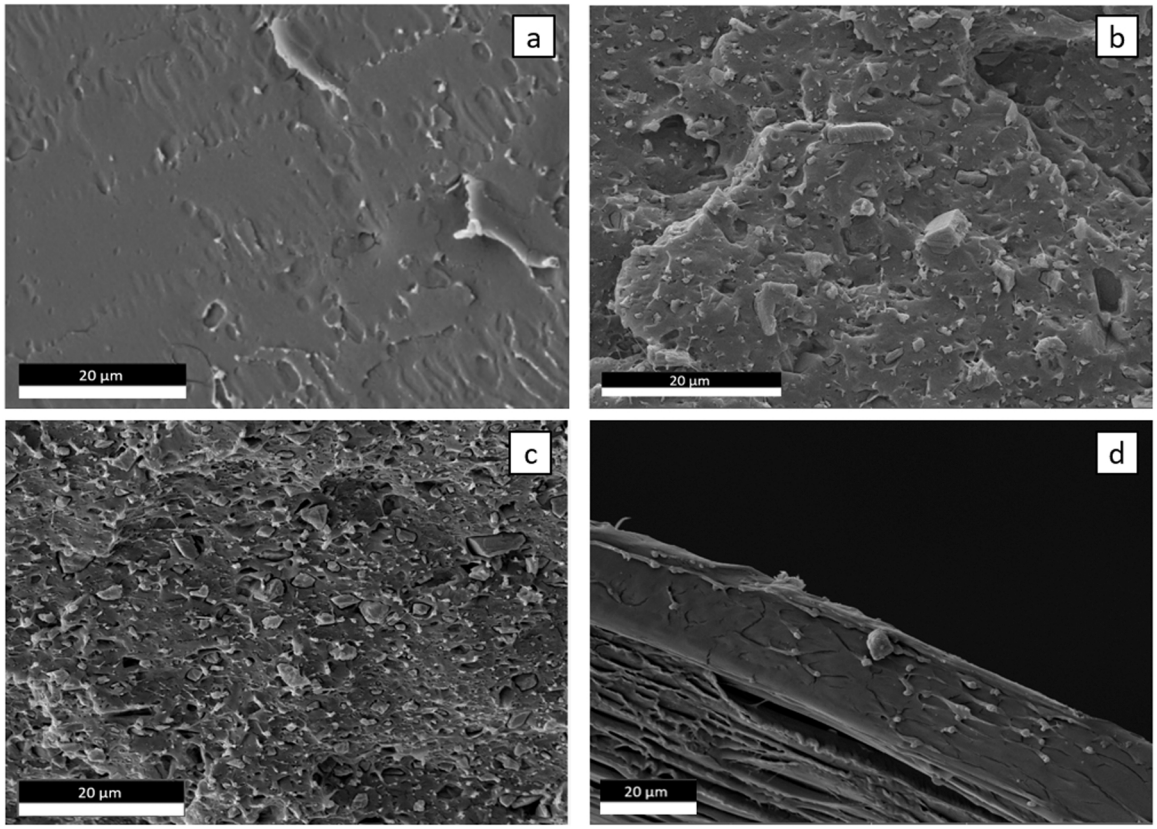

Fig. 1. SEM micrographs of a) PLA1, b) PLA2, c) PLA3 and d) PLA4.

Table 3
Thermal properties and degree of crystallinity (χ) of the bioplastic samples.

	T_g [°C]	T_{cc} [°C]	T_m [°C]	ΔH_{cc} [J/g]	ΔH_m [J/g]	χ [%]
PLA1	64.7	103.5	169.1	3.7	38.6	37.5
PLA2	63.9	118.0	152.4	22.9	23.4	0.5
PLA3	65.6	95.9	170.7	18.3	33.5	17.9
PLA4	59.5	111.9	157.4	15.8	23.2	8.1

on the molecular weight and content of L, D or meso-lactide in the main chain. Also the melting and cold crystallization temperatures of PLA are affected by the D-lactide content [43]. DSC results highlighted differences in the crystallization behaviour of the commercial samples of concern.

Fig. 2 shows the thermal degradation curves for all tested materials. PLA1, PLA2 and PLA4 displayed a single-stage decomposition process (Fig. 2b), with a sharp peak in the range 352–376 °C and a final weight loss between 97% and 100% (Table 4). PLA3 showed a second decomposition stage above 700 °C and a final residue of ~9.3%, which confirms the higher amount of inorganic fillers observed through SEM analysis (Fig. 2a) and the corresponding higher nucleating efficiency. The second peak was likely related to the presence of $CaCO_3$, since it was

Table 4
Onset temperature (T_0), maximum degradation temperature (T_{peak}) and residual mass for each tested material.

	T_0 [°C]	T_{peak} [°C]	Residual mass [%]
PLA1	349	376	0
PLA2	340	361	3.2
PLA3	319	352; 730	9.3
PLA4	327	359	1.8

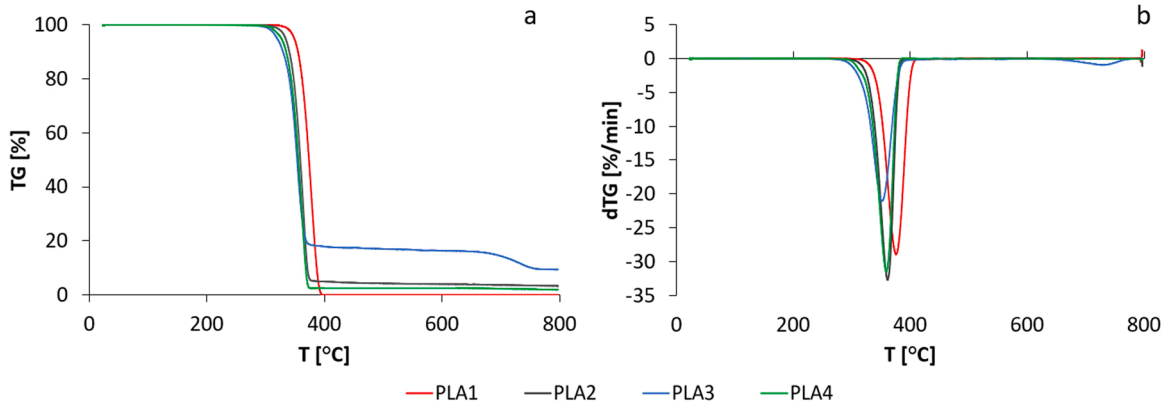

Fig. 2. a) TGA and b) dTG curves for the tested materials.

detected within the typical thermal decomposition range of carbonate phases (700–800 °C) [44].

Based on these results, it may be stated that the PLA1 sample showed a virtually neat composition, also on account of the negligible amount of residual mass at 800 °C. The T_{peak} for PLA2 and PLA4 was 4% lower than for PLA1, which could be linked to a different polymeric grade of PLA or to the presence of additives. It should be noted that the peak temperature for PLA3 was even lower (6%), suggesting an inverse correlation between T_{peak} and the residual mass of material at 800 °C. The presence of additives may have worsened the material's thermal stability as observed by other researchers [45]. InTable 4, the values of T_0 (onset temperature, which corresponds to a 5% sample mass loss), T_{peak} and residual mass for each bioplastic product are reported.

FT-IR spectra are shown in Fig. 3 in the wavenumber range between 1900 and 400 cm^{-1}, where the main absorption bands were detected. All products displayed similar spectra, indicating a rather definite chemical composition characteristic of the PLA polymer [46]. Deformation and asymmetric bendings of -CH- groups were visible at 1382 cm^{-1} and 1360 cm^{-1}, respectively, while at 1452 cm^{-1} the asymmetric bending of $-CH_3$ was also detected. At 1748 cm^{-1} and 696 cm^{-1} the carbonyl group (-C═O) asymmetric stretching was observed, which was interpreted by other authors [18] as a PLA amorphous phase. At 1266 cm^{-1} the -C-O-C stretching and CH bending modes were identified. Two additional bands at 867 cm^{-1} (C-C═O stretching) and 754 cm^{-1} (C═O bending) were observed, corresponding to the amorphous and crystalline phases of PLA respectively [47,48]. However, some differences in the ranges 1245–1158 and 970–900 cm^{-1} were detected. As reported in previous studies [46,49], bands located at 1210 and 922 cm^{-1} are related to absorptions of a highly crystalline polymer. Indeed, the decrese in intensity for the bands at 1210 and 1180 cm^{-1} has been associated in the literature to the amorphization mechanisms. Furthermore, the band centered at 922 cm^{-1} is correlated to the α and σ' crystalline forms, and the band at 955 cm^{-1} is assigned to the amorphous phase. As shown in Fig. 3, PLA1 and PLA4 possessed a higher initial degree of crystallinity. The crystallinity indexes were calculated as the ratio between the band intensities at 1210/1180 (r_1) and 955/922 (r_2). It was observed that both r_1 and r_2 were higher for the PLA cups; more specifically r_1 was ~16% for PLA1 and ~20% for PLA4, while the corresponding values for r_2 were ~23% and ~43%.

3.2. Anaerobic biodegradation tests

The results of the abiotic degradation tests indicated the absence of any detectable biogas production deriving from PLA degradation. Nevertheless, some appreciable physical alteration was observed for the PLA1 item (cup) at the end of the tests, with some significant particle size reduction. The initial 1.5-cm square PLA1 particles were found to have been reduced to needle-like shreds at the end of the abiotic test, and lactate was retrieved in the liquid solution at a concentration of 5.4 g/L, corresponding to 9.8% conversion of the initial organic carbon into lactate as a result of partial PLA dissolution. On the other hand, the powdered material particles at the end of the test were no longer visually identifiable. Unfortunately, due to analytical interferences it was not possible to measure the lactic acid content in this sample to quantify the degree of depolymerization occurred. However, the significant turbidity of the liquid solution in the reactor suggested that at least part of the original polymer was present in solution in the form of colloidal particles rather than oligomers of monomers resulting from depolymerization.

For the PLA2 sample (plate) the abiotic tests produced notably lower effects in terms of particle size reduction and sample dissolution. In this case, no evident physical alteration effect could be identified, and lactate production was only 4.7% of the initial organic carbon. The observed differences between the PLA1 and PLA2 samples are likely due to both the different thickness of the two items and the different mechanical, chemical and thermal properties of the polymeric mixtures tested.

The results of the biodegradation assays were evaluated in terms of the time evolution of bioplastics conversion into biogas during the tests, as well as total cumulative biogas yield attained. In order to compare the different PLA materials tested, the experimental data were reported per unit of initial TOC of the feed material. In particular, Fig. 4 shows the amount of evolved biogas for the different PLA samples, while Fig. 5 reports the associated $t_{x\%}$ values derived from the fitting curve of cumulative biogas production.

It should be mentioned that the positive control material (calcium lactate) displayed a degradation yield of 87 ± 1.6% in 26 days (data not shown), confirming the reliability of the degradation tests performed.

The total duration of the biodegradation tests for the different PLA samples was found to lie, according to the criterion defined by Holliger et al. [35], in the range 38–45 d, with the only exception of PLA3 which continued until day 52. The material disintegration was complete for all the experimental runs.

On the basis of the results shown in Fig. 4, no significant differences in the specific cumulative biogas production were observed among the different tests, with yields ranging between 1620 and 1830 mL/$gTOC_{PLA}$. The methane content was between 52% and 57% of the total cumulative biogas production for the different PLA items and a similar overall degree of biodegradation (86–100%) was attained in all tests (Fig. 6), along with a virtually complete conversion of PLA into biogas. Among the operating parameters tested, decreasing the I/S ratio from 2 to 1 was found to produce no evident effect on the biodegradation process, neither in terms of the overall biogas production nor in terms of total process duration. Particle size did not apparently affect the biodegradation degree, whereas it was found to reflect on the degradation rate. To this regard, the powdered PLA1 sample displayed faster

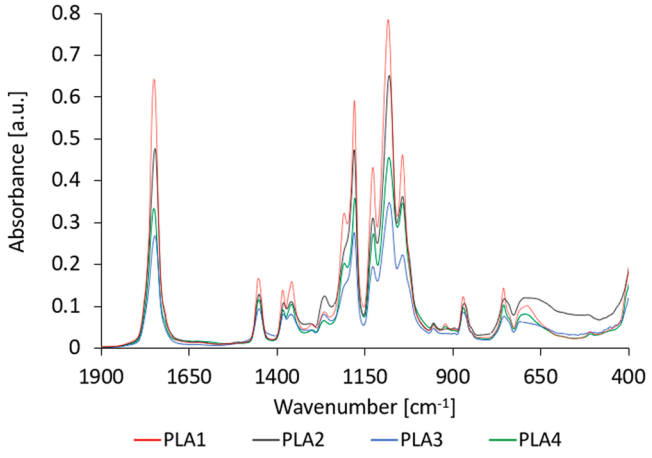

Fig. 3. FT-IR spectra for the test materials.

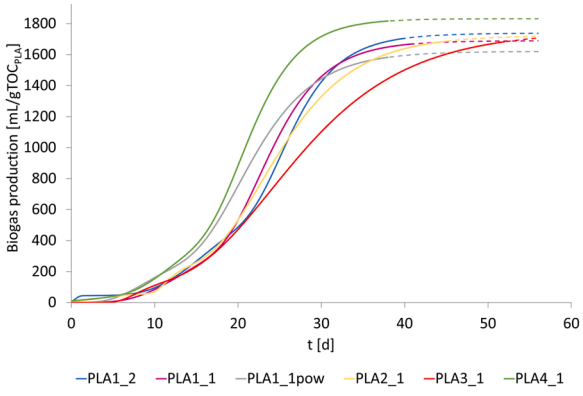

Fig. 4. Specific biogas production for the experimental runs. The dashed lines indicate the Gompertz fitting curves.

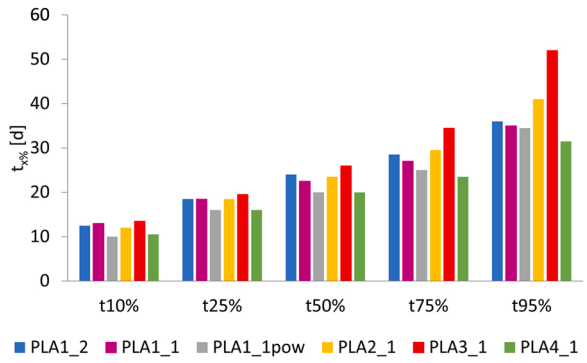

Fig. 5. Evolution of $t_{x\%}$ along the biodegradation process for the different experimental runs. The parameter identifies the time required to reach a predetermined percentage x% of the maximum biogas conversion yield.

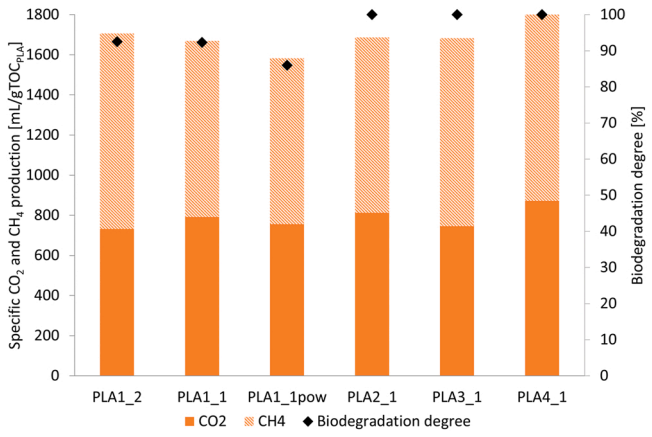

Fig. 6. Biogas composition and biodegradation degree for the experimental runs. Error bars are not shown, since the error ranged between 0.7% and 2.2%.

degradation kinetics particularly during the initial stages of the process, with a decrease in the $t_{10\%}$ and $t_{25\%}$ values by 22% and 14% from those of the 1.5-cm square PLA1 particles (See Fig. 5). However, the differences in the degradation rate between the two samples tended to decrease gradually as biodegradation proceeded, so that the $t_{95\%}$ value was similar for the square and the powdered PLA1 particles. This indicates that, while reducing the initial PLA particle size was capable of accelerating the degradation process (as also reported previously [50]) likely by promoting the material physical disintegration (as also observed during the abiotic tests) and the subsequent biomass accessibility to the substrate, the biodegradation process was almost complete in both cases irrespective of whether powdering was performed.

Comparing the two cup items, PLA4 was found to be degraded, compared to PLA1, 12–16% faster in the initial process stages and 18–21% faster in the final stages. This could be related to the physical differences among the two cups, with PLA1 being thicker and stiffer than PLA4 (see Table 1). In general terms, a good correlation ($R^2 > 0.92$) was observed between the product thickness and $t_{x\%}$ up to x = 75%, which confirms that thickness can influence more the degradation kinetics than the total duration and yield of the process.

On the other hand, for the two PLA plates, the later biodegradation phases were considerably delayed (by 9–15% for PLA2 and by 39–57% for PLA3) compared to PLA1. In this case, rather than the different degree of polymer crystallinity, this result may be due to the different chemical composition of the materials, suggesting that the more additives are embedded in the matrix the more the biodegradation kinetics is affected.

The results of the experimental runs at different degradation times are shown in Fig. 7. In such tests, both the biodegradation and disintegration degree were measured over time. I/S did not particularly affect the kinetics and the biogas yield, but yet affected disintegration, mainly in the first stages of the process. Disintegration for sample PLA1_2_t1 was more than twice that of sample PLA1_1_t1. This could be attributed to some bulking phenomena that could have occurred in the PLA1_1 reactor, where the bioplastic mass was twice the one for the PLA1_2 test, therefore friction and shear stresses could have played a role during the physical alteration processes. Unfortunately, for the PLA2_1_t1 and PLA2_1_t2 runs it was not possible to retrieve information about the disintegration degree due to operational interruptions occurred to the stirring system. However, at day 32, the disintegration degree for PLA2 was 40% lower than for PLA1, confirming that the different material's thickness was a relevant factor during the process. The biodegradation degree was still null at day 15, increased to 35–42% at day 24, attaining 80–100% for PLA1 and 57% for PLA2 at day 32.

In addition, a mass balance of carbon was carried out to track the fate of the organic matter at intermediate stages (t_1, t_2 and t_3) and at the end of the process (Fig. 8). The distribution of organic carbon was calculated considering the whole digestion system, implying that the resulting values derived from the combined contributions of both the bioplastic and the inoculum to the degradation process. The following contributions to the overall amount of carbon were considered: i) gasified carbon, as the sum of carbon in the evolved CO_2 (including the fraction dissolved in the liquid phase) and CH_4; ii) residual particulate carbon, obtained as the difference between the final TOC and DOC masses; iii) dissolved carbon in the form of undegraded carbohydrates and VFAs; iv) residual dissolved carbon, obtained as the difference between total DOC and the directly determined metabolic products (iii). A term referred to as "balance" was added to account for the carbon mass lost due to analytical inaccuracies or to sample heterogeneity and was meant to close the material balance. The values for the balance term ranged from − 2–22% throughout the different runs, indicating a good degree of carbon recovery from the different analytical determinations.

In the first stages of the process, a dramatic reduction in the particulate (−37%) and, mostly, the dissolved (−66%) carbon mass was observed. At time t_1 the main conversion observed for the organic C was into VFAs (12–19%), while gas production was still minor and comparable to that observed for the blank test. In the subsequent biodegradation stages, particulate and dissolved carbon consumption proceeded along with VFA conversion, while the contribution of gas production increased as expected. It should be noted that the residual mass of organic carbon in the digestate at the end of the digestion tests was comparable with the results obtained for the blank runs, confirming that the initial mass of carbon associated to the bioplastic products was fully converted into biogas.

3.3. *Digestate characterization*

SEM analyses were conducted on the digestate samples taken at the end of the biodegradation tests to try and determine the presence of residual microbioplastics. It was possible to detect plastic fragments only in the first stages of the process, when the disintegration degree was still low, while at later process stages no detectable bioplastic fragments could be identified. While this was clearly related to the high degree of biodegradation attained during the tests as commented above, it should also be emphasized that the use of the inoculum may have masked the presence of residual micron- and sub-micron-size particles, which could not be identified through morphological observations. Coupling SEM with EDS analysis was equally unsuccessful for the identification of microparticles, since PLA is mainly composed by carbon and oxygen, which are also the major elements that can be found in the digestate. Fig. 9a shows an example image of the particles detected in the PLA1_1_t1 sample, which was easily distinguished from the surrounding matrix due to its sharp edges and linear cracks, while in Fig. 9b the material's surface erosion can be observed.

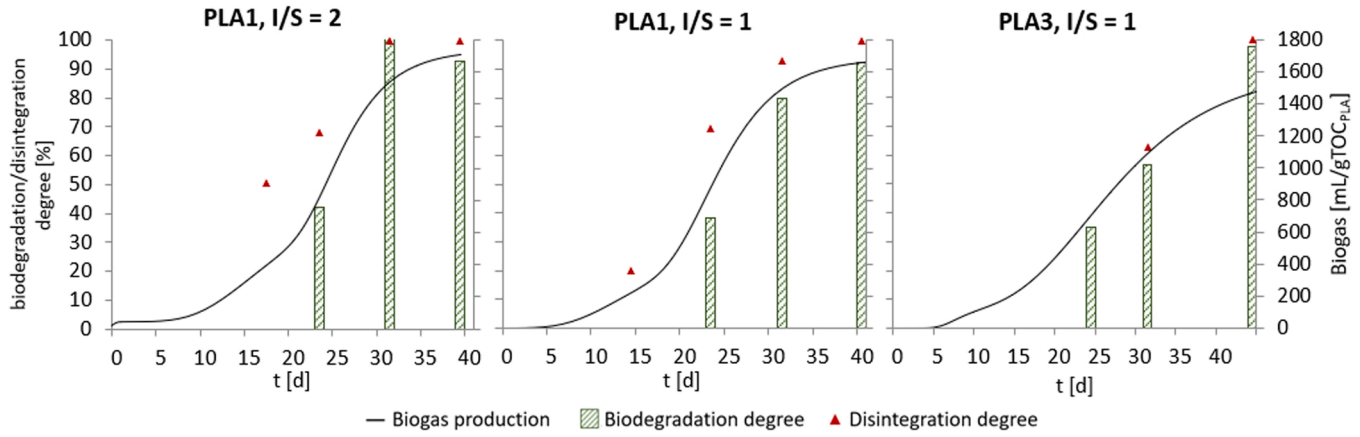

Fig. 7. Biogas production, biodegradation and disintegration degrees for the experimental runs with set of times.

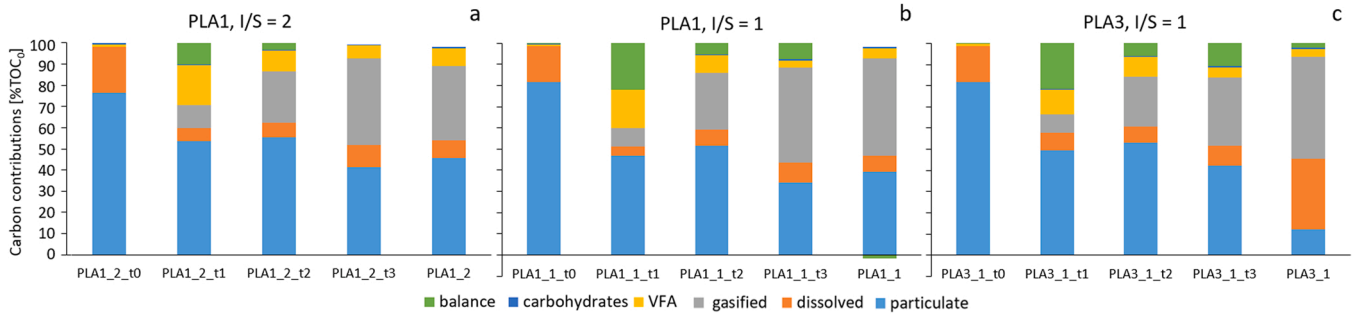

Fig. 8. Carbon mass balance for the experimental runs at different durations (t_1, t_2, t_3 and test completion time): a) PLA1_2; b) PLA1_1; c) PLA3_1.

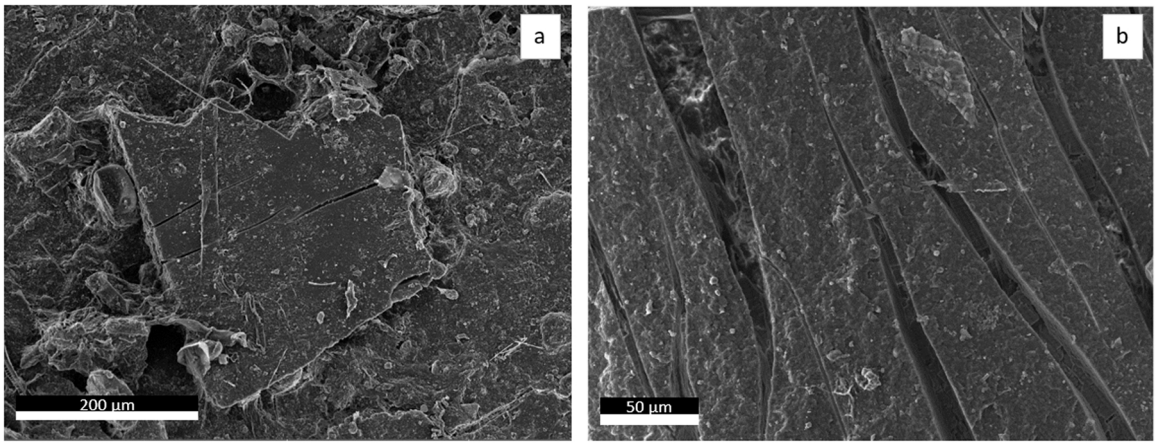

Fig. 9. Overview of a) plastic fragment detected in the digestate of the test PLA1_1_t1 and b) enlarged view of the cracks of one of the fragments.

The thermogravimetric analyses results for the final digestates are shown in Fig. 10 10. While the material's response to thermal degradation was mainly dictated by the inoculum, some differences among the samples could still be identified. The peaks in the range 50–150 °C were attributed to dehydration reactions, as observed by other authors [51–53]. Three further degradation steps were detected: one in the range 200–350 °C and one in the range 400–500 °C corresponding to the decomposition of organic matter [54] and a last one around 650 °C. In the low temperature range, semi-volatile and readily oxidizable compounds are lost [52], including carbohydrates, cellulose and hemicellulose as well as simple aliphatic and aromatic compounds (e.g., lipids and amminoacids) [51,55,56].

The peak intensity in this region was most affected by the degradation process and was taken as an indirect evidence of digestate stabilization [52,54,57]. In particular, a lower intensity of the peak at 250–300 °C may be correlated to a higher degree of stabilization of the material, which was observed for sample PLA1_2 (Fig. 10c). Moreover, the residual weight shown in Fig. 10a was higher for sample PLA1_2, confirming that this was the test where the biodegradation had proceeded to a larger extent as also indicated by the highest specific biogas yield attained.

Fig. 10d shows the thermal behaviour of digestates deriving from different PLA products. It should be noted that the lower intensity of the peak at around 300 °C for sample PLA3_1 was not an evidence of a higher degradation degree, being rather related to the high content of inorganic compounds derived from the untreated polymeric matrix.

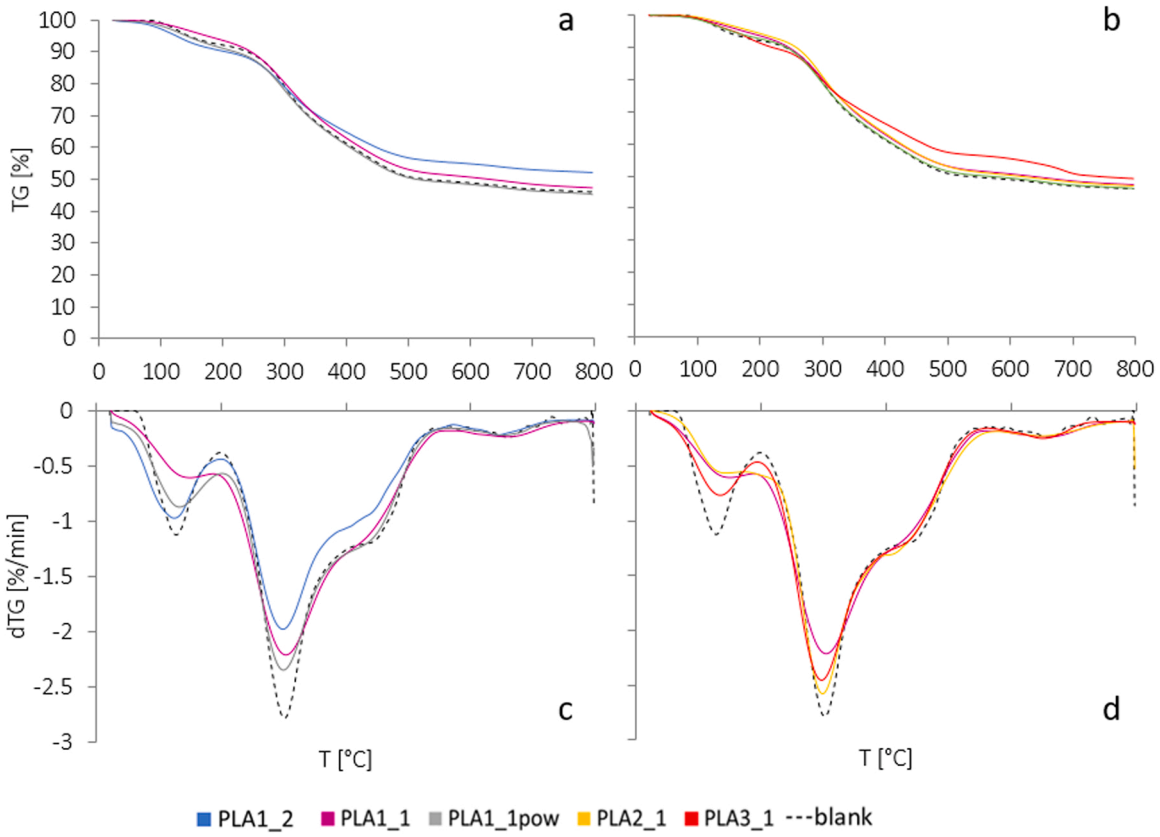

Fig. 10. Comparison of a) TG and c) dTG curves of the experimental runs with PLA1 and b) TG and d) dTG curves of the experimental runs with all the PLA products tested at I/S = 1. In each figure the curve of blank test is shown for comparison.

The second peak (400–550 °C) was attributed to the degradation of a more thermally stable organic matter fraction, including high molecular weight aromatic structures [55,56]. This peak was overlapping with the first one, appearing to be more rounded, and was very similar in terms of characteristic temperature and intensity for all samples, with the exception of PLA1_2 and PLA3_1. Another peak was observed at ~650 °C, which was related to the decomposition of carbonate species [53,54]; the peak was of low intensity for all samples with the exception of PLA3_1, suggesting the presence of undegraded additives in the final matrix. The additional peak identified at 180 °C likely indicated the presence of some chemically bound water that was evaporated only at high temperatures.

The results of the FT-IR analyses are shown in Fig. 11. Even in this case, the response was mainly dictated by the presence of the inoculum,

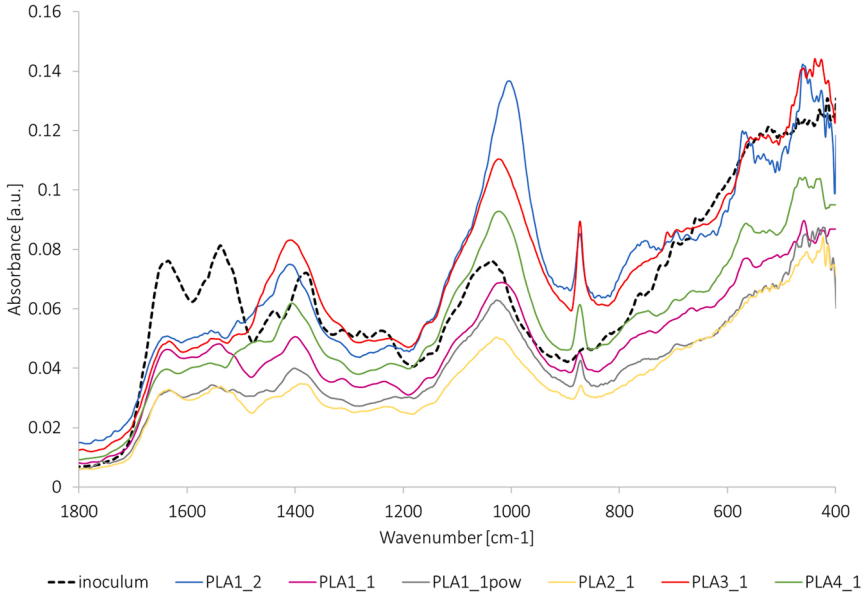

Fig. 11. FT-IR analyses for the final digestates.

however an absorption band at 870 cm^{-1}, corresponding to calcium carbonate, was evident in all tests. The presence of calcium carbonate was evidently a result of PLA degradation, and it testifies that in PLA1 there was also some Ca hidden by the C═O bending peak in the spectrum of the original material. The bands in the range 1495–1410 cm^{-1} could also be associated to carbonates [58].

The FT-IR analyses carried out on the tests at different durations are shown both for the final digestate (Fig. 12a) and for the plastic fragments collected from the disintegration tests (Fig. 12b). It was found that the main peaks identified in the original PLA1 sample were still visible at t_1 and t_2, while they gradually disappeared in the later stages of the process where the inoculum response became predominant (Fig. 12a). This is in agreement with the disintegration analysis, where 100% and ~90% disintegration was measured for samples PLA1_2_t3 and PLA1_1_t3, respectively. On the other hand, the degree of disintegration for sample PLA3_1_t3 was ~60% and the FT-IR analysis confirmed the presence of detectable amounts of the original material. The collected PLA fragments (Fig. 12b) showed a similar IR response regardless of the test duration and this could mean that the residual plastic fragments were virtually unaffected by the degradation process. The band detectable at ~1585 cm^{-1} was attributed to some remaining biofilm, as also observed by other authors [19].

4. Conclusions

The anaerobic mono-digestion tests conducted on single-use PLA-based items showed that the influence of the operating conditions investigated (I/S ratio and material powdering) was negligible for all tests, at least in the range of values selected. All the tested products reached a biodegradation degree of 86–100% in around 42 days, although at different rates depending on the product type, material size and digestion conditions. Thermogravimetric and spectroscopic analyses allowed to understand the chemical composition of the bioplastic products and to correlate it with the degradation process. In particular, the additives concentration in PLA3 was found to be higher than in PLA2, and this resulted in a slower biodegradation rate. The analyses of the final digestates confirmed a virtually complete degradation of the organic matter from biodegradable plastics but revealed the presence of

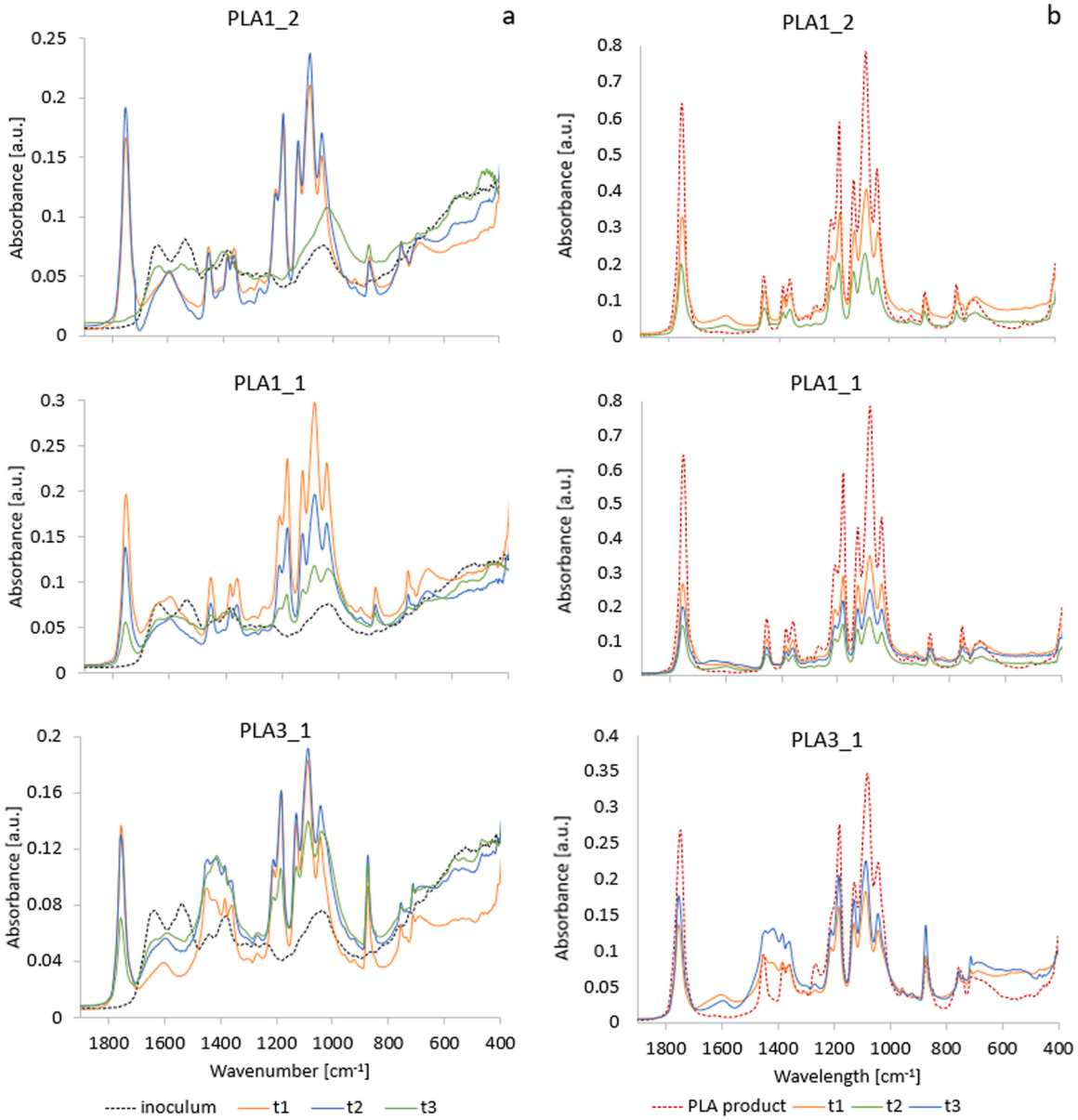

Fig. 12. FT-IR analyses at different digestion times: a) digestate and b) individual plastic fragments retrieved from the digestate.

a residual mass associated to the presence of inorganic additives. The findings of the present study indicate that the behaviour of bioplastic waste needs further and careful evaluation, since there are many factors dictating their response when subjected to controlled degradation conditions. The nature and amount of additives, the polymer crystallinity and the product shape and thickness are some of the aspects that need to be taken into account. Their fate in existing anaerobic digestion and composting facilities should be investigated and understood to avoid mismanagement of bioplastic waste. Although the final degree of biodegradation of the polymer in the tested PLA blends was high for all the experimental runs, it should be emphasized that the biological process required prolonged digestion times that may not be in line with the design values commonly adopted for anaerobic digesters treating municipal and food waste. Furthermore, the residual content of undregraded inorganic components (additives and fillers) from the commercial polymeric blends should be carefully evaluated to assess the quality of the final digestate, particularly in a prospective scenario in which the amount of bioplastic waste collected along with the organic fraction of municipal waste is expected to increase significantly. To this aim, different operating conditions or suitable pretreatments of the feed material must be explored to make biodegradable plastics degradation compatible with conventional full-scale digesters, identify potential technological improvements to be implemented and highlight issues of potential environmental concern.

Environmental implication

The findings of the present work can contribute to building knowledge on bioplastic waste management, and in particular to understand the behaviour of these residues when subjected to biological treatments for valorization. The aim is to gain detailed knowledge about the biodegradability of commercial biodegradable plastics, which in turn can be used to define the potential advantages and disadvantages associated to an extensive use of such products, since they are currently indicated as a potential sustainable alternative to conventional plastics.

CRediT authorship contribution statement

A. Polettini: Conceptualization, Methodology, Supervision, Data curation, Writing – original draft, Writing – review & editing. **R. Pomi**: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing. **A. Rossi**: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. **T. Zonfa**: Methodology, Writing – review & editing. **M. Falzarano**: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. **G. De Gioannis**: Conceptualization, Methodology, Writing – review & editing. **A. Muntoni**: Conceptualization, Methodology, Writing – review & editing. **M. P. Bracciale:** Investigation, Data curation, Conceptualization, Methodology, Writing – review & editing. **F. Sarasini:** Investigation, Data curation, Conceptualization, Methodology, Writing – review & editing. **J. Tirillò:** Investigation, Data curation, Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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