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Is a compostable plastic biodegradable in the sea? A rapid standard protocol to test mineralization in marine conditions

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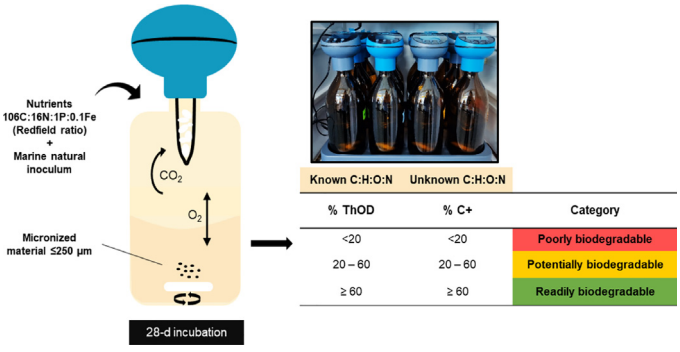
HIGHLIGHTS

- Current plastic degradation standards media do not reflect marine conditions.
- Material pretreatment and marine media reduces incubation from months to 28 days.
- Use of PHB as reference enables testing plastics of unknown composition.
- Compostable plastics made form biopolymers are not biodegradable in marine conditions.

GRAPHICAL ABSTRACT



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ABSTRACT

Due to environmental persistence, lack of a proper land-based waste management, and global circulation, marine ecosystems are especially threatened by plastics. The search for alternatives to conventional oil-based polymers gave rise to novel materials commercialized under different "green" labels based on compostability. However, current international standards are not effective in predicting actual biodegradability of plastic objects in natural scenarios, and degradation of these novel bioplastics in marine conditions is unwarranted. We present a simple and rapid standard protocol based on their biological oxygen demand, intended to support policy-makers and plastic industry in the search for truly marine-biodegradable plastics. Improvements include: development of an environmentally relevant nutrient formulation following Redfield ratio (106C:16 N:1P); use of a natural inoculum representative of marine habitats (sediment pore water); standardization of the test material by grinding to particles below 250 μm to shorten the incubation period, and selection of a truly biodegradable biopolymer (PHB), used as positive control. This protocol was successfully applied to show that commercial compostable plastics are not biodegradable in marine environments.

1. Introduction

The high functionality and relatively low cost of plastics made these materials increasingly ubiquitous in everyday life. However, conventional oil-based plastics are not amenable to biodegradation in the natural environment, and thus show undesirable environmental persistence. The low density, high bulk and increasing presence of plastic in municipal solid waste demands increasing amounts of landfill space (Philp et al., 2013). In addition, about 42% of the plastic produced is intended for packaging, that is for immediate disposal (Geyer et al., 2017), and inappropriate end-of-life management is commonplace (Jambeck et al., 2015). As a consequence, plastic litter is an aesthetical nuisance in natural landscape, and the so derived secondary microplastics dominate marine debris, impact marine

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diversity and demand regulatory and management efforts (Amaral-Zettler et al., 2015).

The search for sustainable alternatives to conventional plastics prompted a proliferation of the so-called 'green plastics', which can be fully or only partially made from biological resources and, thus, have limited environmental benefit, just as it occurs with composites of starch and environmentally persistent polymers (Andrady, 2003). In fact the use of the term 'biodegradable' in labels of plastic products is controversial, and instances of 'greenwashing' have been reported (Philp et al., 2013). Several regulatory bodies (e.g. OECD, ASTM, ISO) issued standards to enable strict assessment of the biodegradability of plastics in different environments, such as soil, composting facilities, aquatic environment and even marine habitats. Bio-based polymers (e.g. polylactic acid, PLA) may be in compliance with those technical standards yet hardly biodegradable in environmentally-relevant conditions (George et al., 2020). According to the European Plastic Strategy (European Commission, 2018), materials under those labels "degrade under specific conditions which may not always be easy to find in the natural environment, [particularly] in the marine environment", and following UNEP "their adoption will not bring about a significant decrease in the quantity of plastic entering the ocean" (Hamprecht et al., 2011).

Biodegradation can be formally defined as the mineralization of organic matter mediated by heterotrophic microorganisms, leading in aerobic conditions to CO_2 and H_2O, and to an increase in the biomass of microorganisms (Subach, 1997). A truly biodegradable polymer must then be amenable to mineralization to CO_2 by natural microorganisms under a set of standardized incubation conditions representative of a given environmental compartment. Potential biodegradability in the sea can thus be assessed in laboratory on the basis of mineralization rates obtained under simulated marine conditions, but current standards are insufficient in their ability to realistically predict the biodegradability of plastic objects such as carrier bags in aquatic environments (Harrison et al., 2018) and there is a clear need for reliable and environmentally relevant standardized methodologies (Lott et al., 2020; Viera et al., 2021). This study presents a standard protocol intended to assist policy makers and industry in the development of truly marine-biodegradable plastics, contributing to bring a significant decrease in the accumulation of plastic litter in the global ocean. The underlying hypothesis of the study are that natural microbial inoculum from marine sediments and nutrient formulation representative of marine conditions will improve the standard method to test marine biodegradability. Novel aspects beyond current standards include development of an environmentally relevant nutrient formulation, use of an inoculum representative of marine coastal habitats, standardization of the material pretreatment, shorter incubation times, and selection of a marine biodegradable polymer to be used as a positive control, enabling classification of materials of unknown or undisclosed composition by comparison to this reference.

2. Materials & methods

We assessed aerobic biodegradation of plastic materials through the Biological Oxygen Demand (BOD), i.e. the amount of oxygen in mg L^{-1} that natural microorganisms consume to mineralize the material in a closed bottle (Jouanneau et al., 2014). With that aim we filled 0.5 L amber glass bottles with 0.8-μm filtered seawater (FSW) sterilized with UV light and enriched with nutrients (N, P and Fe), a marine inoculum (1% of the water volume), the testing polymer or reference material (100 mg L^{-1}), and a stirring magnet. Headspace was allowed in the bottles (biphasic bottles) in order to increase available O_2 and support longer incubation periods. We finally placed all bottles on a magnetic stirring plate inside an incubator at 20 ± 0.1 °C.

We recorded BOD (mg L^{-1}) daily by means of OxiTop® (WTW, Germany) pressure sensor caps that use KOH pellets to capture the evolved CO_2. Incubations lasted for 28 days (OECD, 1992). Each trial included blanks (with FSW, nutrients and inoculum, but no plastic), positive controls (compound of known high biodegradability, and the tested plastic materials run per duplicate. For each trial, the blank BOD is subtracted from the BOD of the treatments. The resulting blank-corrected BOD values are expressed as % of the Theoretical Oxygen Demand (ThOD), i.e. the theoretical amount of oxygen required to fully degrade the whole carbon content of the plastic to CO_2. In addition, for each tested plastic, the BOD is also expressed as percentage of that recorded in a positive control (%C+), consisting of two substances: sucrose (Panreac-AppliChem, pharma grade) in preliminary trials, and polyhydroxybutyrate (PHB) resin (ENMAT Y3000 purchased from Helian Polymers, The Netherlands) in the definitive tests. PHB is a biosynthetic polymer belonging to the family of polyhydroxyalkanoates (PHA), proved biodegradable in marine environment (Tsuji and Suzuyoshi, 2002).

2.1. Nutrients formulation and inoculum

We carried out several trials in order to search for an environmentally-relevant incubation medium representative of the marine environment in terms of nutrients composition and microbial inoculum. As sources of N, P and Fe, we used $NaNO_3$ (pharma grade), $Na_2HPO_4{\cdot}2H_2O$ (analysis grade), and pure $FeCl_3{\cdot}6H_2O$ (ITW Reagents, USA). We compared the nutrient formulation prescribed by conventional standards (ISO 16221, 2001; OECD, 1992) with alternative formulations intended to fit the well-known Redfield ratio 106:16:1:0.1 for C:N:P:Fe (Chester and Jickells, 2012). With that aim, the following treatments were tested: Medium 1 (hereafter termed ISO) consisted of the N, P and Fe molar composition prescribed by OECD and ISO (solutions *a* and *d* in OECD No. 306) but replacing ammonium by nitrate as N source, in order to avoid potential interference from nitrogenous oxygen demand. Medium 2 (Red-N) consisted of the same molar concentration of inorganic N than ISO, but levels of P and Fe adjusted to Redfield. Medium 3 (Red-P) consisted of a 10-fold lower level of P than ISO and levels of N and Fe adjusted to Redfield. Medium 4 (Red-C) consisted of the Redfield ratio adjusted on the basis of the C content in 100 mg L^{-1} of the reference material.

Regarding microbial inoculum, we tested the following sources: seawater column (SWC) from a presumably clean site, SWC from a harbor, effluent from a waste-water treatment plant (WWTP), sediment pore water (SPW), and an aqueous extract of the sediment (termed elutriate, ELU). SWC was carefully sampled by opening a glass bottle at ca. 50 cm depth in order to avoid the surface-floating materials (Fig. 1c). SPW was sampled by digging a ca. 30 cm depth whole in the sand in the intertidal zone and collecting the arising water (Fig. 1c). ELU were obtained by mixing solid sediment samples with FSW (1:4 weight ratio) in an overhead rotator at 60 rpm for 30 min, and taking the supernatant after 12 h decantation. For both SWC and SPW inocula, we counted the viable colony forming units (CFU) after 72 h of incubation in Marine Agar (25 °C, darkness).

University of Vigo central services characterized the inoculum finally selected in terms of nutrients (phosphate, ammonium, nitrite and nitrate) and total organic carbon (TOC) by means of an AA3 Bran+Luebbe AutoAnalyzer and an AnalytikJena multi N/C 3100 respectively.

2.2. Materials tested

We studied 7 types of resins and 4 commercial plastics advertised as compostable (3 carrier bags and cups, see Table A.1), previously micronized using a ZM200 ultracentrifuge mill (Retsch, Verder Scientific) and sieved through a 250 μm metallic mesh. We also tested the effect of PHB presentation (1–3 mm diameter pellets, 0.5 cm^2, 100 μm thick film pieces, and powder sieved to obtain ≤250 μm fraction) on biodegradation rates. In this case, sucrose, a fully biodegradable sugar, was the positive control.

2.3. Quality assurance and assessment criteria

The BOD_{28} in the blanks was always <3 mg L^{-1} and less than 3% of the positive control, with a 99% percentile of 2.4 mg L^{-1}. This is consistent with the theoretical maximum values predicted from the TOC analyses (7.4 mg L^{-1} TOC in the SPW, and 5.5 mg L^{-1} in the FSW), which would

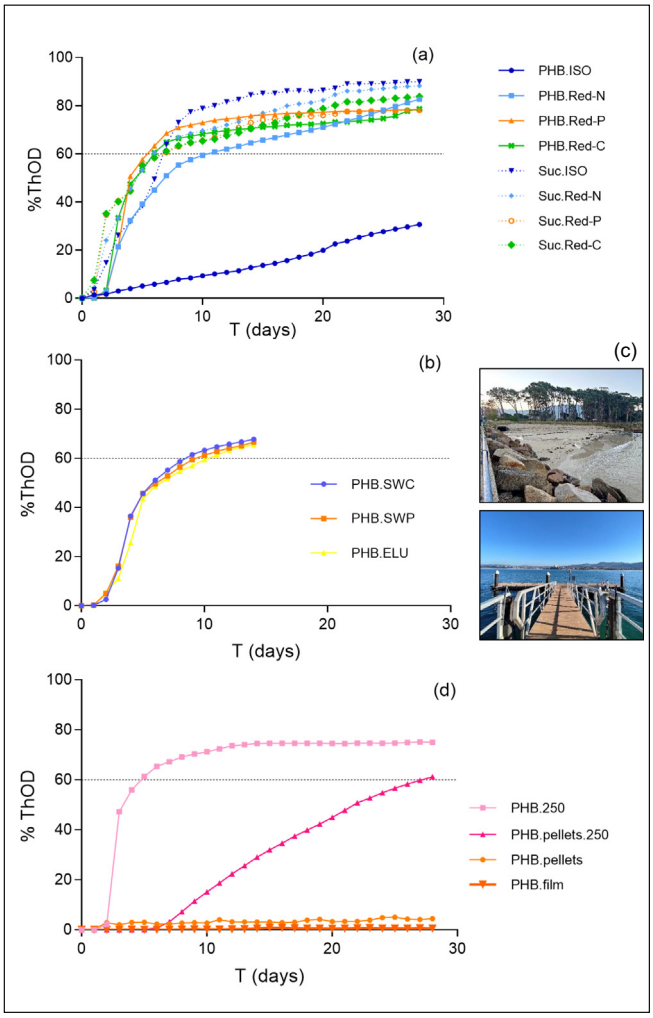


Fig. 1. Tests for optimizing incubation conditions. (a) Biodegradation, expressed as % ThOD, for both sucrose (Suc) and PHB using different nutrient formulations (see text). Notice for PHB the poor performance of the formulation prescribed by ISO compared to those reflecting the Redfield ratio. (b) Biodegradation of PHB using three different marine inocula: water column (SWC), sediment pore water (SPW), and sediment elutriate (ELU). (c) Sampling locations for sediment (above) and water column (below). d) Biodegradation of PHB with different size ranges: powder ≤250 μm (PHB.250), intact PHB pellets of 1–3 mm (PHB.pellets), PHB pellets micronized to ≤250 μm (PHB.pellets.250), and 0.5 cm^2 pieces of 100-μm-thick PHB film (PHB.film).

correspond to a blank ThOD of 6.4 mg O_2/L. Moderate blank BOD translates in high signal to noise ratios that enhance the power of the method to discriminate between materials with different biodegradability. The BOD_{28} in the positive controls was always >60% ThOD, meeting current acceptability criteria (ISO 23977-2, 2020; OECD No. 306).

Regarding assessment criteria, 20% ThOD is used by OSPAR (2020) as a pre-screening criterion to consider a material as potentially biodegradable in the marine environment, and 60% ThOD has been frequently invoked as a threshold for ready biodegradability (ISO 14851, 2019, ISO 16221, 2001; OECD No. 301 and No. 306). Using these benchmarks, plastic materials can be classified according to their marine biodegradability into the following classes: poorly biodegradable (<20% ThOD in 28 days), potentially biodegradable (20 to ≤60% ThOD), and readily biodegradable (>60% ThOD). However, during microbial biodegradation a relevant proportion of the polymer carbon is not mineralized to CO_2 but assimilated by the heterotrophic microbial consortium and converted into biomass, setting an actual maximum BOD between 30 and 50% below ThOD (Krzan et al., 2006). On the other hand, current biodegradable materials are frequently heteropolymers and complex mixtures whose exact atomic composition is unknown. These limitations for the use of ThOD to assess biodegradability will be discussed below, and an alternative more practical classification based on percentage of the actual BOD of the positive control, rather than the theoretical ThOD, will be proposed.

2.4. Statistical methods

The observed 28-days biodegradation curves were fit to a logistic model according to the equation:

$$Y = \frac{BOD_L}{1 + 10^{(\mathrm{Log}\ a - X) * b}}$$

where Y is BOD (mg L^{-1}), X is time (days), BOD_L is the ultimate BOD, corresponding to the asymptote of the curve, *a* is the time at which 50% mineralization is achieved, and *b* is the slope of the curve, which quantifies the biodegradation rate.

We conducted statistical analyses using IBM SPSS (v. 25), and GraphPad Prism (v. 8). Aiming to identify significant differences in biodegradation rate and ultimate biodegradability between materials and inoculum sources, we compared the slopes (*b*) and BOD_L values obtained from the logistic curves.

3. Results & discussion

3.1. Nutrients formulation and inoculum

When sucrose was used as reference material and nutrients were added, more than 50% biodegradation of this substance, assessed as %ThOD, was observed after just 5 days incubation except for the ISO formulation (Table 1), and over 75% was observed after 28 days disregarding nutrient composition. The Redfield-based formulations results were thus comparable to the certified BOD_5 for sucrose in freshwater (0.69 g O_2 per g of substance, i.e. 61%ThOD, AmericanBio (2014) Sucrose safety data-sheet), and to other studies carried out in seawater (more than 60% mineralization assessed as CO_2 evolution after 5 days, Ratto et al., 2001), whereas the performance of the ISO formulation, with N deficit compared to Redfield, was remarkably poorer. Only the absence of nutrients or sterile medium prevented sucrose mineralization (see Table 1). When PHB was used as reference, the poor performance of the ISO formulation was even more evident, promoting biodegradation of barely 5% ThOD after 5 d, and achieving a mean value of only 31% even after 28 d, versus the 78 to 82% for the Redfield-based media (Fig. 1a). Since adjusting nutrients to

Table 1
Biological Oxygen Demand (BOD) after 5 days incubation in seawater of sucrose (0.1 g L^{-1}), using different nutrient formulations and sources of microbial inoculum.

Factor	Treatments	BOD_5 (mg L^{-1})	%ThOD
Nutrients	No nutrients	5.5	4.9
	ISO[a]	43.1	38.4
	Redfield-N	59.9	53.4
	Redfield-P	62.3	55.4
	Redfield-C	62	55.2
Inoculum	Sterilized FSW	0.55	<1
	NSW (clean site)	51.5	45.9
	NSW (polluted site)	52.0	46.4
	WWTP effluent	55.0	49.0
	SPW[b]	59.5	53.0

[a] Notice the poor performance of the ISO nutrient formulation compared to the three formulations based on the Redfield ratio. FSW: filtered seawater; NSW: natural seawater; WWTP: wastewater treatment plant.

[b] Notice also that the inoculum with which sucrose reach the highest degradation after 5 days is sediment pore water (SPW). FSW: filtered seawater; NSW: natural seawater; WWTP: wastewater treatment plant.

the Redfield ratio remarkably enhanced biodegradation, the Red-C nutrient composition was adopted for further testing.

The use of nitrate rather than the ammonium prescribed in conventional standards as N source, in combination with the reduced incubation (28 days), prevents nitrogenous oxygen demand, normally occurring after long exposure periods (Sullivan et al., 2010). In fact, the ammonia: nitrate ratios recorded after the 28-days exposure in C+ bottles were remarkably higher than the ratio recorded in the SPW inoculum (Table A.2), supporting lack of a significant nitrification activity during incubations. Additional trials with N-allylthiourea, a nitrification inhibitor, also supported this. On the other hand, since the marine water is naturally buffered by the carbonate system that includes a mixture of a weak acid (carbonic acid) and associated anions (bicarbonate and carbonate) (Chester and Jickells, 2012), the use of different ammonium salts, prescribed in conventional standards derived from freshwater applications where pH must be buffered, is unnecessary. This allows also an easier adjustment of the nutrient composition to the demands of marine microorganisms reflected in the well-known Redfield ratio, where N must be over one order of magnitude more abundant than P.

The nutrient formulation prescribed by ISO and other international standards seems inappropriate to support heterotrophic microbial growth in marine conditions, and alternative formulations based on the Redfield C:N:P ratio support remarkably higher biodegradation rates, especially in the case of using a biopolymer as C source. This problem illustrates the deficiencies of current technical standards for biodegradability of plastics, often not representative of natural environments (Viera et al., 2021).

Concerning bacterial inoculum, the initial short-term trials conducted with sucrose showed that SPW outperformed in terms of supported BOD_5 the other sources of inoculum (Table 1), and it was adopted as standard inoculum for further testing. This is in line with previous findings that proved faster plastic degradation in sediment-contact exposures compared to the water column (Beltrán-Sanahuja et al., 2020; Lott et al., 2020). In addition, the bacterial density recorded by agar plating in the SPW ($1.40{\cdot}10^4$ UFC mL^{-1}) was ca. one order of magnitude higher compared to the SWC inoculum ($3.03{\cdot}10^3$ UFC mL^{-1}). However in the 28-days incubations using PHB as reference (Fig. 1b), SWC and SPW performed similarly, whereas mineralization in bottles inoculated with ELU showed a slight initial delay, although no significant differences in the slope or the asymptote (BOD_L) of the logistic curves among inocula were found (Table A.3).

Not only bacterial density but also composition may explain differences in performance among inocula. Both field and laboratory experiments support that plastics based on biopolymers degrade faster in contact with the seafloor than floating in the water (Tosin et al., 2012; Briassoulis et al., 2019). Doi et al. (1992) isolated two strains of PHB degrading actinomycetes from marine sediment. Considering all that evidence, we recommend SPW from marine sediment as inoculum for standard biodegradation tests.

3.2. Material pretreatment: influence of particle size

As we can see in Fig. 1d, PHB ground to ≤250 μm (light pink line) show substantial biodegradation (Table A.3). In contrast, intact pellets and film pieces (orange symbols) showed very low biodegradation rates (<5% ThOD after 28 days). In addition, the pattern of biodegradation depended also on the polymer stock. The powdered stock reached over 60% ThOD in less than 10 days, whereas the micronized pellets (pink triangles) showed a much slower mineralization, as indicated by the significantly lower slope (Table A.3) but eventually exceeding the 60% ThOD benchmark after 28 days, and showing similar BOD_L values.

The different biodegradation velocity between the pellet and powder stocks may be due to differences in chemical additives. Pure PHB has very poor mechanical properties, and it is normally blended with plasticizers (Seggiani et al., 2015). Since pelletization of pure PHB is more demanding that production of a powdered stock, we can speculate that additives not present in the powder retard biodegradation of the pellets, and this could explain the remarkably longer lag phase for the micronized pellets compared to the powder. Also, production of pellets from thermoplastics involve the use of blowing agents some of which include metal salts potentially toxic for the microorganisms (Drobny, 2014).

The remarkably higher biodegradation of the micronized pellets compared to the intact ones can be related to the increased weight-specific surface area. Degradation of solid PHBV (polyhydroxybutyrate-valerate copolymer) specimens in seawater progressed through the surface (Deroiné et al., 2014). Previous studies using particles of biodegradable polymers concluded that the higher the surface area, the higher the biodegradation rates (César et al., 2009; Chinaglia et al., 2018). Chinaglia et al. (2018) reported that biodegradation of polybutylene sebacate, another biodegradable and compostable polymer, initially progressed as a zero-order kinetics since it was limited by the exposed area of the plastic particles. Depolymerases secreted by microorganisms act on the polymer surface (Park et al., 2021), and their efficiency should also be dependent on the area of the plastic particles. By grinding samples to ≤250 μm we can propose a protocol analogous in duration to the standard OECD method for the assessment of environmental persistence of chemicals (OECD No. 301), 28 days, much shorter than existing standards for plastics (60 days to 6 months). This greatly increases the throughput of the method, and decreases costs and chance of technical failures during very long incubation periods.

3.3. PHB: a suitable reference material

Previous studies have proved the biodegradability of polyhydroxy alcanoates in aquatic ecosystems both under laboratory conditions (Tsuji and Suzuyoshi, 2002) and in situ (Brandl and Püchner, 1991; Sekiguchi et al., 2011), reporting substantial weight loss or tensile strength decrease after incubation periods within the order of months. Our results confirm the biodegradability of PHB in seawater, and greatly reduce the time needed for the assessment provided the material is previously ground to particle sizes below 250 μm. This allows the use of PHB as reference material in the assessment of plastics biodegradability. Including micronized PHB as reference material or positive control (C+) at each run of the test enables the expression of results as %C+, and the assessment of novel polymeric materials of unknown C:H:O:N composition for which calculation of the ThOD, as required in current ISO standards, is not possible.

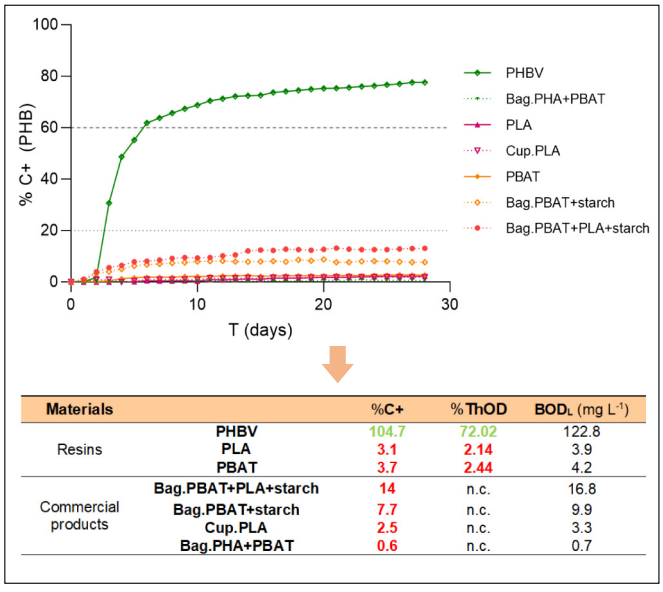
In addition, the use of materials with high free energy content such as PHB as reference to test biodegradability of other synthetic polymers may be more suitable for studies of biopolymers than the traditional use as reference of polysaccharides with lower free energy such as cellulose. The latter are chiefly composed by carbon atoms in a more oxidized state that are extensively converted to CO_2 with very little production of cell biomass whereas, in the former, a substantial proportion of the carbon constitutes a source of new cell biomass rather than fuel for cell respiration, and that portion is not mineralized to CO_2 (Krzan et al., 2006 and citations therein). Therefore, the use of cellulose as reference material and %ThOD as criteria may underestimate the biodegradability of materials with high free energy content, such as some synthetic biodegradable polymers.

The 20% and 60% ThOD benchmarks used by OSPAR (2020) and ISO 14851 (2019) can be applied to the novel end-point % C+, to establish the three categories of biodegradability (poorly biodegradable, potentially biodegradable and readily biodegradable) depicted in the Table of Contents. ThOD is a theoretical parameter that assumes all C atoms from the organic matter are mineralized to CO_2, but it has been experimentally shown that disappearance of up to 90% dissolved organic carbon corresponded in simultaneous tests of oxygen demand to just ca. 50% ThOD. On this basis, several authors criticized the 60% ThOD benchmark as unnecessarily stringent (Boethling et al., 2003, Martin et al., 2017 and references within). In fact, in the present study, a chemical as much biodegradable as sucrose hardly exceeded 60% ThOD, and other readily biodegradable compound, aniline, used as reference by Martin et al. (2017) did not reach 60% ThOD in 28 days. Using %C+ rather than %ThOD would ease this strict benchmark and would contribute to consistently classify clearly non-persistent substances as readily biodegradable.

3.4. Application to commercial bioplastics

A rapid, high throughput, and environmentally relevant method to assess the potential biodegradability of novel polymeric materials in marine conditions is presented. The method includes sample pretreatment to standardize particle size to ≤250 μm. Further micronization to smaller particle size fails to speed up biodegradation (Table A.3). A bacterial inoculum obtained from marine SPW increases the bacterial density compared to SWC inoculum and enhances rapid degradation of sucrose. When we tested resins and commercial products labeled as biodegradable and/or compostable with this novel protocol, only the PHB (reference material) and PHBV powders resulted to be readily biodegradable in marine conditions (Fig. 2). None of the other resins and commercial products reached by far similar levels of biodegradability. The PLA resin and PLA cups showed a biodegradation just 3.1% and 2.5% that of PHB, which is consistent with previous reports. Using surface analysis, Chamas et al. (2020) described degradation rates of PLA in seawater as low as those for polyethylene, and Nazareth et al. (2019) did not find any evidence of degradation for PLA cups in seawater after 180 days.

In the case of the two bags that listed starch in their composition (Table A.1), their biodegradation was just 8 and 14% that of the PHB. This may be due to mineralization of the starch component, since degradation of cornstarch-PHBV blends correlate with the starch content (Imam et al., 1999). Moreover, the bag that listed PHAs (PHB and PHBV) but no starch in its composition showed a surprisingly low biodegradability (0.6%), supporting the hypothesis that starch content is responsible for the mineralization recorded in the other bags. However, the low mineralization of this bag is surprising considering that it lists PHAs among its components, and both PHB and PHBV resins proved to be marine biodegradable. The PHB homopolymer exhibits high crystallinity that limits its commercial use (Orts et al., 2008), and it is normally blended with other biopolymers such as PLA or PBAT (polybutylene-adipate-terephthalate) that showed very poor marine biodegradability in our tests (Fig. 2), and stabilizers are added to extend product's shelf life (Owonubi et al., 2018).



Materials		%C+	%ThOD	BOD_L (mg L^{-1})
Resins	PHBV	104.7	72.02	122.8
	PLA	3.1	2.14	3.9
	PBAT	3.7	2.44	4.2
Commercial products	Bag.PBAT+PLA+starch	14	n.c.	16.8
	Bag.PBAT+starch	7.7	n.c.	9.9
	Cup.PLA	2.5	n.c.	3.3
	Bag.PHA+PBAT	0.6	n.c.	0.7

Fig. 2. Marine biodegradability of the bioplastics tested according to the methods developed in the present study. Biodegradability is expressed as the percentage of the BOD_{28} compared to PHB as reference material (%C+), and as Theoretical Oxygen Demand (%ThOD). The ultimate BOD predicted by a logistic model is also shown (BOD_L). Notice that none of the compostable products made from biopolymers showed relevant biodegradability in seawater. n.c. not calculable. Red: poorly biodegradable; green: readily biodegradable.

4. Conclusions

The nutrient formulation prescribed by ISO and other international standards seems inappropriate to support heterotrophic microbial growth in marine conditions, and alternative formulations based on the Redfield C:N:P ratio support remarkably higher biodegradation rates, especially in the case of using a biopolymer as C source.

The use of micronized PHB as positive control at each test run allows the expression of results compared to this reference, in contrast with the conventional %ThOD that requires knowledge of the elemental composition of the problem material. This allows classification of plastics whose composition is not disclosed.

This study presents advances towards the development of a more rapid and environmentally relevant standard method to test biodegradability of plastics in marine conditions typical of temperate coastal habitats. Further research should address the influence of lower temperatures typical of deep sea, and microbial consortia obtained from other habitats. This method has proved to be suitable for assessing actual marine biodegradability of commercial products made from biopolymers and their corresponding resins in a much shorter time than current standards available, what is useful as a first biodegradability screening for problem materials. The method showed that PLA and PBAT are not readily biodegradable in marine conditions. From the four commercial products made of biopolymers (PHA, PBAT, PLA) and labeled as compostable, tested with this novel protocol, none of them showed relevant marine biodegradability and only those including starch in its composition showed 8 to 14% mineralization in seawater, likely due to the starch. PHB raw material showed inherent marine biodegradability, but chemical additives for processing the resin and preserving the final product may suppress this advantage. Labels shown in commercial plastic materials should be based on standards that reflect a real and more accurate degradation in the environment and avoid general statements such as 'biodegradable' that may lead to incorrect disposal by consumers.

CRediT authorship contribution statement

Sara López-Ibáñez: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Writing-Original Draft, Writing-Review & Editing, Visualization, Funding acquisition.

Ricardo Beiras: Conceptualization, Methodology, Validation, Formal Analysis, Writing-Original Draft, Writing-Review & Editing, Supervision, Project administration, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.154860.

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