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

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

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11

12 **Abstract**

13 The biodegradation of PHB, PHBV, PBS, PBAT, PCL, PLA, and a PLA-PCL blend was
14 systematically compared under aerobic and anaerobic aqueous conditions assessing
15 biodegradation kinetics, extent, carbon fate and particle size influence (in the range of 100–1000
16 μm). Under standard test conditions, PHB and PHBV were biodegraded anaerobically ($83.9\pm 1.3\%$
17 and $81.2\pm 1.7\%$, respectively) in 77 days or aerobically ($83.0\pm 1.6\%$ and $87.4\pm 7.5\%$) in 117 days,
18 while PCL was only biodegraded ($77.6\pm 2.4\%$) aerobically in 177 days. Apparent biomass growth
19 accounted for up to 30.5% of the total initial carbon depending on the bioplastic and environment.
20 Maximum aerobic and anaerobic biodegradation rates were improved up to 331 and 405%,
21 respectively, at the lowest particle size tested (100–250 μm). This study highlights the usefulness
22 of a more detailed analysis of biodegradation kinetics and carbon fate to improve both the
23 development and testing of biodegradable materials/products in the context of a circular
24 bioeconomy.

25 **Keywords:** Aerobic biodegradation; biodegradation tests; bioplastics; circular economy; end-of-life
26 management; organic recycling.

27

28 **1. Introduction**

29 Owing to their biodegradability and circularity potential, the use of biodegradable bioplastics
30 (BBs) has gained increasing momentum to face the global plastic pollution problem (Dorigato,
31 2021; García-Depraect et al., 2021). Although the BBs produced worldwide (1.2 million tons in
32 2020) currently represent a small share (~1%) of the global production of plastics, a significant
33 market growth for BBs of almost 50% is expected by 2025 (European Bioplastics, 2021). The
34 growth on the use of BBs is fostered by the European Union and beyond through the
35 implementation of different measures devoted to adopting a sustainable plastics economy while
36 mitigating plastic pollution. The European Commission launched the Circular Economy Action
37 Plan (CEAP) and the European Green Deal, which included different recently published and
38 forthcoming initiatives on single-use plastics, plastic packaging, microplastics, as well as bio-
39 based, biodegradable and compostable plastics (European Commission, 2021). The
40 implementation of such policy framework in a comprehensive way will require an improved
41 knowledge on bioplastics, addressing fundamental and priority issues such as their
42 biodegradability, recyclability, toxicological safety (Ding et al., 2021; Malafaia et al., 2021) and
43 their direct or indirect impact on surrounding environments (Bandini et al., 2020; Sanz-Lázaro et
44 al., 2021; Zhou et al., 2021).

45 Despite the extent and rate of biodegradation of several bioplastics have been previously
46 investigated in different environments under both aerobic and anaerobic conditions, the results
47 obtained are not conclusive and more studies are needed to deepen our understanding of BBs
48 biodegradation (Bátori et al., 2018; Chamas et al., 2020; Folino et al., 2020; Shrestha et al.,
49 2020). For instance, a recent literature review on the anaerobic biodegradation of poly(lactic acid)

50 (PLA) and poly(ϵ -caprolactone) (PCL) has highlighted the significant variability in their
51 biodegradation level, without a clear correlation to temperature or incubation time (Quecholac-
52 Piña, 2020). Although biodegradability tests are conducted under standardized laboratory
53 conditions following International (ISO), European (CEN) and American (ASTM) standards, for
54 example, there are several factors affecting the rate and extent of biodegradation that vary
55 among the reported studies. Biotic and abiotic factors depend on the specific experimental
56 conditions as well as the nature of the microorganisms involved. Hence, more systematic,
57 comparative testing and mechanistic data on the biodegradation of BBs is still needed to engineer
58 biodegradable materials/products, to develop end-of-life management processes, and to gain a
59 detailed and comprehensive understanding of the mechanisms underlying bioplastic
60 biodegradation. In this context, each BB material must be evaluated under the same testing
61 scheme in order to achieve unbiased comparisons of biodegradability results (SEPEA, 2020).

62 The biodegradation extent of plastics is typically followed by measuring the oxygen demand or
63 carbon dioxide (CO_2) evolution, or the amount of CO_2 and methane (CH_4) evolved when the
64 plastic material is either aerobically or anaerobically biodegraded. Thus, biodegradation standard
65 tests only account for the mineralized carbon, overlooking the carbon fixed in the form of cell
66 biomass (Chinaglia et al., 2018; García-Depraect et al., 2021). The terms “mineralization” and
67 “biodegradation” are commonly used interchangeably in the literature for the sake of simplicity;
68 however, they are related to different processes. Yet, mineralization represents the last stage
69 following biodeterioration and biofragmentation, but is in fact an essential step in the
70 biodegradation process. Mineralization provides information about the actual metabolic capability
71 of a microorganism or a community to convert the polymer monomers or fragments that have
72 been generated in the preceding two stages into biomass, gasses (CO_2 and/or CH_4), water and
73 potentially other metabolites [(further details on biodegradation mechanism can be found, for
74 example, in the review by García-Depraect et al. (2021)]. In this context, an overall carbon

75 balance analysis (for the different carbon sinks) could provide a more meaningful and
76 comprehensive information of biodegradation (Chiellini et al., 2007; Pagga et al., 2001; Urstadt et
77 al., 1995). The carbon balance approach may in fact be vital to reliably quantify biodegradation
78 and can thus allow for a sustained judgement regarding the polymer fate and completeness of
79 biodegradation in a defined environment as compared to the current methods defined in the
80 international and local biodegradation test standards (ISO, ASTM, CEN, etc.). However, very little
81 is known about the fate of carbon during the biodegradation of the various BBs. Moreover,
82 despite it is well recognized that particle size and surface area are important interdependent
83 factors affecting the process of biodegradation of plastics, only few studies have investigated their
84 impact on biodegradation (Chinaglia et al., 2018; Yagi et al., 2012), and comparatively less
85 attention has been devoted to aqueous environments. Therefore, a complementary experimental
86 framework other than the guidelines set by the existing biodegradation test standards for
87 bioplastics is required to get a deeper understanding of their biodegradation and the correlation
88 between the different factors influencing biodegradation (García-Depraect et al., 2021).

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

100 **2. Materials and methods**

101 **2.1. Materials**

102 The BBs used in this study were PHB (ENMAT™ Y3000P), PHBV (ENMAT™ Y1000P, 3
103 mol% HV), PBS (BioPBS™ FZ91PM/FZ91PB), PBAT (Mvera® B5037), PLA (LUMINY® L105),
104 PCL (Capa® 6500D), and PLA/PCL 80/20 blend (PLA Luminy® L105/PCL Capa® 6500D). All BBs
105 were purchased from the Technological Institute of Packaging, Transportation and Logistics
106 (ITENE, Spain). In addition, microcrystalline cellulose with a particle size distribution ($\geq 80\%$) of
107 20-160 μm (Merck Ltd., Germany, CAS number 9004-34-6) and high density polyethylene
108 (HDPE) (Sigma-Aldrich, USA, product number 427985) were used as the reference materials for
109 positive and negative controls, respectively. Polymer data according to its technical data sheet is
110 summarized in the **e-supplementary material**.

111 The plastic materials, which were initially in a pellet form, were grinded in a commercial
112 blender (Cecotec Titanium 2000 pro, Spain) equipped with titanium blades. Repeated crushing
113 (~3 min on, ~5 min off) using dry ice as a cooling strategy was employed to avoid melting and
114 recrystallization, as reported elsewhere (Yagi et al., 2012). Finally, the polymer powders were
115 sieved using an electromagnetic sieve (CISA RP-20, Spain) with stainless-steel sieves of 100,
116 250, 500 and 1000 μm and then dried at room temperature. The different powder fractions were
117 stored in closed packaging under dark and dry conditions at room temperature until usage.
118 According to the recommendations of the standards ISO 14852 and ISO 14853, the tested BBs
119 (and HDPE) were used in powdered form with a particle size of 100-250 μm .

120 **2.2. Aerobic biodegradation test**

121 Polymer biodegradation under aqueous aerobic conditions was determined according to the
122 standard ISO 14852: *Determination of the ultimate aerobic biodegradability of plastic materials in*
123 *an aqueous medium — Method by analysis of evolved carbon dioxide*. In brief, the
124 biodegradability test was carried out in 2.1 L gas-tight glass bioreactors (1 L of working volume)

125 containing either 150 mg/L of the tested BBs, cellulose (positive control) or HDPE (negative
126 control), 64.8 mL of the activated sludge inoculum (corresponding to a final concentration of 0.5 g
127 total solids (TS)/L), and 935.2 mL of a mineral salt medium freshly prepared with the following
128 composition (in mg/L): KH_2PO_4 , 85.0; K_2HPO_4 , 217.5; $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 334.0; NH_4Cl , 5.0;
129 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 22.5; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 36.4; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.25. All chemicals used were of analytical
130 grade. Blank tests without any carbon source addition were also performed in parallel to correct
131 for the background (endogenous) CO_2 production. The bioreactors were closed with rubbers
132 septa and aluminum caps, and then incubated under gentle agitation (at 4.5 rpm) in a roller
133 shaker (Wheaton Scientific Products, USA) placed in a controlled-temperature room (25 ± 1 °C)
134 under diffuse light conditions. Activated sludge kindly supplied by the sewage treatment plant of
135 Valladolid (Spain) was employed as inoculum within 1 day after collection. This inoculum was not
136 previously adapted to the biodegradation of the target plastics as the only carbon and energy
137 source. The initial pH of the cultivation broths in the bioreactors was 7.0 ± 0.1 . The CO_2 and O_2
138 concentration in the headspace was measured periodically until the mineralization curve
139 plateaued. When the O_2 concentration in the headspace decreased below 5%, the headspace
140 was aerated with an air compressor for 5 min to prevent O_2 limitation during the biodegradability
141 test. All tests were performed in triplicate.

142 The degree of biodegradation (D_T), expressed in percentage, at time t (in days) was calculated
143 by comparing the cumulative net carbon evolved as CO_2 gas in the bioreactor headspace (in mg)
144 from the test (or reference) material with its corresponding theoretical amount (ThCO_2 , in mg), as
145 shown in Eq. (1); where, $\sum(\text{CO}_2)_{Test}$ is the accumulated mass of CO_2 evolved (in mg) in the
146 bioreactor containing the test (or reference) material between the start of the test and time t , and
147 $\sum(\text{CO}_2)_{Blank}$ is the accumulated mass of CO_2 evolved (in mg) in the blank bioreactor between
148 the start of the test and time t . ThCO_2 was calculated according to Eq. (2), where m is the mass
149 (in mg) of the test material, X_C is the carbon content of the test material (express as a mass

150 fraction) determined from its stoichiometric formula, MM_{CO_2} is the molecular mass of CO_2 , and
 151 MM_C is the molecular mass of carbon. At the end of the biodegradability test, the net mass of
 152 dissolved inorganic carbon (DIC, as CO_2 in the liquid phase), after subtracting the mean blank
 153 values, was added to the cumulative net carbon evolved as CO_2 in the headspace. The final
 154 biodegradation degree (D_F) was then calculated by comparing the total amount of carbon
 155 converted to CO_2 (mCO_{2T}) with its $ThCO_2$, as shown in Eq. (3). Additionally, for the sake of
 156 comparison, biodegradation was also estimated by measuring the consumption of O_2 according
 157 to the standard test method ISO 14851.

$$158 \quad D_T = \frac{\Sigma(CO_2)_{Test} - \Sigma(CO_2)_{Blank}}{ThCO_2} \times 100 \quad (1)$$

$$159 \quad ThCO_2 = m \times X_c \times \frac{MM_{CO_2}}{MM_C} \quad (2)$$

$$160 \quad D_F = \frac{mCO_{2T}}{ThCO_2} \times 100 \quad (3)$$

161 **2.3. Anaerobic biodegradation test**

162 Polymer biodegradation under anaerobic conditions was determined according to the standard
 163 ISO 14853: *Plastics — Determination of the ultimate anaerobic biodegradation of plastics*
 164 *materials in an aqueous system — Method by measurement of biogas production*. The
 165 biodegradability test was performed in 2.1 gas-tight glass bioreactors using the same agitation
 166 apparatus and conditions as described in Section 2.1. First, an aliquot of anaerobic inoculum was
 167 added to obtain the desired concentration of 1 g TS/L. Then, 150 mg/L of the test material,
 168 cellulose or HDPE was introduced accordingly, and finally the bioreactors were filled up with a
 169 defined mineral salt medium up to a total volume of 1 L (the initial pH was 7.1). The bioreactors
 170 were then flushed with pure helium gas (Abello Linde, Barcelona, Spain) for 5 min to ensure
 171 anaerobic conditions (which were corroborated by gas chromatographic analyses and the lack of
 172 color of resazurin, a redox indicator), and incubated in the dark under mesophilic conditions ($36 \pm$
 173 1 °C). The mineral salt medium consisted of the following (g/L): KH_2PO_4 , 0.27; $Na_2HPO_4 \cdot 12H_2O$,

174 1.12; NH₄Cl, 0.53; CaCl₂·2H₂O, 0.075; MgCl₂·6H₂O, 0.1; FeCl₂·4H₂O, 0.02; resazurin, 0.001;
 175 Na₂S·9H₂O, 0.1. All reagents were of analytical grade. The methanogenic inoculum herein used
 176 was obtained from the mesophilic anaerobic sludge digester of Valladolid sewage treatment
 177 plant. This inoculum was not acclimated for plastics biodegradation and was preincubated for 7
 178 days at 36 ± 1 °C, without addition of any nutrient and carbon source, in order to reduce the
 179 background gas production. Prior to use, the inoculum was washed twice (mineral salt medium,
 180 10000 rpm for 10 min at 4 °C) and suspended in fresh mineral salt medium to reduce its
 181 inorganic carbon content (< 20 mg/L) in the final test suspension. Blank (inoculum and mineral
 182 salt medium), positive (cellulose) and negative (HDPE) control tests were also performed. All
 183 assays were conducted in triplicate. After 1 h of incubation at 36 ± 1 °C, gas pressure in the
 184 headspace was measured with a manometer and the excess gas was vented in order to reach
 185 equilibrium. The pressure and concentration of CO₂ and CH₄ in the bioreactor headspace were
 186 measured weekly until the mineralization curve plateaued.

187 The degree of biodegradation (D_T , %) at time t (in days) was estimated by comparing the net
 188 mass of carbon evolved in the headspace (as CO₂ and CH₄) from the test (or reference) material
 189 with the mass, in mg, of carbon of the test/reference material (m_v), as shown in Eq. (4); where
 190 $\Sigma(C_{biogas})_{Test}$ and $\Sigma(C_{biogas})_{Blank}$ are the cumulative mass of gaseous carbon as CO₂ and
 191 CH₄ evolved (in mg) in the bioreactors containing the test (or reference) material and in the blank
 192 bioreactors, respectively, between the start of the test and time t . The final biodegradation degree
 193 (D_F) was calculated using Eq. (5); where mC_T is the total amount, in mg, of organic carbon
 194 converted to inorganic carbon and CH₄ at the end of the test (final net mass of inorganic carbon
 195 in the liquid phase plus the cumulative net carbon evolved in the headspace).

$$196 \quad D_T = \frac{\Sigma(C_{biogas})_{Test} - \Sigma(C_{biogas})_{Blank}}{m_v} \times 100 \quad (4)$$

$$197 \quad D_F = \frac{mC_T}{m_v} \times 100 \quad (5)$$

198 **2.4. Carbon mass balance**

199 A series of experiments was additionally conducted with the aim of estimating carbon fate
200 during the biodegradation of BBs under aerobic and anaerobic conditions. The BBs tested were
201 those significantly biodegraded in the aerobic (i.e., PHB, PHBV, and PCL) and anaerobic (i.e.,
202 PHB and PHBV) biodegradability tests above described. The experiments under aerobic
203 conditions were carried out in 2.1 L gas tight glass bioreactors (0.2 L working volume) at 25 ± 1
204 °C and under diffuse light conditions. Each bioreactor was filled with 5 mL of fresh activated
205 sludge inoculum (resulting in 20 mg volatile suspended solids (VSS)/L), 200 mg of the tested BBs
206 (corresponding to 1.0 g volatile solids (VS)/L), and 195 mL of mineral salt medium (KH_2PO_4 , 3.75
207 g/L; $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 8.73 g/L; NH_4Cl , 0.2 g/L; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 22.5 mg/L; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 36.4 mg/L;
208 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 25 mg/L). A particle size lower than 100 μm was employed to enhance BB
209 bioavailability, as recommended by Pagga et al. (2001). Triplicate assays for each tested BB and
210 blanks (only inoculum and medium) were carried out. The cultures were aerated with air when the
211 oxygen concentration in the headspace decreased below 10% (v/v.) The carbon derived from the
212 apparent biomass (measured as VSS) was calculated assuming that the stoichiometric formula of
213 biomass is $\text{C}_5\text{H}_7\text{NO}_2$.

214 The apparent biomass yield on bioplastic ($Y_{X/S}$, mg VSS/mg bioplastic) was calculated by
215 dividing the apparent net biomass growth (in mg VSS) by the mass of test material (in mg). The
216 CO_2 yield on bioplastic ($Y_{\text{CO}_2/S}$, mg CO_2 /g bioplastic) was calculated by dividing the total net
217 carbon converted to CO_2 (in mg) by the mass of test material (in g). During aerobic
218 biodegradation, the degraded bioplastic carbon was diverted to CO_2 in the gas phase, DIC,
219 dissolved organic carbon (DOC), and new biomass.

220 The assessment of carbon fate under anaerobic conditions was carried out in triplicate under
221 similar experimental conditions to those described for the anaerobic biodegradability test (Section
222 2.2), but with a polymer concentration of 1 g VS/L and using a mineral salt medium with higher
223 buffer capacity by increasing the concentration of KH_2PO_4 and $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ from 0.27 and

224 1.12 g/L to 1.35 and 5.6 g/L, respectively. The anaerobically biodegraded bioplastic carbon was
 225 diverted to CO₂ and CH₄, DIC, DOC and new biomass. The carbon fixed in the form of biomass
 226 was assumed to be 10% of the total initial carbon (Chernicharo, 2015). Theoretical CO₂ (ThCO₂)
 227 and CH₄ (ThCH₄) production were calculated following Buswell's equation (Buswell and Neave,
 228 1930). At STP conditions (0 °C, 1 atm), the maximum biogas yields are 1041 NmL/g PHB and
 229 1083 NmL/g PHBV with a theoretical CH₄ content of 56 and 58%, respectively.

230 **2.5. Influence of particle size on BB biodegradation**

231 The effect of particle size on the bioplastic biodegradation extent and rate was investigated
 232 under aerobic and anaerobic conditions for PHB, PHBV and PCL (the latter only under aerobic
 233 conditions). Similar experiments to the aerobic and anaerobic biodegradation tests previously
 234 described in sections 2.2 and 2.3, respectively, were performed in triplicate using mineral salt
 235 media with high buffer capacity and nutrients concentrations, and 1.0 g VS/L of polymer
 236 concentration (Section 2.4). Three particle sizes were assessed, namely 100–250, 250–500 and
 237 500–1000 µm. Biodegradability data were fitted to the modified Gompertz model (Eq. (6)), as
 238 recommended by Ryan et al. (2017), in order to compare the biodegradation kinetics:

$$239 \quad C = P \times \exp\left\{-\exp\left[\frac{R \times e}{P}(\lambda - t) + 1\right]\right\} \quad (6)$$

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

244 **2.6. Analytical methods**

245 The time course of cumulative gas carbon produced during biodegradation (as CO₂ and CH₄)
 246 was measured by manometric and gas-chromatographic methods, using a pressure transducer
 247 (IFM electronic PN7097, Germany) and a gas chromatograph (Agilent 8860, The Netherlands)
 248 equipped with a thermal conductivity detector (GC-TCD) following procedures described

249 elsewhere (Posadas et al., 2014). Solid concentrations (including VSS as a measure of biomass
250 concentration) were analyzed according to standard methods (APHA, 2005). DIC and DOC were
251 measured by a total organic carbon analyzer (Shimadzu TOC-VCSH, Japan) in filtered samples
252 (0.45 μm). Finally, the concentrations of nitrite and nitrate were quantified by HPLC-IC in filtered
253 samples (0.22 μm) according to Posadas et al. (2014).

254 **3. Results and discussion**

255 **3.1. Biodegradation of bioplastics under aerobic conditions in aqueous medium**

256 Fig. 1 shows the mineralization curves for the tested materials under aerobic conditions. After
257 117 days of testing, the degrees of mineralization of PHB, PHBV and PCL were $79.6 \pm 0.4\%$,
258 $84.5 \pm 9.3\%$ and $75.7 \pm 2.3\%$, respectively. Note that considering the amount of DIC, the final
259 biodegradation degrees were $83.0 \pm 1.6\%$ (PHB), $87.4 \pm 7.5\%$ (PHBV) and $77.6 \pm 2.4\%$ (PCL).
260 These results would be considered as "passed to be biodegradable" according to the ISO
261 standard test pass and fail criteria as they reach at least 90% conversion into CO_2 in comparison
262 to the positive control (cellulose, Figure 1). The profile of the biodegradation curves for these BBs
263 was comparable, showing lag phases of 11–13 days likely due to the fact that microorganisms
264 present in the activated sludge used as inoculum were not adapted to the BBs and required some
265 time to synthesize enzymes such as PHA (polyhydroxyalkanoate) depolymerases, cutinases,
266 lipases and esterases for depolymerization (Pathak and Navneet, 2017). After this lag phase, a
267 rapid CO_2 production was observed up to day 45, with PCL experiencing a slightly higher
268 biodegradation rate than the other BBs but lower than that of cellulose. Comparatively, no
269 significant biodegradation was observed for PBS, PBAT, PLA and the PLA-PCL blend, indicating
270 that those bioplastics are non-biodegradable under the tested aerobic conditions in aqueous
271 medium. As expected, the biodegradation of cellulose reached $86.8 \pm 2.3\%$ in a shorter period
272 (68 days), while neither significant CO_2 production nor O_2 consumption was observed in the
273 bioreactor containing HDPE, thereby meeting the validity criteria of the standard method ISO

274 14852 (the degree of biodegradation of the reference material (cellulose) shall be > 60% at the
275 end of the test, with variations of less than 20% between triplicates; no significant amount of
276 evolved CO₂ (> 10%) shall be observed in the negative control).

277 The final biodegradation levels estimated from the measurement of O₂ consumption (88.6 ±
278 2.5%, 88.3 ± 3.7% and 78.9 ± 4.4% for PHB, PHBV and PCL, respectively) did not differ
279 substantially from those observed via CO₂ monitoring, but were all found to be slightly higher (see
280 e-supplementary materials). This suggests that both methods are well suited for estimating the
281 biodegradability of plastics under aerobic conditions in aqueous medium. It is, however,
282 interesting that the determined biodegradability of PHB showed the highest discrepancy of about
283 5% (83% vs. 88%) amongst the tested polymers, indicating that other oxygen depleting
284 processes may have occurred concomitantly. Indeed, nitrification occurred during the assay (with
285 measured concentrations of nitrate ranging from 51.4 to 90.0 mg/L depending on the material),
286 implying that the oxygen demand from nitrogen oxidation should be considered to accurately
287 measure the aerobic biodegradability of plastics. Kunioka and co-workers reported in a
288 composting study that PCL biodegrades more readily into CO₂ rather than forming higher
289 amounts of biomass and metabolites, as it was found for PBS (Kunioka et al., 2009). The authors
290 concluded that the monomer (6-hydroxyhexanoic acid) of PCL is readily incorporated into the
291 beta-oxidation cycle, which requires molecular oxygen as redox partner. PBS, however, was
292 found here not be biodegradable under aqueous conditions, probably due to the difference in
293 microbiota, emphasizing the need for caution in biodegradability comparison between
294 environments and conditions, respectively. Nevertheless, although most material-based carbon
295 (78–88%) was aerobically converted into CO₂ through microbial respiration, it is expected that the
296 ultimate biodegradation of PHB, PHBV and PCL could be even higher since the carbon fraction
297 diverted to new biomass was not determined at this point. Indeed, as will be discussed in Section
298 3.3, the apparent amount of carbon that is channeled into the formation of new biomass under

299 aerobic conditions could amount to as high as 30%, thereby highlighting the importance of
300 measuring the entire carbon flow endpoint for each polymer during biodegradation, which
301 otherwise would underestimate biodegradability significantly.

302 The biodegradable nature of PCL under aerobic aquatic conditions herein observed agrees
303 with the results reported by others (Mezzanotte et al., 2005; Pagga et al., 2001). To the best of
304 the author's knowledge, this is the first time that the aerobic biodegradation behavior of PHB and
305 PHBV have been reported. The differences in biodegradation rate and profile as well as extent of
306 the tested polymers in aqueous medium herein observed, can be assigned to their chemical
307 structure, morphology and difference in formulation of the resin material, respectively (García-
308 Depraect et al., 2021). For instance, the synthetic, aliphatic-aromatic co-polyester PBAT is
309 produced from the poly-condensation reaction of adipic acid, terephthalic acid and 1,4-
310 butanediol, which implies that the efficient biodegradation of PBAT requires the enzymatic
311 depolymerization and further metabolization of its constitutive monomers/oligomers. The content
312 of aromatic monomers, such as terephthalic acid, increases the hydrophobic character and the
313 rigidity (packing, crystallinity) of the polymer structure making it more resistant to enzymatic
314 hydrolysis (Zumstein et. al., 2017). Contrarily, natural aliphatic polyesters PHB and PHBV are
315 well known to be highly biodegradable in managed and unmanaged environments (Meereboer et
316 al., 2020). Also, the synthetic polymer PCL, a linear aliphatic polyester composed of 6-
317 hydroxyhexanoic acid, can be biodegraded in aqueous aerobic environments. This could be
318 assigned to its structural similarity, particularly of a trimer of 6-hydroxyhexanoate, to cutin
319 degradation products (a natural polymeric compound found in the plant cuticle) (Suzuki et al.,
320 2021). Finally, (bio)plastics are typically formulated and contain low amounts (0.1–10% and
321 higher) of additives (Hahladakis et al., 2018) (in order to produce pellets, so called base resin,
322 through extrusion, for example). These formulation additives may be non-biodegradable and

323 inhibitory, respectively, to the biodegradation process and are likely to vary between the tested
324 bioplastics. Their content, however, could not be determined in this study.

325 **3.2. Biodegradation of bioplastics under anaerobic conditions in aqueous medium**

326 The mineralization curves based on the ratio between the net cumulative gas carbon
327 produced (CO_2 and CH_4) and the theoretical amount of evolved biogas are shown in Fig. 2.
328 Following 77 days of incubation, the biodegradability levels of PHB and PBHV on a gas carbon
329 basis were $74.9 \pm 1.9\%$ and $71.1 \pm 2.4\%$, respectively. However, when considering the amount
330 of soluble inorganic carbon (DIC) at the end of the test, the degree of biodegradability accounted
331 for $83.9 \pm 1.3\%$ and $81.2 \pm 1.7\%$. The time course of anaerobic biodegradation for PHB and
332 PHBV exhibited the same trend, with a long lag phase of ~ 18 days followed by an active
333 biodegradation phase until day ~ 49 , and a gradual decline in the biodegradation rate afterwards.
334 The use of a non-pre-exposed anaerobic sludge as inoculum source could explain the long lag
335 phases observed. It has been argued that PHBV with a 3% HV content has a similar crystallinity
336 to PHB, and thus may display a similar biodegradation behavior (Meereboer et al., 2020). On the
337 other hand, the percentages of biodegradation of cellulose and HDPE (positive and negative
338 reference materials, respectively) were $86.7 \pm 2.4\%$ and $1.4 \pm 0.3\%$ in 56 and 77 days,
339 respectively; the latter is considered as no mineralization. The final pH values ranged between
340 6.8 and 7.0, regardless of the tested condition or reference material, which are conducive to the
341 anaerobic digestion process. Therefore, it can be concluded that the anaerobic biodegradability
342 test herein performed was valid in compliance with ISO 14853.

343 The degrees of mineralization of PLA, PBS and PCL were $4.6 \pm 1.9\%$, 3.1 ± 1.6 and $4.5 \pm$
344 0.3% , respectively, while no ($< 2\%$) mineralization was observed for PBAT and the PLA/PCL
345 blend, similarly to HDPE. PCL experienced a low microbial degradation under anaerobic
346 conditions, but it was easily mineralized under aerobic conditions (as discussed in Section 3.1),
347 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 synthesized 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 $95.9 \pm 1.9\%$ and $93.7 \pm 2.8\%$ for PHB and PHBV, respectively, with 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 14852 to quantify the biodegradability of plastics and found that the carbon assimilated as

373 biomass contributed significantly to the degree of biodegradation by up to 40%. In this study, the
374 apparent amount of carbon polymer diverted into biomass varied significantly between the
375 polymers and test conditions (aerobic/anaerobic) ranging from 10.0% to 30.5% (Table 1). Note,
376 as the actual biomass in the activated sludge cannot be measured directly, it was thus indirectly
377 calculated from VSS analysis assuming that the stoichiometric formula of biomass is $C_5H_7NO_2$
378 (see section 2.4), and thus the term apparent biomass fraction will be used. The sum of the
379 soluble organic and inorganic fraction as well as the respiratory gasses together provide a good
380 estimation for the carbon balance approach.

381 ***Aerobic biodegradation.*** The carbon mass balance analysis performed from the BBs
382 biodegradation assays in aqueous medium under aerobic and anaerobic conditions is
383 summarized in Table 1. The assessment of carbon fate under aerobic conditions lasted for 16-
384 days. When PHB, PHBV and PCL were biodegraded in an aerobic aqueous environment, most
385 carbon (up to 73.4%) was diverted to the generation of CO_2 gas, while biomass growth accounted
386 for 13.6–30.5% depending on the bioplastic. The amount of carbon trapped in the liquid phase in
387 the form of dissolved inorganic carbon (DIC) or soluble organic compounds (DOC) was, on
388 average, below 8.3% and 3.2%, respectively, regardless of the BBs. This shows that DIC and
389 DOC together contribute up to 10% to the overall carbon balance and biodegradability value,
390 respectively, and should thus be part of a test standard.

391 The carbon mass balance for PHB, PHBV and PCL accounted for $99.1 \pm 0.8\%$, $95.2 \pm 0.8\%$
392 and $105.5 \pm 1.5\%$ of the initial carbon present in the polymers, respectively. The apparent
393 biomass growth, which equals to the total, apparent biomass concentration measured at the end
394 of the test minus the initial seed biomass concentration, was estimated as 143.3 ± 32.1 , $156.7 \pm$
395 11.5 and 363.3 ± 5.8 mg VSS/L for PHB, PHBV and PCL, respectively. This translates into
396 average cell yields ($Y_{X/S}$) of 0.14, 0.15 and 0.36 mg VSS/mg bioplastic for PHB, PHBV and PCL,
397 respectively. Interestingly, the average CO_2 yield on substrate ($Y_{CO_2/S}$) was 1600 mg CO_2/g

398 bioplastic, regardless of the BBs, which accounted for 80.3, 77.2 and 70.4% of the theoretical
399 total CO₂ production from PHB, PHBV and PCL, respectively.

400 The higher (apparent) biomass fraction produced from PCL (30.5%) under aerobic aqueous
401 conditions was about double to that of PHB (13.5%) and PHBV (14.3%). A possible explanation
402 for higher apparent biomass could be that PCL and its monomer 6-hydroxyhexanoic acid
403 undergoes beta-oxidation, providing the energy for ATP synthesis and acetyl-CoA that can be
404 used for cell growth besides further oxidation via the TCA cycle (Jimenez-Diaz et al., 2017).
405 Based on a previous study, PCL can be considered highly biodegradable under aerobic
406 composting conditions (Funabashi et al., 2007). Yet, the 7-10% less CO₂ production compared to
407 the PHAs (63.8% vs. 73.4% and 70.5%, Table 1) together with the similar DIC and DOC values
408 (about 10% in total), may not fully explain the two-fold increase in apparent biomass. The total
409 (apparent) carbon recovery of over 100% (105.5%) for PCL may suggest that the PCL
410 formulation contained about 5% of non-biodegradable additives (Hahladakis et al., 2018) that
411 could have remained in the sludge.

412 For all three polymers, the DOC analyses herein conducted did not allow to distinguish
413 between microbial metabolic products (e.g., proteins) or degradation intermediates dissolved in
414 the aqueous phase. Yet, it is reasonable to assume that the residual polymer was negligible in
415 this study, not only because of the total carbon recovery values being close to 100% (95.2–
416 105.5%), but also standard resin material of PHB, PHBV and PCL have been used that contain
417 minimal amounts of additives and are minimally processed (personal communication by supplier).
418 Moreover, it is well known that the PHAs used in this study are readily biodegradable (Meereboer
419 et al., 2020; Mezzanotte et al., 2005). Nevertheless, for new polymer formulations and final
420 products (e.g., packaging), it should be recommended, for studies and official standard testing
421 alike, to assess the risk of not fully biodegraded and non-biodegradable residuals that origin from
422 insufficient incubation time and non-biodegradable components, respectively.

423 **Anaerobic biodegradation.** The distribution of carbon during the anaerobic biodegradation of
424 PHB and PHBV, however, was found to be comparable. Most carbon ($\approx 77\%$) at the end of the
425 assay was present in the gas phase in the form of CO_2 and CH_4 , the latter accounting for $58.3 \pm$
426 0.4% and $55.3 \pm 0.3\%$ of the total carbon content of PHB and PHBV, respectively. The maximum
427 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
428 similar or even higher than those using food waste (Demichelis et al., 2017) or liquid swine
429 manure codigested with agroindustrial wastes (Schievano et al., 2014) as the substrate. The
430 percentage of the plastic carbon present as DIC at the end of the assay was $12.4 \pm 0.7\%$ for PHB
431 and $10.7 \pm 0.1\%$ for PHBV, while the dissolved organic compounds represented around 1.5%
432 regardless of the BBs, which is theoretically equivalent to a final acetate concentration of 37.5
433 mg/L (corresponding to “healthy” anaerobic digestion). The plastic carbon present in the biomass
434 was assumed to be 55.8 and 58.1 mg for PHB and PHBV, respectively, (equivalent to 10% of the
435 total initial carbon) (Chernicharo, 2015). Biomass shares of approximately $5\text{--}15\%$ of the total
436 biodegradable organic matter are typically determined in well balanced anaerobic digestion
437 systems (Chernicharo, 2015). The average carbon recoveries were in the range of 97.2 to
438 100.7% , with a small standard deviation, thereby suggesting that PHB and PHBV formulation
439 used here were (almost) completely biodegraded under anaerobic conditions. Note, although the
440 employed biodegradable materials are in form of standard resins and minimally processed, they
441 may contain small amounts of additives needed for producing the resins as well as designed for
442 dedicated applications and processing, which would explain the gap to 100% anaerobic
443 biodegradability in this study.

444 The results of this aerobic and anaerobic biodegradation study confirmed that the
445 measurement of gas carbon generation (CO_2 and CH_4) in standard biodegradation tests alone is
446 not a reliable proxy to estimate the extent of biodegradation of plastics at the end of the test, but
447 rather for the biodegradation rate, as previously suggested in other works (Pagga et al., 2001;

448 Urstadt et al., 1995). Test standards for biodegradability assessment typically utilize cellulose as
449 a reference to represent a 100% biodegradable material, which does not account for differences
450 in metabolism and carbon sinks depending on the materials used and may well under- or
451 overestimate the fraction of biomass produced as clearly demonstrated in this study. In addition,
452 nor do today's test standards quantify soluble and insoluble carbon matter in the remaining media,
453 which amounted to about 11% for all biodegradable materials tested in this study (see Table 1).
454 Therefore, a more accurate approach is recommended to evaluate the biodegradation of BBs via
455 a detailed carbon mass balance considering not only the carbon in the form of gas products and
456 biomass, but also the fractions of DIC, DOC and residual polymer (if any).

457 **3.4. Influence of particle size on BB biodegradation**

458 After grinding and sieving the BBs in its resin form, the biodegradation of the resulting sieved
459 fractions, i.e., 100–250, 250–500, 500–1000 μm , was evaluated under aerobic and anaerobic
460 conditions in aqueous medium. The specific surface area for the tested particle sizes was
461 mathematically calculated by assuming the particles shape as spheres with a diameter equal to
462 the median of each particle range, as reported elsewhere (Chinaglia et al., 2018). The theoretical
463 average specific surface areas were 274.3, 128.0 and 64.0 cm^2/g material for the fractions of
464 100–250, 250–500, 500–1000 μm , respectively, regardless of the BBs.

465 The effect of particle size on the extent and rate of biodegradation is shown in Fig. 3 for
466 aerobic conditions and Fig. 4 for anaerobic conditions. Regardless of the BBs and environment
467 tested, the results clearly show that the lower the particle size (higher specific surface area), the
468 higher the mineralization rate. When the BBs were biodegraded under aerobic conditions, there
469 were no clear differences among treatments in the final extent of mineralization, sustaining a net
470 total CO_2 production in the gas phase of 1424.5 ± 93.1 , 1378.3 ± 81.7 and 1683.5 ± 58.9 mg for
471 PHB, PHBV and PCL, respectively, which corresponded to $69.6 \pm 4.5\%$, $64.7 \pm 3.8\%$ and $72.7 \pm$
472 2.5% of the respective theoretical amount of CO_2 evolved. Likewise, a similar behavior in the

473 biodegradation curves of PHB and PHBV was observed under anaerobic conditions (Fig. 4a and
474 b). However, the degree of biodegradation of the PHB with a particle size of 500-1000 μm was
475 slightly lower compared to the other powder fractions. Hence, the net total carbon production
476 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
477 ± 13.1 , 423.3 ± 3.2 and 342.8 ± 38.6 mg for PHBV, using particles sizes of 100–250, 250–500
478 and 500–1000 μm , respectively. Evident from Fig. 3 and 4, linear kinetic profiles indicate that the
479 lower available specific surface area is rate limiting for the biodegradation process and could be
480 explained by pseudo-zero order kinetics. Under the assumption of biodegradation requiring an
481 initial surface erosion step that is executed by extracellular enzymes, the biodegradation rate is
482 dependent on the specific surface area that contains available, enzymatically cleavable polymer
483 bonds to produce metabolizable products for mineralization (Chinaglia et al., 2018).

484 The effect of particle size on biodegradation kinetics is given in Table 2. The kinetic data fitting
485 resulted in calculated R^2 values higher than 0.985, indicating that the modified Gompertz model
486 adequately describes the BB biodegradation process (see e-supplementary materials). The
487 cumulative mineralization rate was inversely correlated with the particle size regardless of the
488 environment and bioplastic. The lower particle size herein tested supported up to 331 and 405%
489 higher maximum aerobic and anaerobic biodegradation rates, respectively, than those obtained
490 with a particle size of 500–1000 μm . Particle size did not have a markedly influence on the lag
491 phase (λ) among all the tested BBs. Note that the model slightly underestimated the lag time
492 when tested the fraction with lower particle size (100–250 μm) under aerobic conditions (see e-
493 supplementary materials). However, bioplastics with a larger particle size still required longer
494 times to be aerobically/anaerobically biodegraded because less surface area was available for
495 bacterial biodegradation. For instance, using particle sizes of 100–250, 250–500 and 500–1000
496 μm , the anaerobic biodegradation of PHB and PHBV reached a plateau after 35, 42 and 65 days
497 and 35, 56 and 65 days, respectively. Under aerobic conditions, the smallest particle size of

498 PHAs required around 52 days of incubation to reach a plateau in the biodegradation, while the
499 largest particle size needed a 3-month degradation time. Likewise, the biodegradation of PCL
500 (100–250 μm) plateaued after 65 days of incubation, while 79 and 86 days were needed for
501 particle size ranges of 250–500 and 500–1000 μm , respectively.

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

522 polymer porosities and specific surface area, thus facilitating their accessibility to enzymatic
523 attack and lower residence times (García-Depraect et al., 2021).

524 **3.5 Implications of the study and further research needs**

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

545 Additionally, this study provides implications to promote a practical and enhanced end-of-life
546 management of bioplastics, especially via anaerobic digestion which is currently applied in many

547 developed and developing countries facing the plastic pollution problem. The development and
548 evaluation of pretreatment aided anaerobic digesters co-fed with (bio)plastics is a relevant topic
549 for research in the field. Thus, the assessment of how bioplastics affect in the long term the
550 operational performance and microbiology of the process is highly recommended. The possibility
551 that persistent micro- and nanoplastics and additives are released during the biodegradation of
552 bioplastics, and their potential toxicological effect on the environment and human health, should
553 also be assessed in further studies, as recently pointed out by Liao and Chen (2021). Finally,
554 novel integrated upcycling strategies for the microbial production, degradation and valorization of
555 BBs should be pursued in the context of a circular bioeconomy.

556 **4. Conclusions**

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

566 E-supplementary data for this work can be found in e-version of this paper online.

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571

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697 **Figure captions:**

698 **Fig. 1.** Time course of the degree of biodegradation of PHB, PHBV, PBS, PBAT, PCL, PLA,
699 PLA/PCL blend, HDPE (negative control) and cellulose (positive control) under aerobic conditions
700 in aqueous medium. Each data point corresponds to the average and standard deviation from
701 triplicate assays. A particle size of 100–250 μm was used in all the cases, except for cellulose.

702

703 **Fig. 2.** Time course of the degree of biodegradation of PHB, PHBV, PBS, PBAT, PCL, PLA,
704 PLA/PCL blend, HDPE (negative control), and cellulose (positive control) under anaerobic
705 conditions in aqueous medium. Each data point corresponds to the average and standard
706 deviation from triplicate assays. A particle size of 100–250 μm was used in all the cases, except
707 for cellulose.

708

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

712

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

716

717 **Table 1.** Carbon balance calculated from the biodegradation of biodegradable bioplastics under
 718 aerobic/anaerobic conditions in aqueous medium.

Carbon, %	Aerobic conditions			Anaerobic conditions	
	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

719 Note: n.d., not determined; app., apparent. *Under aerobic conditions, the carbon derived
 720 from the apparent biomass (measured as VSS) was calculated assuming that the stoichiometric
 721 formula of biomass is C₅H₇NO₂. Under anaerobic conditions, the carbon fixed in the form of
 722 biomass was assumed to be 10% of the total initial carbon (Chernicharo, 2015).

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

Material	Sieving range, μm	<i>P</i> , mg C/g-material		<i>R</i> , 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	-

724 Notes: AE, aerobic conditions; AN, anaerobic conditions. Data on cumulative gas carbon production from the different bioplastics were adjusted to the
 725 modified Gompertz model.

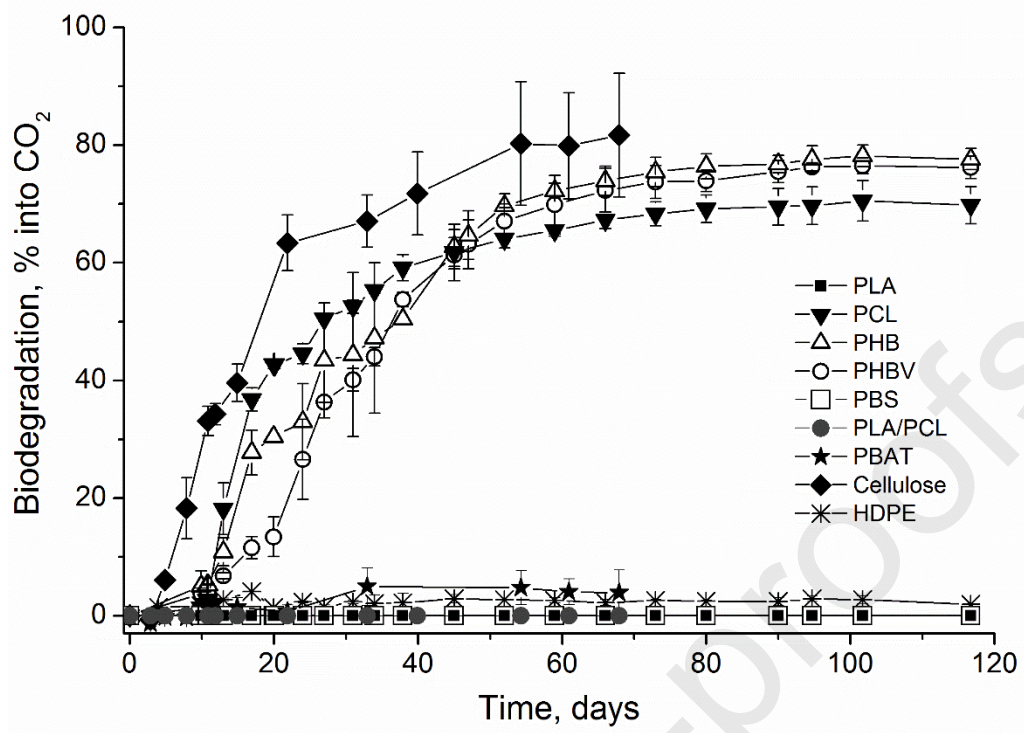
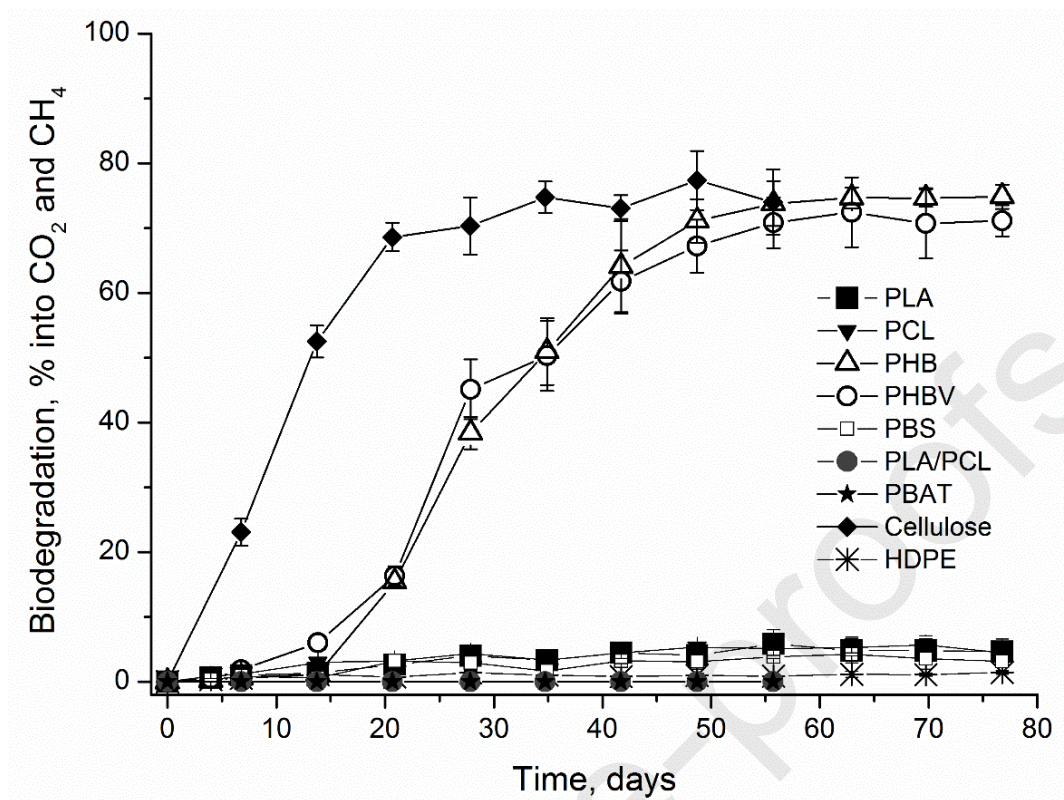


Fig. 1

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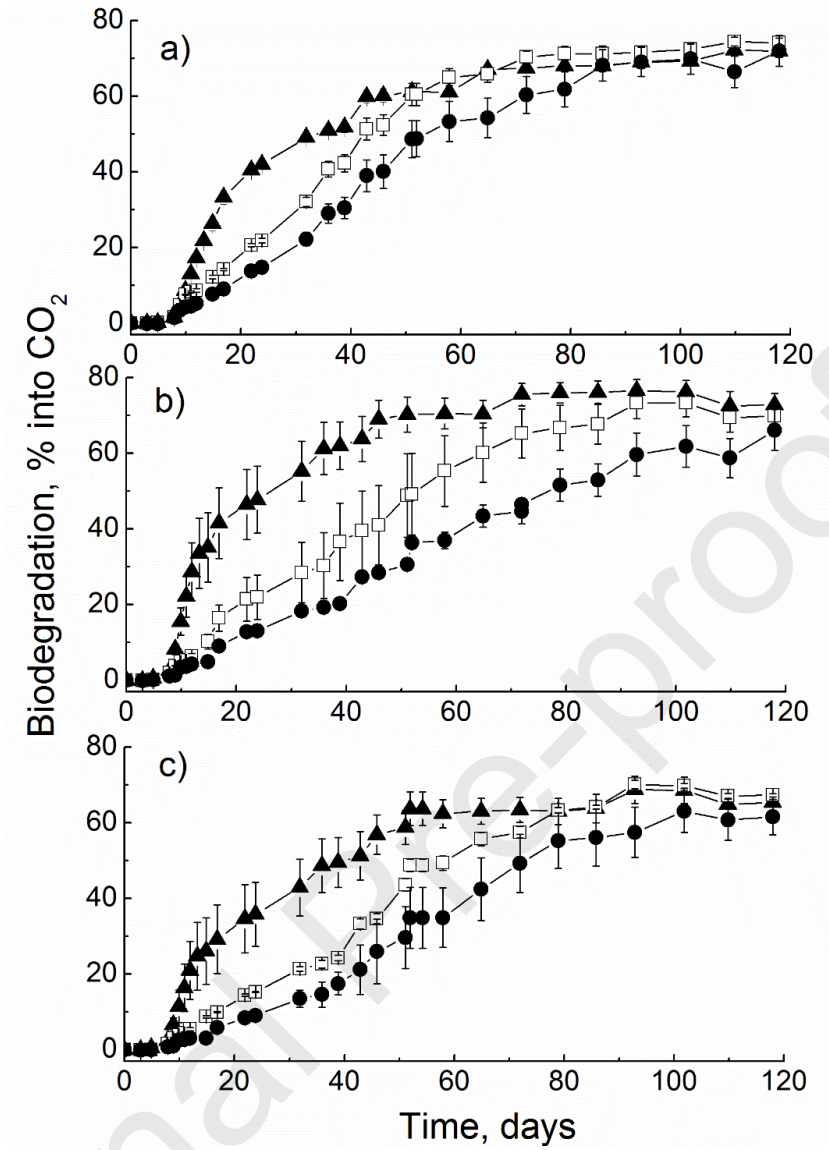


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Fig. 2.



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Fig. 3.

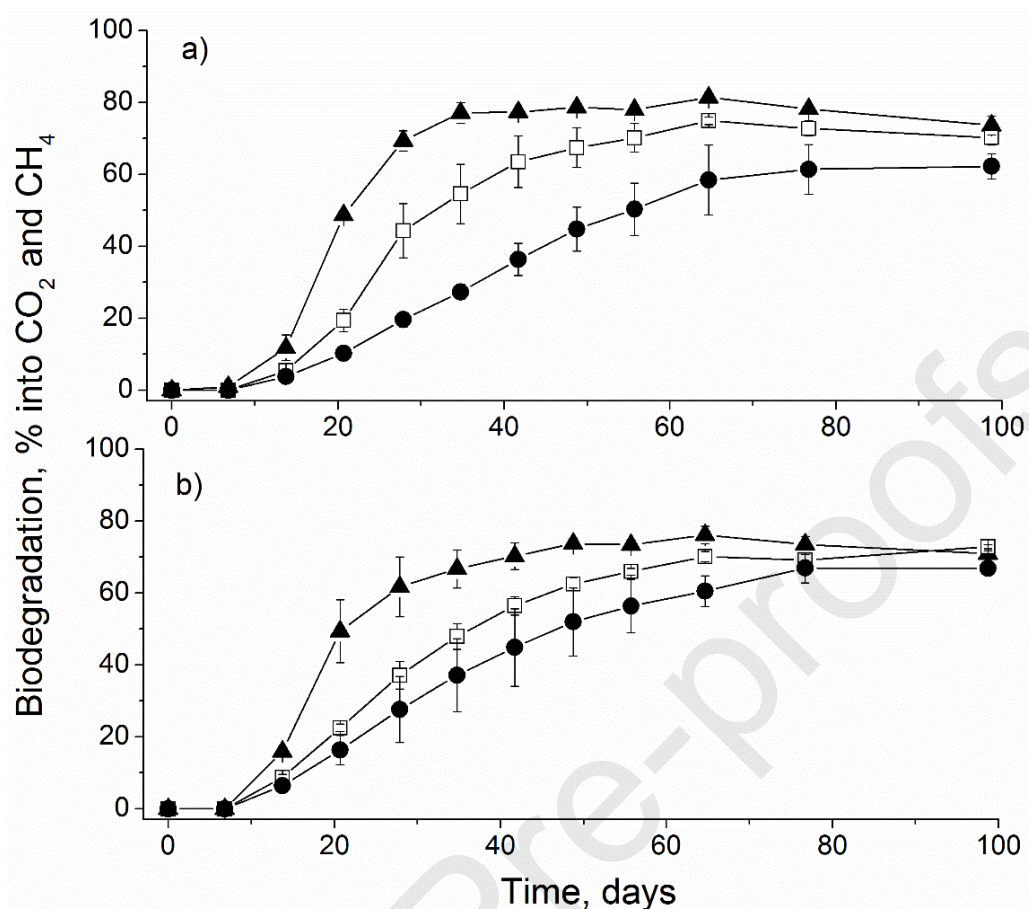


Fig. 4.

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736 **CRedit author statement**737 **Octavio García-Depraect:** Conceptualization; Methodology; Investigation; Writing -738 Original Draft; **Raquel Lebrero:** Conceptualization; Supervision; Project739 administration; Writing - Review & Editing **S.R.-V.:** Investigation; Writing - Review &740 Editing **S.B.:** Conceptualization; Writing - Review & Editing **F.S.-B.:**741 Conceptualization; Writing - Review & Editing **L.J.M.-M.:** Investigation; Writing -742 Review & Editing **R.A.B.:** Conceptualization; Funding acquisition; Writing - Review &743 Editing **T.B.:** Conceptualization; Funding acquisition; Writing - Review & Editing744 **R.M.:** Conceptualization; Funding acquisition; Project administration; Methodology;

745 Supervision; Writing - Review & Editing

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748 **Declaration of interests**

749

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

752

753 The authors declare the following financial interests/personal relationships which may be
754 considered as potential competing interests:

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761 **Highlights**

762 • Only PHAs were aerobically/anaerobically biodegradable under aqueous

763 conditions.

764 • PHB and PHBV yielded up to 496 and 480 Nm³ of CH₄ per ton, respectively.

765 • C-balance analysis for the different carbon sinks estimates polymer

766 biodegradability.

767 • Mineralization rate depended on the total specific surface area of polymer.

768 • The modified Gompertz model accurately described bioplastic biodegradation.

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