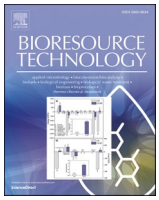
Bioresource Technology 344 (2022) 126265

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech



Biodegradation of bioplastics under aerobic and anaerobic aqueous conditions: Kinetics, carbon fate and particle size effect

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HIGHLIGHTS

- Only PHAs were aerobically/anaerobically biodegradable under aqueous conditions.
- PHB and PHBV yielded up to 496 and 480 Nm^3 of CH_4 per ton, respectively.
- C-balance analysis for the different carbon sinks estimates polymer biodegradability.
- Mineralization rate depended on the total specific surface area of polymer.
- The modified Gompertz model accurately described bioplastic biodegradation.

ARTICLE INFO

Keywords:
Aerobic biodegradation
Biodegradation tests
Bioplastics
Circular economy
End-of-life management
Organic recycling

ABSTRACT

The biodegradation of PHB, PHBV, PBS, PBAT, PCL, PLA, and a PLA-PCL blend was compared under aerobic and anaerobic aqueous conditions assessing biodegradation kinetics, extent, carbon fate and particle size influence (in the range of 100–1000 μm). Under standard test conditions, PHB and PBHV were biodegraded anaerobically (83.9 ± 1.3% and 81.2 ± 1.7%, respectively) in 77 days or aerobically (83.0 ± 1.6% and 87.4 ± 7.5%) in 117 days, while PCL was only biodegraded (77.6 ± 2.4%) aerobically in 177 days. Apparent biomass growth accounted for 10 to 30.5% of the total initial carbon depending on the bioplastic and condition. Maximum aerobic and anaerobic biodegradation rates were improved up to 331 and 405%, respectively, at the lowest particle size tested (100–250 μm). This study highlights the usefulness of analysing biodegradation kinetics and carbon fate to improve both the development and testing of biodegradable materials, and waste treatments in the context of a circular bioeconomy.

1. Introduction

Owing to their biodegradability and circularity potential, the use of biodegradable bioplastics (BBs) has gained increasing momentum due to the global plastic pollution problem (Dorigato, 2021; García-Depraect et al., 2021). Although the BBs produced worldwide (1.2 million tons in 2020) currently represent a small share (~1%) of the global production of plastics, a significant market growth for BBs of almost 50% is expected by 2025 (European Bioplastics, 2021). The growth on the use of BBs is fostered by the European Union and beyond through the implementation of different measures devoted to adopting a sustainable plastics economy while mitigating plastic pollution. The European Commission launched the Circular Economy Action Plan (CEAP) and the European Green Deal, which included different recently published and forthcoming initiatives on single-use plastics, plastic packaging, microplastics, as well as bio-based, biodegradable plastics (European Commission, 2021). The implementation of such policy framework in a comprehensive way will require an improved knowledge on bioplastics, addressing fundamental and priority issues such as their biodegradability, recyclability, toxicological safety (Ding et al., 2021; Malafaia et al., 2021) and their direct or indirect impact on surrounding environments (Bandini et al., 2020; Sanz-Lázaro et al., 2021; Zhou et al.,

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https://doi.org/10.1016/j.biortech.2021.126265
Received 27 August 2021; Received in revised form 26 October 2021; Accepted 28 October 2021
Available online 2 November 2021

2021).

Despite of the extent and rate of biodegradation of several bioplastics have been previously investigated in different environments under both aerobic and anaerobic conditions, the results obtained are not conclusive and more studies are needed to deepen our understanding of BBs biodegradation (Bátori et al., 2018; Chamas et al., 2020; Folino et al., 2020; Shrestha et al., 2020). For instance, a recent literature review on the anaerobic biodegradation of poly(lactic acid) (PLA) and poly (ε-caprolactone) (PCL) has highlighted the significant variability in their biodegradation level, without a clear correlation to temperature or incubation time (Quecholac-Piña et al., 2020). Although biodegradability tests are conducted under standardized laboratory conditions following International (ISO), European (CEN) and American (ASTM) standards, for example, there are several factors affecting the rate and extent of biodegradation that vary among the reported studies. Biotic and abiotic factors depend on the specific experimental conditions as well as the nature of the microorganisms involved. Hence, more systematic, comparative testing and mechanistic data on the biodegradation of BBs is still needed to engineer biodegradable materials/products, to develop end-of-life management processes, and to gain a detailed and comprehensive understanding of the mechanisms underlying bioplastic biodegradation. In this context, each BB material must be evaluated under the same testing scheme in order to achieve unbiased comparisons of biodegradability results (SAPEA, 2020).

The biodegradation extent of plastics is typically followed by measuring the oxygen demand or carbon dioxide (CO_2) evolution, or the amount of CO_2 and methane (CH_4) evolved when the plastic material is either aerobically or anaerobically biodegraded. Thus, biodegradation standard tests only account for the mineralized carbon, overlooking the carbon fixed in the form of cell biomass (Chinaglia et al., 2018; García-Depraect et al., 2021). The terms "mineralization" and "biodegradation" are commonly used interchangeably in the literature for the sake of simplicity; however, they are related to different processes. Yet, mineralization represents the last stage following biodeterioration and biofragmentation, but is in fact an essential step in the biodegradation process. Mineralization provides information about the actual metabolic capability of a microorganism or a community to convert the polymer monomers or fragments (that have been generated in the preceding two stages) into biomass, gasses (CO_2 and/or CH_4), water and potentially other metabolites [further details on biodegradation mechanism can be found, for example, in the review by García-Depraect et al. (2021)]. In this context, an overall carbon balance analysis (for the different carbon sinks) could provide a more meaningful and comprehensive information of biodegradation (Chiellini et al., 2007; Pagga et al., 2001; Urstadt et al., 1995). The carbon balance approach may in fact be vital to reliably quantify biodegradation and can thus allow for a sustained judgement regarding the polymer fate and completeness of biodegradation in a defined environment as compared to the current methods defined in the international and local biodegradation test standards (ISO, ASTM, CEN, etc.). However, direct biomass determintation remains difficult in environmental samples, such as activated sludge, and thus, little is known about the fate of carbon during the biodegradation of the various BBs. Moreover, despite it is well recognized that particle size and surface area are important interdependent factors affecting the rate of surface erosion process (biodegradation), only few studies have investigated their impact on biodegradation (Chinaglia et al., 2018; Yagi et al., 2012), and comparatively less attention has been devoted to aqueous environments. Therefore, a complementary experimental framework other than the guidelines set by the existing biodegradation test standards for bioplastics is required to get a deeper understanding of their biodegradation and the correlation between the different factors influencing biodegradation (García-Depraect et al., 2021).

This study aimed at extending the standard test method through a more detailed analysis including kinetics, carbon fate and effect of particle size. The biodegradation rate and extent of a diverse set of polyester-based bioplastics under aerobic and anaerobic aqueous conditions was investigated. The tested bioplastics included poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), poly (butylene succinate) (PBS), poly(butylene adipate-co-terephthalate) (PBAT), PLA, PCL and a PLA-PCL blend, which are the most commonly used BBs (European Bioplastics, 2021). The fate of carbon during BBs biodegradation and the effect of three different particle sizes on BBs biodegradation kinetics were also investigated. The findings obtained in this study are useful in the design, testing, and up-cycling of BBs in the context of a more sustainable, circular, and resource-efficient bioeconomy.

2. Materials and methods

2.1. Materials

The BBs used in this study were PHB (ENMAT^{TM} Y3000P), PHBV (ENMAT^{TM} Y1000P, 3 mol% HV), PBS (BioPBS^{TM} FZ91PM/FZ91PB), PBAT (Mvera® B5037), PLA (LUMINY® L105), PCL (Capa® 6500D), and PLA/PCL 80/20 blend (PLA Luminy® L105/PCL Capa® 6500D). All BBs were purchased from the Technological Institute of Packaging, Transportation and Logistics (ITENE, Spain). In addition, microcrystalline cellulose with a particle size distribution ($\geq$80%) of 20–160 μm (Merck Ltd., Germany, CAS number 9004–34-6) and high density polyethylene (HDPE) (Sigma-Aldrich, USA, product number 427985) were used as the reference materials for positive and negative controls, respectively. Polymer data according to its technical data sheet is summarized in the e-supplementary material.

The plastic materials, which were initially in a pellet form, were grinded in a commercial blender (Cecotec Titanium 2000 pro, Spain) equipped with titanium blades. Repeated crushing (~3 min on, ~5 min off) using dry ice as a cooling strategy was employed to avoid melting and recrystallization, as reported elsewhere (Yagi et al., 2012). Finally, the polymer powders were sieved using an electromagnetic siever (CISA RP-20, Spain) with stainless-steel sieves of 100, 250, 500 and 1000 μm and then dried at room temperature. The different powder fractions were stored in closed packaging under dark and dry conditions at room temperature until usage. According to the recommendations of the standards ISO 14,852 and ISO 14853, the tested BBs (and HDPE) were used in powdered form with a particle size of 100–250 μm.

2.2. Aerobic biodegradation test

Polymer biodegradation under aqueous aerobic conditions was determined according to the standard ISO 14852: *Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by analysis of evolved carbon dioxide*. In brief, the biodegradability test was carried out in 2.1 L gas-tight glass bioreactors (1 L of working volume) containing either 150 mg/L of the tested BBs, cellulose (positive control) or HDPE (negative control), 64.8 mL of the activated sludge inoculum (corresponding to a final concentration of 0.5 g total solids (TS)/L), and 935.2 mL of a mineral salt medium freshly prepared with the following composition (in mg/L): KH_2PO_4, 85.0; K_2HPO_4, 217.5; $Na_2HPO_4{\cdot}2H_2O$, 334.0; NH_4Cl, 5.0; $MgSO_4{\cdot}7H_2O$, 22.5; $CaCl_2{\cdot}2H_2O$, 36.4; $FeCl_3{\cdot}6H_2O$, 0.25. All chemicals used were of analytical grade. Blank tests without any carbon source addition were also performed in parallel to correct for the background (endogenous) CO_2 production. The bioreactors were closed with rubbers septa and aluminum caps, and then incubated under gentle agitation (at 4.5 rpm) in a roller shaker (Wheaton Scientific Products, USA) placed in a controlled-temperature room (25 $\pm$ 1 °C) under diffuse light conditions. Activated sludge kindly supplied by the sewage treatment plant of Valladolid (Spain) was employed as inoculum within 1 day after collection. This inoculum was not previously adapted to the biodegradation of the target plastics as the only carbon and energy source. The initial pH of the cultivation broths in the bioreactors was 7.0 $\pm$ 0.1. The CO_2 and O_2 concentration in the headspace was measured periodically until the mineralization curve plateaued. When the O_2 concentration in

the headspace decreased below 5%, the headspace was aerated with an air compressor for 5 min to prevent O_2 limitation during the biodegradability test. All tests were performed in triplicate.

The degree of biodegradation (D_T), expressed in percentage, at time t (in days) was calculated by comparing the cumulative net carbon evolved as CO_2 gas in the bioreactor headspace (in mg) from the test (or reference) material with its corresponding theoretical amount ($ThCO_2$, in mg), as shown in Eq. (1); where, $\sum (CO_2)_{Test}$ is the accumulated mass of CO_2 evolved (in mg) in the bioreactor containing the test (or reference) material between the start of the test and time t, and $\sum (CO_2)_{Blank}$ is the accumulated mass of CO_2 evolved (in mg) in the blank bioreactor between the start of the test and time t. $ThCO_2$ was calculated according to Eq. (2), where m is the mass (in mg) of the test material, X_C is the carbon content of the test material (express as a mass fraction) determined from its stoichiometric formula, MM_{CO2} is the molecular mass of CO_2, and MM_C is the molecular mass of carbon. At the end of the biodegradability test, the net mass of dissolved inorganic carbon (DIC, as CO_2 in the liquid phase), after subtracting the mean blank values, was added to the cumulative net carbon evolved as CO_2 in the headspace. The final biodegradation degree (D_F) was then calculated by comparing the total amount of carbon converted to CO_2 (mCO_{2T}) with its $ThCO_2$, as shown in Eq. (3). Additionally, for the sake of comparison, biodegradation was also estimated by measuring the consumption of O_2 according to the standard test method ISO 14851.

$$D_T = \frac{\sum (CO_2)_{Test} - \sum (CO_2)_{Blank}}{ThCO_2} \times 100 \quad (1)$$

$$ThCO_2 = m \times X_c \times \frac{MM_{CO_2}}{MM_C} \quad (2)$$

$$D_F = \frac{mCO_{2T}}{ThCO_2} \times 100 \quad (3)$$

2.3. Anaerobic biodegradation test

Polymer biodegradation under anaerobic conditions was determined according to the standard ISO 14853: *Plastics — Determination of the ultimate anaerobic biodegradation of plastics materials in an aqueous system — Method by measurement of biogas production*. The biodegradability test was performed in 2.1 gas-tight glass bioreactors using the same agitation apparatus and conditions as described in Section 2.1. First, an aliquot of anaerobic inoculum was added to obtain the desired concentration of 1 g TS/L. Then, 150 mg/L of the test material, cellulose or HDPE was introduced accordingly, and finally the bioreactors were filled up with a defined mineral salt medium up to a total volume of 1 L (the initial pH was 7.1). The bioreactors were then flushed with pure helium gas (Abello Linde, Barcelona, Spain) for 5 min to ensure anaerobic conditions (which were corroborated by gas chromatographic analyses and the lack of color of resazurin, a redox indicator), and incubated in the dark under mesophilic conditions (36 ± 1 °C). The mineral salt medium consisted of the following (g/L): KH_2PO_4, 0.27; $Na_2HPO_4{\cdot}12H_2O$, 1.12; NH_4Cl, 0.53; $CaCl_2{\cdot}2H_2O$, 0.075; $MgCl_2{\cdot}6H_2O$, 0.1; $FeCl_2{\cdot}4H_2O$, 0.02; resazurin, 0.001; $Na_2S{\cdot}9H_2O$, 0.1. All reagents were of analytical grade. The methanogenic inoculum herein used was obtained from the mesophilic anaerobic sludge digester of Valladolid sewage treatment plant. This inoculum was not acclimated for plastics biodegradation and was preincubated for 7 days at 36 ± 1 °C, without addition of any nutrient and carbon source, in order to reduce the background gas production. Prior to use, the inoculum was washed twice (mineral salt medium, 10000 rpm for 10 min at 4 °C) and suspended in fresh mineral salt medium to reduce its inorganic carbon content (<20 mg/L) in the final test suspension. Blank (inoculum and mineral salt medium), positive (cellulose) and negative (HDPE) control tests were also performed. All assays were conducted in triplicate. After 1 h of incubation at 36 ± 1 °C, gas pressure in the headspace was measured with a manometer and the excess gas was vented in order to reach equilibrium. The pressure and concentration of CO_2 and CH_4 in the bioreactor headspace were measured weekly until the mineralization curve plateaued.

The degree of biodegradation (D_T, %) at time t (in days) was estimated by comparing the net mass of carbon evolved in the headspace (as CO_2 and CH_4) from the test (or reference) material with the mass, in mg, of carbon of the test/reference material (m_v), as shown in Eq. (4); where $\sum (C_{biogas})_{Test}$ and $\sum (C_{biogas})_{Blank}$ are the cumulative mass of gaseous carbon as CO_2 and CH_4 evolved (in mg) in the bioreactors containing the test (or reference) material and in the blank bioreactors, respectively, between the start of the test and time t. The final biodegradation degree (D_F) was calculated using Eq. (5); where mC_T is the total amount, in mg, of organic carbon converted to inorganic carbon and CH_4 at the end of the test (final net mass of inorganic carbon in the liquid phase plus the cumulative net carbon evolved in the headspace).

$$D_T = \frac{\sum (C_{biogas})_{Test} - \sum (C_{biogas})_{Blank}}{m_v} \times 100 \quad (4)$$

$$D_F = \frac{mC_T}{m_v} \times 100 \quad (5)$$

2.4. Carbon mass balance

A series of experiments was additionally conducted with the aim of estimating carbon fate during the biodegradation of BBs under aerobic and anaerobic conditions. The BBs tested were those significantly biodegraded in the aerobic (i.e., PHB, PHBV, and PCL) and anaerobic (i.e., PHB and PHBV) biodegradability tests above described. The experiments under aerobic conditions were carried out in 2.1 L gas tight glass bioreactors (0.2 L working volume) at 25 ± 1 °C and under diffuse light conditions. Each bioreactor was filled with 5 mL of fresh activated sludge inoculum (resulting in 20 mg volatile suspended solids (VSS)/L), 200 mg of the tested BBs (corresponding to 1.0 g volatile solids (VS)/L), and 195 mL of mineral salt medium (KH_2PO_4, 3.75 g/L; $Na_2HPO_4{\cdot}2H_2O$, 8.73 g/L; NH_4Cl, 0.2 g/L; $MgSO_4{\cdot}7H_2O$, 22.5 mg/L; $CaCl_2{\cdot}2H_2O$, 36.4 mg/L; $FeCl_3{\cdot}6H_2O$, 25 mg/L). A particle size lower than 100 μm was employed to enhance BB bioavailability, as recommended by Pagga et al. (2001). Triplicate assays for each tested BB and blanks (only inoculum and medium) were carried out. The cultures were aerated with air when the oxygen concentration in the headspace decreased below 10% (*v/v*).) The carbon derived from the apparent biomass (measured as VSS) was calculated assuming that the stoichiometric formula of biomass is $C_5H_7O_2N$ (van Haandel and van der Lubbe, 2012).

The apparent biomass yield on bioplastic ($Y_{X/S}$, mg VSS/mg bioplastic) was calculated by dividing the apparent net biomass growth (in mg VSS) by the mass of test material (in mg). The CO_2 yield on bioplastic ($Y_{CO2/S}$, mg CO_2/g bioplastic) was calculated by dividing the total net carbon converted to CO_2 (in mg) by the mass of test material (in g). During aerobic biodegradation, the degraded bioplastic carbon was diverted to CO_2 in the gas phase, DIC, dissolved organic carbon (DOC), and new biomass.

The assessment of carbon fate under anaerobic conditions was carried out in triplicate under similar experimental conditions to those described for the anaerobic biodegradability test (Section 2.2), but with a polymer concentration of 1 g VS/L and using a mineral salt medium with higher buffer capacity by increasing the concentration of KH_2PO_4 and $Na_2HPO_4{\cdot}12H_2O$ from 0.27 and 1.12 g/L to 1.35 and 5.6 g/L, respectively. The anaerobically biodegraded bioplastic carbon was diverted to CO_2 and CH_4, DIC, DOC and new biomass. The carbon fixed in the form of biomass was assumed to be 10% of the total initial carbon (Chernicharo, 2007). Theoretical CO_2 ($ThCO_2$) and CH_4 ($ThCH_4$) production were calculated following Buswell's equation (Buswell and Neave, 1930). At STP conditions (0 °C, 1 atm), the maximum biogas yields are 1041 NmL/g PHB and 1083 NmL/g PHBV with a theoretical CH_4 content of 56 and 58%, respectively.

2.5. Influence of particle size on BB biodegradation

The effect of particle size on the bioplastic biodegradation extent and rate was investigated under aerobic and anaerobic conditions for PHB, PHBV and PCL (the latter only under aerobic conditions). Similar experiments to the aerobic and anaerobic biodegradation tests previously described in sections 2.2 and 2.3, respectively, were performed in triplicate using mineral salt media with high buffer capacity and nutrients concentrations, and 1.0 g VS/L of polymer concentration (Section 2.4). Three particle sizes were assessed, namely 100–250, 250–500 and 500–1000 μm. Biodegradability data were fitted to the modified Gompertz model (Eq. (6)), as recommended by Ryan et al. (2017), in order to compare the biodegradation kinetics:

$$C = P \times \exp\left\{ -exp\left[\frac{R \times e}{P}(\lambda - t) + 1\right]\right\} \quad (6)$$

where C is the cumulative carbon in the gas phase (mg C/g bioplastic) at incubation time t (days), P is the maximum conversion of plastic carbon to gaseous carbon (mg C/g-bioplastic), R is the maximum rate of mineralization (mg C/g-bioplastic·day), λ is the lag time (in days) of gas carbon products release, and e is the Euler's constant (2.7182).

2.6. Analytical methods

The time course of cumulative gas carbon produced during biodegradation (as CO_2 and CH_4) was measured by manometric and gas-chromatographic methods, using a pressure transducer (IFM electronic PN7097, Germany) and a gas chromatograph (Agilent 8860, The Netherlands) equipped with a thermal conductivity detector (GC-TCD) following procedures described elsewhere (Posadas et al., 2014). Solid concentrations (including VSS as a measure of biomass concentration) were analyzed according to standard methods (APHA, 2005). DIC and DOC were measured by a total organic carbon analyzer (Shimadzu TOC-VCSH, Japan) in filtered samples (0.45 μm). Finally, the concentrations of nitrite and nitrate were quantified by HPLC-IC in filtered samples (0.22 μm) according to Posadas et al. (2014).

3. Results and discussion

3.1. Biodegradation of bioplastics under aerobic conditions in aqueous medium

Fig. 1 shows the mineralization curves for the tested materials under aerobic conditions. After 117 days of testing, the degrees of mineralization of PHB, PHBV and PCL were 79.6 ± 0.4%, 84.5 ± 9.3% and 75.7 ± 2.3%, respectively. Note that considering the amount of DIC, the final biodegradation degrees were 83.0 ± 1.6% (PHB), 87.4 ± 7.5% (PHBV) and 77.6 ± 2.4% (PCL). These results would be considered as "passed to be biodegradable" according to the ISO standard test pass and fail criteria as they reach at least 90% conversion into CO_2 in comparison to the positive control (cellulose, Fig. 1) within less than 3 months (90 days). The profile of the biodegradation curves for these BBs was comparable, showing lag phases of 11–13 days likely due to the fact that microorganisms present in the activated sludge (inoculum) were not adapted to the BBs and required adaptation time to synthesize enzymes such as PHA (polyhydroxyalkanoate) depolymerases, cutinases, lipases and esterases for depolymerization (Pathak and Navneet, 2017). After this lag phase, a rapid CO_2 production was observed up to day 45, with PCL experiencing a slightly higher biodegradation rate than the other BBs but lower than that of cellulose. Comparatively, no significant biodegradation was observed for PBS, PBAT, PLA and the PLA-PCL blend, indicating that those bioplastics are non-biodegradable under the tested aerobic conditions in aqueous medium. As expected, the biodegradation of cellulose reached 86.8 ± 2.3% in a shorter period (68 days), while neither significant CO_2 production nor O_2 consumption was

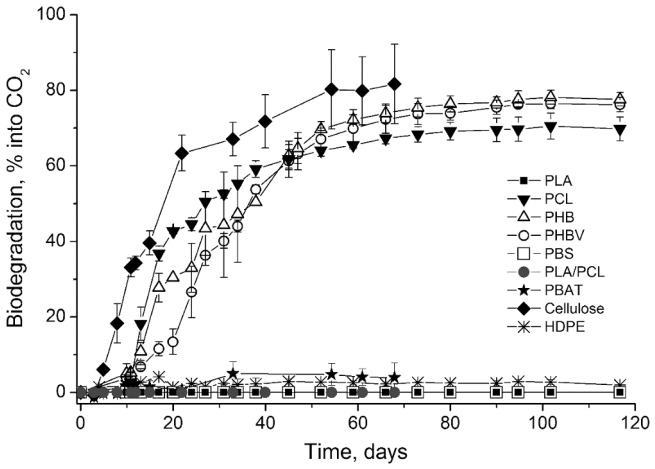

Fig. 1. Biodegradation profiles (expressed as % conversion into CO_2) of PHB, PHBV, PBS, PBAT, PCL, PLA, PLA/PCL blend, HDPE (negative control) and cellulose (positive control) under aerobic conditions in aqueous medium. Each data point corresponds to the average and standard deviation from triplicate assays. A particle size of 100–250 μm was used in all the cases, except for cellulose.

observed in the bioreactor containing HDPE, thereby meeting the validity criteria of the standard method ISO 14,852 (the degree of biodegradation of the reference material (cellulose) shall be >60% at the end of the test, with variations of <20% between triplicates; no significant amount of evolved CO_2 (<10%) shall be observed in the negative control).

The final biodegradation levels estimated from the measurement of O_2 consumption (88.6 ± 2.5%, 88.3 ± 3.7% and 78.9 ± 4.4% for PHB, PHBV and PCL, respectively) did not differ substantially from those observed via CO_2 monitoring, but were all found to be slightly higher (see e-supplementary materials). This suggests that both methods are well suited for estimating the biodegradability of plastics under aerobic conditions in aqueous medium. It is, however, interesting that the determined biodegradability of PHB showed the highest discrepancy of about 5% (83% vs. 88%) amongst the tested polymers, indicating that other oxygen depleting processes may have occurred concomitantly. Indeed, nitrification occurred during the assay (with measured concentrations of nitrate ranging from 51.4 to 90.0 mg/L depending on the material), implying that the oxygen demand from nitrogen oxidation should be considered to accurately measure the aerobic biodegradability of plastics. Kunioka and co-workers reported in a composting study that PCL biodegrades more readily into CO_2 rather than forming higher amounts of biomass and metabolites, as it was found for PBS (Kunioka et al., 2009). The authors concluded that the monomer (6-hydroxyhexanoic acid) of PCL is readily incorporated into the beta-oxidation cycle, which requires molecular oxygen as redox partner. PBS, however, was found here not be biodegradable under aqueous conditions, probably due to the difference in microbiota, emphasizing the need for caution in biodegradability comparison between environments and conditions, respectively. Nevertheless, although most material-based carbon (78–88%) was aerobically converted into CO_2 through microbial respiration, it is expected that the ultimate biodegradation of PHB, PHBV and PCL should be even higher since the carbon fraction diverted to new biomass was not determined at this point. Indeed, as will be discussed in Section 3.3, the apparent amount of carbon that is channeled into the formation of new biomass under aerobic conditions could amount to as high as 30%, thereby highlighting the importance of measuring the entire carbon flow endpoint for each polymer during biodegradation, which otherwise would underestimate biodegradability significantly.

The biodegradable nature of PCL under aerobic aquatic conditions herein observed agrees with the results reported by others (Mezzanotte

et al., 2005; Pagga et al., 2001). To the best of the author's knowledge, this is the first time that the aerobic biodegradation behavior of PHB and PHBV have been reported. The differences in biodegradation rate and profile as well as extent of the tested polymers in aqueous medium herein observed, can be assigned to their chemical structure, morphology and difference in formulation of the resin material, respectively (García-Depraect et al., 2021). For instance, the synthetic, aliphatic–aromatic co-polyester PBAT is produced from the polycondensation reaction of adipic acid, terephthalic acid and 1,4- butanediol, which implies that the efficient biodegradation of PBAT requires the enzymatic depolymerization and further metabolization of its constitutive monomers/oligomers. The content of aromatic monomers, such as terephthalic acid, increases the hydrophobic character and the rigidity (packing, crystallinity) of the polymer structure making it more resistant to enzymatic hydrolysis (Zumstein et al., 2017). Contrarily, natural aliphatic polyesters PHB and PHBV are well known to be highly biodegradable in managed and unmanaged environments (Meereboer et al., 2020). Also, the synthetic polymer PCL, a linear aliphatic polyester composed of 6-hydroxyhexanoic acid, can be biodegraded in aqueous aerobic environments. This could be assigned to its structural similarity, particularly of a trimer of 6-hydroxyhexanoate, to cutin degradation products (a natural polymeric compound found in the plant cuticle) (Suzuki et al., 2021). Finally, (bio)plastics are typically formulated and contain low amounts (0.1–10% and higher) of additives (Hahladakis et al., 2018) (in order to produce pellets, so called base resin, through extrusion, for example). These formulation additives may be non-biodegradable and inhibitory, respectively, to the biodegradation process and are likely to vary between the tested bioplastics. Their content, however, could not be determined in this study.

3.2. Biodegradation of bioplastics under anaerobic conditions in aqueous medium

The mineralization curves based on the ratio between the net cumulative gas carbon produced (CO_2 and CH_4) and the theoretical amount of produced biogas are shown in Fig. 2. Following 77 days of incubation, the biodegradability levels of PHB and PBHV on a gas carbon basis were 74.9 ± 1.9% and 71.1 ± 2.4%, respectively. However, when considering the amount of soluble inorganic carbon (DIC) at the end of the test, the degree of biodegradation amounted to 83.9 ± 1.3% and 81.2 ± 1.7%. The time course of anaerobic biodegradation for PHB and PHBV exhibited the same trend, with a long lag phase of ~18 days followed by an active biodegradation phase until day ~ 49, and a gradual decline in the biodegradation rate afterwards. The use of a non-pre-exposed anaerobic sludge as inoculum source could explain the long lag phases observed. It has been argued that PHBV with a 3% 3-hydroxy valerate content has a similar crystallinity to PHB, and thus may display a similar biodegradation behavior (Meereboer et al., 2020). The percentages of biodegradation of cellulose and HDPE (positive and negative reference materials, respectively) were 86.7 ± 2.4% and 1.4 ± 0.3% in 56 and 77 days, respectively; the latter is considered as no mineralization. The final pH values ranged between 6.8 and 7.0, regardless of the tested condition or reference material, which are conducive to the anaerobic digestion process. Therefore, it can be concluded that the anaerobic biodegradability test herein performed was valid in compliance with ISO 14853.

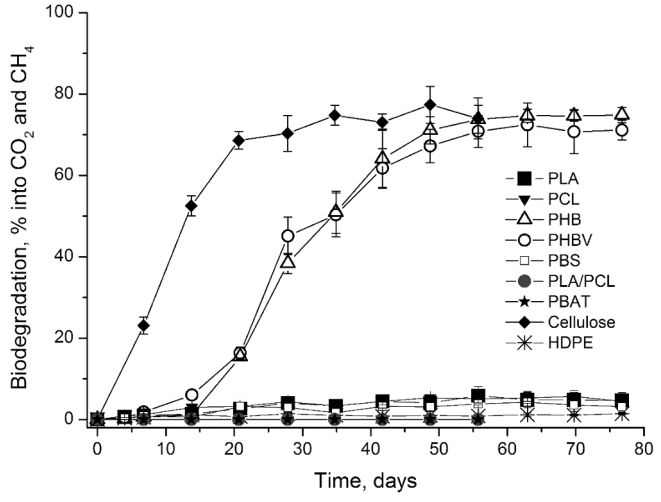

Fig. 2. Biodegradation profiles (expressed as % conversion into CO_2 and CH_4) of PHB, PHBV, PBS, PBAT, PCL, PLA, PLA/PCL blend, HDPE (negative control), and cellulose (positive control) under anaerobic conditions in aqueous medium. Each data point corresponds to the average and standard deviation from triplicate assays. A particle size of 100–250 μm was used in all the cases, except for cellulose.

The degrees of mineralization of PLA, PBS and PCL were 4.6 ± 1.9%, 3.1 ± 1.6 and 4.5 ± 0.3%, respectively, while no (<2%) mineralization was observed for PBAT and the PLA/PCL blend, similarly to HDPE. PCL experienced a low microbial degradation under anaerobic conditions, but it was easily mineralized under aerobic conditions (as discussed in Section 3.1), which was in accordance with earlier studies (Abou-Zeid et al., 2001; Massardier-Nageotte et al., 2006, Hubackova et al., 2013). Assuming the hydrolyzed monomer 6-hydroxyhexanoic acid undergoes beta-oxidation (Kunioka et al., 2009), oxygen is required as redox partner and thus the PCL monomer could not be further metabolized under anaerobic conditions. In addition, the lack of suitable extracellular, hydrolytic enzymes for initial PCL degradation into monomers has been reported (Gan et al., 1997) and is assumed to be exacerbated in the absence of molecular oxygen (O_2) since anaerobic microorganisms grow slower and typically encode fewer enzymes (Siracusa, 2019). Likewise, PLA has been shown to be susceptible to biodegradation only at thermophilic temperatures, which are close to its glass transition temperature (T_g) (Yagi et al., 2009). These high temperatures trigger chemical hydrolysis and facilitate the attachment of microorganisms/enzymes onto the polymer surface by increasing polymer hydrophilicity (Itävaara et al., 2002). PBS, which is an aliphatic BBs synthetized from succinic acid and 1,4-butanediol, has also been shown to undergo very little or no biodegradation under anaerobic conditions (Cho et al., 2011; Yagi et al., 2014). Comparatively, polyhydroxyalkanoates can be degraded by many microorganisms using extra- and intracellular PHAs depolymerases because they are produced naturally by living cells/organisms, thus their biodegradation process is easier and more natural. Empirical estimations on carbon fate indeed indicate ultimate biodegradation values of 95.9 ± 1.9% and 93.7 ± 2.8% for PHB and PHBV, respectively, with about 10% of the carbon present in the PHAs diverted to biomass formation (see Section 3.3).

3.3. Distribution of carbon during bioplastics biodegradation under aerobic and anaerobic conditions in aqueous medium

The relevance of conducting an accurate carbon balance, including not only the determination of CO_2, DIC and DOC, but also the carbon assimilated in the form of biomass to accurately measure the ultimate biodegradability of bioplastics, was already pointed out by Pagga et al. (2001). The authors made an international ring-test to investigate the suitability of the standard ISO 14,852 to quantify the biodegradability of plastics and found that the carbon assimilated as biomass contributed significantly to the degree of biodegradation by up to 40%. In this study, the apparent amount of carbon polymer diverted into biomass varied significantly between the polymers and test conditions (aerobic/anaerobic) ranging from 10.0% to 30.5% (Table 1). Note, as the actual biomass in the activated sludge cannot be measured directly, it was thus indirectly calculated from VSS analysis assuming that the stoichiometric formula of biomass is $C_5H_7NO_2$ (see section 2.4), and thus the term apparent biomass fraction will be used. The sum of the directly

Table 1
Carbon balance for the biodegradation of biodegradable bioplastics under aerobic/anaerobic conditions in aqueous medium.

	Aerobic conditions			Anaerobic conditions	
Carbon, %	PHB	PHBV	PCL	PHB	PHBV
C-CO_2	73.4 ± 2.6	70.5 ± 0.9	63.8 ± 0.9	18.7 ± 0.4	19.5 ± 0.4
C-CH_4	–	–	–	58.3 ± 0.5	55.3 ± 0.3
C-DIC	8.3 ± 0.4	7.1 ± 0.8	7.7 ± 1.1	12.4 ± 0.7	10.7 ± 0.1
C-DOC	3.7 ± 0.2	3.3 ± 0.4	3.4 ± 0.7	1.2 ± 0.2	1.7 ± 0.0
*C-biomass, app.	13.6 ± 3.1	14.3 ± 1.1	30.5 ± 0.5	10.0	10.0
Residual C-polymer	n.d.	n.d.	n.d.	n.d.	n.d.
C-total (recovery)	99.1 ± 0.8	95.2 ± 0.8	105.5 ± 1.5	100.7 ± 0.7	97.2 ± 0.3

Note: n.d., not determined; app., apparent. *Under aerobic conditions, the carbon derived from the apparent biomass (measured as VSS) was calculated assuming that the stoichiometric formula of biomass is $C_5H_7O_2N$ (van Haandel and van der Lubbe, 2012). Under anaerobic conditions, the carbon fixed in the form of biomass was assumed to be 10% of the total initial carbon (Chernicharo, 2007).

measured soluble organic and inorganic fraction as well as the respiratory gasses together with the aparent biomass provide a good estimation for the carbon balance approach.

3.3.1. Aerobic biodegradation

The carbon mass balance analysis performed from the BBs biodegradation assays in aqueous medium under aerobic and anaerobic conditions is summarized in Table 1. The assessment of carbon fate under aerobic conditions lasted for 16-days. When PHB, PHBV and PCL were biodegraded in an aerobic aqueous environment, most carbon (up to 73.4%) was diverted to the generation of CO_2 gas, while apparent biomass growth accounted for 13.6–30.5% depending on the bioplastic. The amount of carbon trapped in the liquid phase in the form of dissolved inorganic carbon (DIC) or soluble organic compounds (DOC) was, on average, 8.3% and 3.4%, respectively, regardless of the BBs. This shows that DIC and DOC together contribute aproximately 11% to the overall carbon balance and biodegradability value, respectively, and should thus be part of a test standard.

The carbon mass balance for PHB, PHBV and PCL accounted for 99.1 ± 0.8%, 95.2 ± 0.8% and 105.5 ± 1.5% of the initial carbon present in the polymers, respectively. The apparent biomass growth, which equals to the total, apparent biomass concentration measured at the end of the test minus the initial seed biomass concentration, was estimated to 143.3 ± 32.1, 156.7 ± 11.5 and 363.3 ± 5.8 mg VSS/L for PHB, PHBV and PCL, respectively. This translates into average cell yields ($Y_{X/S}$) of 0.14, 0.15 and 0.36 mg VSS/mg bioplastic for PHB, PHBV and PCL, respectively. Interestingly, the average CO_2 yield on substrate ($Y_{CO2/S}$) was 1600 mg CO_2/g bioplastic, regardless of the BBs, which accounted for 80.3, 77.2 and 70.4% of the theoretical total CO_2 production from PHB, PHBV and PCL, respectively.

The higher (apparent) biomass fraction produced from PCL (30.5%) under aerobic aqueous conditions was about double to that of PHB (13.5%) and PHBV (14.3%). A possible explanation for higher apparent biomass could be that PCL and its monomer 6-hydroxyhexanoic acid undergoes beta-oxidation, providing the energy for ATP synthesis and acetyl-CoA that can be used for cell growth besides further oxidation via the TCA cycle (Jimenez-Diaz et al., 2019). Based on a previous study, PCL can be considered highly biodegradable under aerobic composting conditions (Funabashi et al., 2007). Yet, the 7–10% less CO_2 production compared to the PHAs (63.8% vs. 73.4% and 70.5%, Table 1) together with the similar DIC and DOC values (about 11% in total), may not fully explain the two-fold increase in apparent biomass. The total (apparent) carbon recovery of over 100% (105.5%) for PCL may suggest that the PCL formulation contained about 5% of non-biodegradable additives (Hahladakis et al., 2018) that could have remained in the sludge.

For all three polymers, the DOC analyses herein conducted did not allow to distinguish between microbial metabolic products (e.g., proteins) or degradation intermediates dissolved in the aqueous phase. Yet, it is reasonable to assume that the residual polymer was negligible in this study, not only because of the total carbon recovery values being close to 100% (95.2–105.5%), but also standard resin material of PHB, PHBV and PCL have been used that contain minimal amounts of additives and are minimally processed (personal communication by supplier). Moreover, it is well known that the PHAs used in this study are readily biodegradable (Meereboer et al., 2020; Mezzanotte et al., 2005). Nevertheless, for new polymer formulations and final products (e.g., packaging), it should be recommended, for studies and official standard testing alike, to assess the risk of not fully biodegraded and non-biodegradable residuals that origin from insufficient incubation time and non-biodegradable components, respectively. Nevertheless, the here presented carbon balance approach can provide satisfactory carbon recovery values, and thus would provide a meaningful methodological addition to the existing standard test methods.

3.3.2. Anaerobic biodegradation

The distribution of carbon during the anaerobic biodegradation of PHB and PHBV was found to be comparable. Most carbon (≈77%) at the end of the assay was present in the gas phase in the form of CO_2 and CH_4, the latter accounting for 58.3 ± 0.4% and 55.3 ± 0.3% of the total carbon content of PHB and PHBV, respectively. The maximum CH_4 yields were 495.8 ± 4.0 NmL CH_4/g PHB and 480.1 ± 15.5 NmL CH_4/g PHBV, which are similar or even higher than those using food waste (Demichelis et al., 2017) or liquid swine manure codigested with agro-industrial wastes (Schievano et al., 2014) as the substrate. The percentage of the plastic carbon present as DIC at the end of the assay was 12.4 ± 0.7% for PHB and 10.7 ± 0.1% for PHBV, while the dissolved organic compounds represented around 1.5% regardless of the BBs, which is theoretically equivalent to a final acetate concentration of 37.5 mg/L (corresponding to “healthy” anaerobic digestion). The plastic carbon present in the biomass was assumed to be 55.8 and 58.1 mg for PHB and PHBV, respectively, (equivalent to 10% of the total initial carbon) (Chernicharo, 2007). Biomass shares of approximately 5–15% of the total biodegradable organic matter are typically determined in well balanced anaerobic digestion systems (Chernicharo, 2007). The average carbon recoveries were in the range of 97.2 to 100.7%, with a small standard deviation, thereby suggesting that PHB and PHBV formulation used here were (almost) completely biodegraded under anaerobic conditions. Note, although the employed biodegradable materials are in form of standard resins and minimally processed, they may contain small amounts of additives needed for producing the resins as well as designed for dedicated applications and processing, which would explain the gap to 100% anaerobic biodegradability in this study.

The results of this aerobic and anaerobic biodegradation study confirmed that the measurement of gas carbon generation (CO_2 and CH_4) in standard biodegradation tests alone is not a reliable proxy to estimate the extent of biodegradation of plastics at the end of the test, but rather for the biodegradation rate, as previously suggested in other works (Pagga et al., 2001; Urstadt et al., 1995). Test standards for biodegradability assessment typically utilize cellulose as a reference to represent a 100% biodegradable material, which does not account for differences in metabolism and carbon sinks depending on the materials used and may well under- or overestimate the fraction of biomass produced as clearly demonstrated in this study. In addition, nor do todays test standards quantify soluble and insoluble carbon matter in the remaining media, which amounted to about 11% for all biodegradable materials tested in this study (see Table 1). Therefore, a more accurate approach is recommended to evaluate the biodegradation of BBs via a

detailed carbon mass balance considering not only the carbon in the form of gas products and biomass, but also the fractions of DIC, DOC and residual polymer (if any).

3.4. Influence of particle size on BB biodegradation

After grinding and sieving the BBs in its resin form, the biodegradation of the resulting sieved fractions, i.e., 100–250, 250–500, 500–1000 μm, was evaluated under aerobic and anaerobic conditions in aqueous medium. The specific surface area for the tested particle sizes was mathematically calculated by assuming the particles shape as spheres with a diameter equal to the median of each particle range, as reported elsewhere (Chinaglia et al., 2018). The theoretical average specific surface areas were 274.3, 128.0 and 64.0 cm^2/g material for the fractions of 100–250, 250–500, 500–1000 μm, respectively, regardless of the BBs.

The effect of particle size on the extent and rate of biodegradation is shown in Fig. 3 for aerobic conditions and Fig. 4 for anaerobic conditions. Regardless of the BBs and environment tested, the results clearly show that the lower the particle size (higher specific surface area), the higher the mineralization rate. When the BBs were biodegraded under aerobic conditions, there were no clear differences among treatments in the final extent of mineralization, sustaining a net total CO_2 production in the gas phase of 1424.5 ± 93.1, 1378.3 ± 81.7 and 1683.5 ± 58.9 mg for PHB, PHBV and PCL, respectively, which corresponded to 69.6 ± 4.5%, 64.7 ± 3.8% and 72.7 ± 2.5% of the respective theoretical amount of CO_2 evolved. Likewise, a similar behavior in the biodegradation curves of PHB and PHBV was observed under anaerobic conditions (Fig. 4a and b). However, the degree of biodegradation of the PHB with a

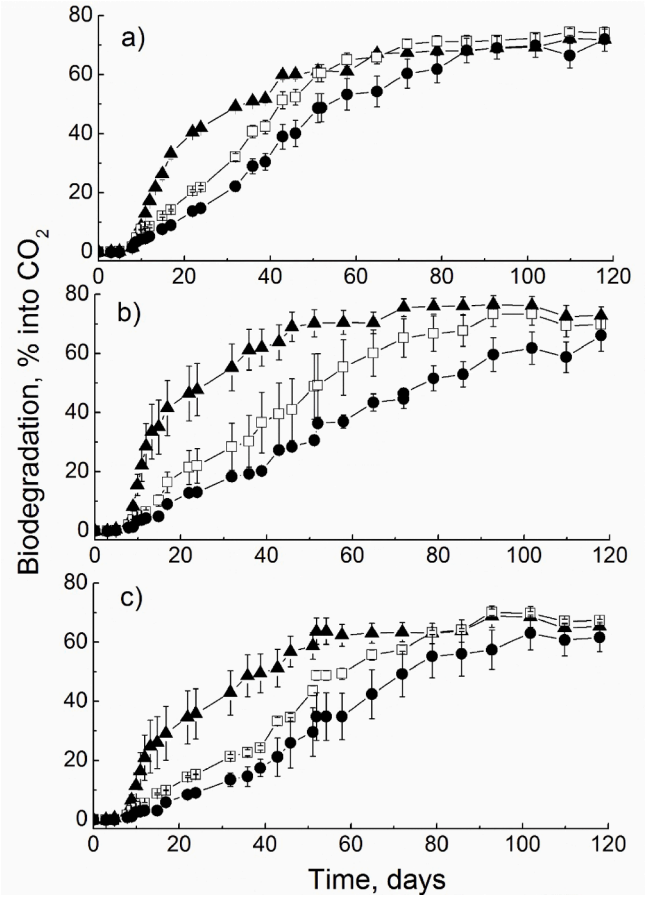

Fig. 3. Effect of particle size on the aerobic biodegradation of a) PCL, b) PHB and c) PHBV. Particle sizes: 100–250 μm (filled triangles), 250–500 μm (open squares) and 500–1000 μm (filled circles).

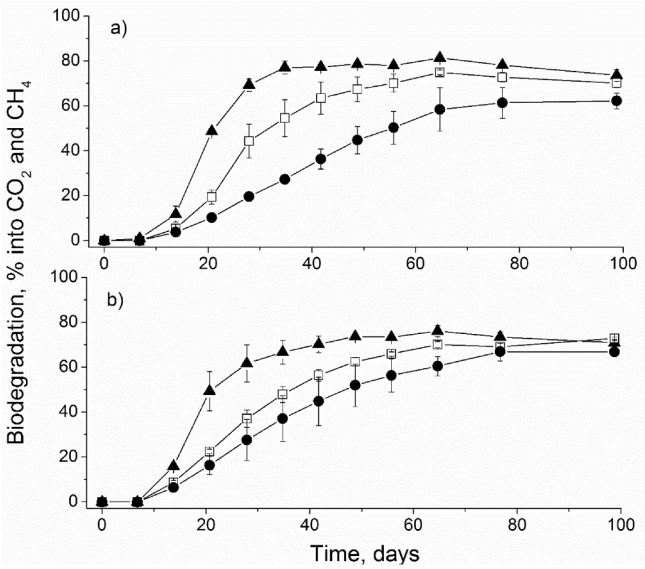

Fig. 4. Effect of particle size on the anaerobic biodegradation of a) PHB and b) PHBV. Particle sizes: 100–250 μm (filled triangles), 250–500 μm (open squares) and 500–1000 μm (filled circles).

particle size of 500–1000 μm was slightly lower compared to the other powder fractions. Hence, the net total carbon production present in the gas phase for PHB was 436.7 ± 1.5, 406.5 ± 11.7 and 347.4 ± 19.4 mg; and 426.7 ± 13.1, 423.3 ± 3.2 and 342.8 ± 38.6 mg for PHBV, using particles sizes of 100–250, 250–500 and 500–1000 μm, respectively. Evident from Figs. 3 and 4, linear kinetic profiles indicate that the lower available specific surface area is rate limiting for the biodegradation process and could be explained by pseudo-zero order kinetics. Under the assumption of biodegradation requiring an initial surface erosion step that is executed by extracellular enzymes, the biodegradation rate is dependent on the specific surface area that contains available, enzymatically cleavable polymer bonds to produce metabolizable products for mineralization (Chinaglia et al., 2018).

The effect of particle size on biodegradation kinetics is given in Table 2. Kinetic data fitting resulted in calculated R^2 values higher than 0.985, indicating that the modified Gompertz model adequately describes the BB biodegradation process (see e-supplementary materials). The cumulative mineralization rate was inversely correlated with the particle size regardless of the environment and bioplastic. The lower particle size herein tested supported up to 331 and 405% higher maximum aerobic and anaerobic biodegradation rates, respectively, than those obtained with a particle size of 500–1000 μm. Particle size did not have a markedly influence on the lag phase (λ) among all the tested BBs. Note that the model slightly underestimated the lag time of the lower particle size (100–250 μm) under aerobic conditions (see e-supplementary materials). However, bioplastics with a larger particle size still required longer times to be aerobically/anaerobically biodegraded because less surface area was available for bacterial biodegradation. For instance, using particle sizes of 100–250, 250–500 and 500–1000 μm, the anaerobic biodegradation of PHB and PHBV reached a plateau after 35, 42 and 65 days and 35, 56 and 65 days, respectively. Under aerobic conditions, the smallest particle size of PHAs required around 52 days of incubation to reach a plateau in the biodegradation, while the largest particle size needed a 3-month degradation time. Likewise, the aerobic biodegradation of PCL (100–250 μm) plateaued after 65 days of incubation, while 79 and 86 days were needed for particle size ranges of 250–500 and 500–1000 μm, respectively.

Based on the obtained biodegradation results, small particle sizes are clearly advantageous to reduce the assay time for biodegradation test standards and to eliminate surface-limiting effects, in order to assess the

Table 2
Comparison of the modelled biodegradation kinetics for the tested bioplastics at different particle sizes and conditions (aerobic, anaerobic).

Material	Particle size range, μm	*P*, mg C/g-material		*R*, mg C/g-material·day		λ, days		R^2	
		AE	AN	AE	AN	AE	AN	AE	AN
PHB	100–250	400.3 ± 7.2	436.1 ± 5.7	17.4 ± 6.7	32.1 ± 1.5	4.4 ± 1.0	11.9 ± 0.7	0.9855 ± 4.8E-03	0.9978 ± 7.2E-04
	250–500	420.3 ± 9.6	406.0 ± 6.5	6.9 ± 1.6	17.9 ± 5.7	8.6 ± 1.8	13.9 ± 2.5	0.9955 ± 1.0E-03	0.9969 ± 1.2E-03
	500–1000	380.9 ± 44.6	364.5 ± 30.3	4.6 ± 0.3	7.8 ± 1.6	11.6 ± 1.6	14.5 ± 1.6	0.9956 ± 8.4E-04	0.9982 ± 2.0E-03
PHBV	100–250	375.8 ± 10.1	424.2 ± 9.6	11.1 ± 3.8	28.1 ± 12.2	3.6 ± 0.8	9.9 ± 1.4	0.9873 ± 6.9E-03	0.9979 ± 1.2E-03
	250–500	426.1 ± 9.4	416.4 ± 1.2	6.4 ± 0.3	12.2 ± 1.2	12.4 ± 0.3	10.3 ± 0.6	0.9953 ± 2.5E-04	0.9982 ± 1.1E-03
	500–1000	403.7 ± 25.9	400.9 ± 13.7	5.6 ± 1.3	9.6 ± 3.3	19.0 ± 0.5	11.5 ± 0.4	0.9965 ± 1.0E-03	0.9990 ± 3.2E-04
PCL	100–250	420.5 ± 4.9	–	14.1 ± 0.8	–	5.0 ± 0.1	–	0.9881 ± 1.0E-03	–
	250–500	472.1 ± 7.5	–	9.7 ± 0.6	–	9.2 ± 0.3	–	0.9981 ± 1.4E-04	–
	500–1000	450.7 ± 22.6	–	7.7 ± 1.0	–	12.1 ± 0.8	–	0.9978 ± 3.4E-04	–

Notes: AE, aerobic conditions; AN, anaerobic conditions. Data on cumulative gas carbon production from the different bioplastics were adjusted to the modified Gompertz model. *P*, carbon yield; *R*, rate of gaseous carbon (CO_2, CH_4) formation; λ, lag phase; R^2: goodness of fit.

intrinsic biodegradability of a material, as previously outlined by Chinaglia et al. (2018), who assessed the effect of particle size on the biodegradation of the polyester poly(1,4-butylene sebacate) under controlled composting conditions at laboratory scale. On the other hand, such biodegradability results, originating from standard tests and typically communicated via a certificate by a material supplier, provide only limited applicability. For example, the anaerobic digestion of BBs with energy recovery represents a promising end-of-life opportunity but only for some BBs such as PHAs (Abraham et al., 2021). However, the fastest anaerobic biodegradation time to reach full conversion of PHA required in this study, of 35–42 days, using the smallest particle sizes (100–250 μm) would still require residence times in anaerobic digesters significantly longer than the typical residence times applied in sludge, urban solid waste or livestock manure digesters under mesophilic conditions (20–30 days). In addition, grinding the plastic waste to such small particle size fraction as tested in this study is impractical and probably prohibitive from an energy balance point of view for scale-up. In this context, commercial grinders at full scale can shred plastics into pieces with higher lengths (few cm), which are expected to require comparatively longer digestion times due to a surface area limitation, pointing out the importance of applying other robust, cost-effective, and efficient BBs pretreatments. The application of mechanical, thermal, or chemical pretreatments can also help to reduce the degree of crystallinity and the molecular mass of the bioplastics while increasing polymer porosities and specific surface area, thus facilitating their accessibility to enzymatic attack and lower residence times (García-Depraect et al., 2021).

3.5. Implications of the study and further research needs

This work aims at extending the biodegradation standard test methods for bioplastics through a more detailed analysis including kinetics, carbon fate and effect of particle size. To the best of our knowledge, this is the first study applying this comprehensive analysis to systematically investigate and compare the biodegradation rate and extent of seven common polyester-based bioplastics under aerobic and anaerobic aqueous conditions. The results obtained highlight the usefulness of the carbon balance approach to improve both the development and testing of biodegradable materials/products. Importantly, the increase in the biomass growth should be considered to determine polymer fate and to accurately assess the ultimate biodegradability. Moreover, it should be discussed to which extent test standards and methods should and can be optimized to achieve full biodegradation, as they should rather attempt to mimic the receiving environment of the bioplastic waste product, which, however, can vary significantly and thus may make it difficult to develop a representative test standard. It was beyond the scope of this study to propose and validate a new method for determining the biodegradability of plastic materials but should be taken into consideration in future studies. Further improvements in the biodegradability of bioplastics require not only the design of tailor-made BBs but also of in-depth mechanistic studies on biodegradation. More research is necessary to provide further insights in the BBs-degrading microorganisms and their related enzymes and biodegradation mechanisms. To achieve superior biodegradability features, constitutive monomers or oligomers derived either via enzymatic or chemical reactions, should be further metabolized by microorganisms. In this context, the development of efficient engineered biocatalysts is a current research gap.

Additionally, this study provides implications to promote a practical and enhanced end-of-life management of bioplastics, especially via anaerobic digestion which is currently applied in many developed and developing countries facing the plastic pollution problem. The development and evaluation of pretreatment aided anaerobic digesters co-fed with (bio)plastics is a relevant topic for research in the field. Thus, the assessment of how bioplastics affect in the long term the operational performance and microbiology of the process is highly recommended. The possibility that persistent micro- and nanoplastics and additives are released during the biodegradation of bioplastics, and their potential toxicological effect on the environment and human health, should also be assessed in further studies, as recently pointed out by Liao and Chen (2021). Finally, novel integrated upcycling strategies for the microbial production, degradation and valorization of BBs should be pursued in the context of a circular bioeconomy.

4. Conclusions

The biodegradation kinetics, extent, and carbon fate of several bioplastics was investigated under aerobic/anaerobic conditions in aqueous medium. According to test standards, PCL was biodegraded only under aerobic conditions, while PHB and PHBV were biodegraded regardless of the conditions. The C-balance analysis estimated the different carbon sinks (gasses, biomass, soluble compounds), thus, would add a valuable analysis to the existing biodegradation tests. Lower particle sizes favored higher mineralization rates, reducing the assay duration, but may remain unrealistic/impractical for waste treatment applications. Conclusively, combining C-balance and kinetic analysis can aid to improve the development and testing of biodegradable materials and waste treatments.

CRediT authorship contribution statement

Octavio García-Depraect: Conceptualization, Methodology, Investigation, Writing – original draft. **Raquel Lebrero:** Conceptualization, Supervision, Project administration, Writing – review & editing. **Sara Rodriguez-Vega:** Investigation, Writing – review & editing. **Sergio Bordel:** Conceptualization, Writing – review & editing. **Fernando Santos-Beneit:** Conceptualization, Writing – review & editing. **Leonardo J. Martínez-Mendoza:** Investigation, Writing – review & editing. **Rosa Aragão Börner:** Conceptualization, Funding acquisition, Writing – review & editing. **Tim Börner:** Conceptualization, Funding acquisition, Writing – review & editing. **Raúl Muñoz:** Conceptualization,

Funding acquisition, Project administration, Methodology, Supervision, 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.

Acknowledgments

This project was financed by Société des Produits Nestlé S.A. The financial support from the Regional Government of Castilla y León and the EU-FEDER programme (CLU 2017-09 and UIC 315) is also gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2021.126265.

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