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The rate of biodegradation of PHA bioplastics in the marine environment: A meta-study

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ABSTRACT

There is a reasonably extensive body of literature recording mass loss of polyhydroxyalkanoates (PHAs) (a class of biodegradable plastics) in the natural marine environment. However, to date, this research has been very disparate. Thus, it remains unclear what the timeframe for the biodegradation of such marine biodegradable plastics actually is. The aim of this work was to determine the rate of biodegradation of PHA in the marine environment and apply this to the lifetime estimation of PHA products. This provides the clarification required as to what 'marine biodegradation of PHA' means in practicality and allows the risks and benefits of using PHA to be transparently discussed. It was determined that the mean rate of biodegradation of PHA in the marine environment is 0.04–0.09 $mg \cdot day^{-1} \cdot cm^{-2}$ ($p = 0.05$) and that, for example, a PHA water bottle could be expected to take between 1.5 and 3.5 years to completely biodegrade.

1. Introduction

Polyhydroxyalkanoates (PHAs) are normally presented as marine biodegradable plastics (Deroiné et al., 2014a; Volova et al., 2010). Supporting this claim is a reasonably extensive body of literature recording mass loss over time of PHA in the natural marine environment. However, to date, this research has been disparate, making it hard to draw overarching conclusions regarding PHA biodegradation rate or to estimate lifetimes. With production levels of PHA set to quadruple in the next five years (European Bioplastics, 2018), PHA is an important polymer to understand from a marine lifetime estimation point of view, to avoid implementing what may be a solution to recalcitrant plastics in theory, but a problem in practice. More broadly, an understanding of estimated lifetimes of marine biodegradable plastics is required in order to facilitate an informed discussion as to whether biodegradable plastics should be included in bans and taxes on plastic. This paper determines an average rate of biodegradation of PHA in the marine environment based on the relevant available literature so that lifetime estimation of PHA products can be undertaken, allowing the risks and benefits to be more transparently discussed.

One hundred million tonnes of plastic waste are predicted to enter the oceans between the years 2010 to 2025 (Dilkes-Hoffman et al., 2019; Jambeck et al., 2015). This has led to growing concern over the impacts of plastics in the marine environment (UNEP, 2016). Plastics that enter the oceans have a wide range of environmental and economic impacts including threat to marine organisms (through ingestion, entanglement, or habitat destruction), dispersal of invasive organisms and pollutants, and disruption of the tourism and fishing industries (Codina-García et al., 2013; Kedzierski et al., 2018; Moore, 2008). One of the key issues is that conventional plastics break down very slowly and only in the presence of UV radiation, heat, and/or oxygen (Andrady, 2015). Thus, these plastics persist in the environment for hundreds to thousands of years, with degradation in the marine environment being particularly slow due to low temperatures in the ocean and minimal UV exposure once submerged (Andrady, 2015). One of the proposed solutions is to produce marine biodegradable plastics, such as PHAs, that have shorter lifetimes in the marine environment. However, it remains unclear what the timeframe for the biodegradation of such marine biodegradable plastics actually is.

Understanding the lifetime of biodegradable polymers starts with understanding the mechanisms through which biodegradation can occur. In this paper, biodegradation is taken to mean the complete breakdown of materials through biological activity, such as through the action of microorganisms such as bacteria, archaea, fungi and algae. PHAs are biodegraded under both aerobic and anaerobic conditions by PHA degraders present in most natural environments, including the marine environment (Jendrossek and Handrick, 2002; Shah et al., 2008). Under aerobic conditions the resulting products should ultimately be biomass, CO_2 and water, whilst under anaerobic conditions the resulting products should be biomass, CO_2, methane and water (Gu,

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2003).

The biodegradation of PHA is known to primarily occur through surface erosion via enzyme catalysed hydrolysis (Guerin et al., 2010; Laycock et al., 2017), meaning that when considered in simple terms, the rate of mass loss of a PHA object is related to the surface area accessible to enzymatic attack, and even whilst mass loss occurs, bulk material properties are normally preserved (Doi et al., 1992; Mergaert et al., 1995; Rutkowska et al., 2008; Sashiwa et al., 2018; Tsuji and Suzuyoshi, 2002a, 2002b). Attempting to understand the interplay of factors that influences the rate of biodegradation at any specific time point is when complexity is introduced. Properties of the polymer such as crystallinity, side-chain length, shape, and surface morphology as well as properties of the biodegradation environment such as temperature, UV exposure, nutrient levels, strength of mechanical forces, types of bacteria present, pH and oxygen levels can all influence the rate of biodegradation (Deroiné et al., 2014b; Laycock et al., 2017; Woolnough et al., 2013). Furthermore, as biodegradation proceeds, the surface of the polymer changes, pores can form and a shift of the mechanism towards bulk degradation and autocatalytic hydrolysis rather than purely surface degradation can occur (Ho et al., 2002; Laycock et al., 2017; Tsuji and Suzuyoshi, 2002a, 2002b). All of these factors act in synergy leading to a complex interplay which influences the rate of biodegradation.

Unfortunately, the data required to tease out the influence of the individual factors often doesn't exist, making the development of complex models hard. However, the lack of the information required to inform a complex model does not mean useful understanding can not be developed from combining the literature. It is important to consider the timescales that one is interested in. Over longer timescales and when focused on macro properties such as time to complete biodegradation, the importance of each individual parameter diminishes and bulk parameters can be considered appropriate.

Thus, in order to determine average rates of biodegradation of PHA for the purposes of this paper, a simple approach to biodegradation has been adopted. A simplified biodegradation process has been conceptualised with three key steps and a rate for each defined (Fig. 1) (Haider et al., 2018; Lucas et al., 2008). It is acknowledged that precise differentiation between each step in the process, as presented here, is not entirely accurate (all steps occur in a concurrent and iterative manner), but simplification is required for the purpose of communication. One of the three steps that has been defined is biofilm formation (also known as biodeterioration), the rate of which has been designated R_B. The biofilm is a unique and complex association of microbes formed from surface-associated microbial cells that are embedded in a self-produced extracellular polymeric substance (EPS) matrix consisting of polysaccharides, proteins and entrapped organic and inorganic particles (Donlan, 2002; Flemming, 1998). A lag time (ranging from a few days up to a few weeks) is often observed before a steady rate of biodegradation is reached, as it takes time for a biofilm to form and the microbial population to adapt (Imam et al., 1999; Woolnough et al., 2008). Another step that has been defined is enzyme catalysed, hydrolytic depolymerisation, the rate of which has been designated R_D. This is when extracellular depolymerases catalyse the hydrolytic bond cleavage of the polymer, eventually leading to the formation of oligomers, dimers and monomers. Finally, the uptake of small molecules by the cell during bioassimilation for either growth and reproduction or mineralisation has been combined into a single parameter. The resulting products of this process are increased cell biomass and simple end products like CO_2 in aerobic environments and methane in anaerobic environments. The rate of the bioassimilation and mineralisation has been designated R_M. R_B and R_D are considered to be rate limiting, meaning that the effects of any factors on R_M can be considered to be less significant (Chinaglia et al., 2018; Hong and Yu, 2003; Spyros et al., 1997).

Two key methods by which biodegradation is normally monitored are physical methods (such as mass loss) or respirometric methods (such as CO_2 evolution and biological oxygen demand). Respirometric methods are used in laboratory studies to provide a complete picture of the polymer biodegradation, as they are the only way to prove that the final step of mineralisation has occurred. This is normally the focus of international biodegradation standards - such as the five active standards that exist for monitoring the biodegradation of a polymer in the marine environment (Harrison et al., 2018). These standards present a framework for proving that biodegradation occurs in the marine environment, and for comparing the rate at which biodegradation occurs between different materials. A recent and comprehensive review of these standards has been completed by Harrison et al. (2018). However, whilst a material that passes these standards can claim to be marine biodegradable, this does not provide the information required to allow for prediction of in situ rates of biodegradation, giving no indication of how long an item made out of that material would actually take to break down in the open environment. This is why physical methods, such as monitoring mass loss, are employed, as they are the only practical way to monitor biodegradation in the target environment.

Results from both respirometric methods and mass loss experiments can be used to calculate rates of biodegradation. Using data from respirometric methods allows for the rate of biodegradation to be calculated as a function of R_B (biofilm formation), R_D (depolymerisation) and R_M (bioassimilation and mineralisation) and for the effect of specific factors to be tested. Using data from mass loss experiments only allows for the rate of biodegradation to be calculated as a function of R_B and R_D and is less sensitive to the effect of different factors (Mohan Krishna and Srivastava, 2010; Shah et al., 2008). There are a few points to be noted for calculating rates based on the different methods. Firstly, results from CO_2 evolution experiments should only be used for estimations of rates up until the point at which 80% of the polymer carbon has been evolved as CO_2. At this point, mass loss is likely to have been completed even if not all of polymer has been evolved as CO_2 due to some of it being converted into biomass (Kasuya et al., 1998). Using data from beyond the 80% conversion timepoint to calculate rates or undertake lifetime estimations can lead to the estimation of longer times than are actually required for disappearance of the material. Secondly, for PHA the rate of mass loss (a function of R_B and R_D) is a suitable proxy for the rate of biodegradation even if it does not account for mineralisation. It has been suggested that the rate limiting step in the biodegradation of PHA is the biofilm formation phase and attachment of enzymes to the polymer surface leading to catalytic depolymerisation (Hong and Yu, 2003; Spyros et al., 1997). For the majority of the biodegradation process when direct mineralisation of the polymer is the focus, not mineralisation of the formed biomass, the assimilation and mineralisation of PHA is assumed to be rapid ($R_M \ll R_B$ & R_D; Chinaglia et al., 2018). Combining this assumption with the understanding that PHA is degraded via a surface erosion mechanism and only macro changes in polymer integrity over long timescales are of interest leads to the conclusion that the rate of biodegradation of PHA can be suitably estimated as the linear rate of mass loss over time.

The main aim of this work was to determine the rate of biodegradation of PHA in the natural marine environment and apply this to the lifetime estimation of various PHA products. This can be achieved by drawing together the existing literature on PHA weight loss in the natural environment in order to understand what the upper and lower boundaries of the biodegradation rate are. The aim of this paper is not to develop a theoretical model for determining the rate of biodegradation of PHA under a specific set of conditions.Two secondary aims were to: a) compare the biodegradation rate of PHA in a marine environment to the biodegradation rate in soil, compost and anaerobic digestion; and b) collate information on the currently known factors influencing the biodegradation rate to determine what initial conclusions can be drawn at this point and identify what targeted studies need to be performed in order to better understand the significant parameters. This analysis was used to identify some of the gaps in understanding that still exist and to suggest initial improvements for further

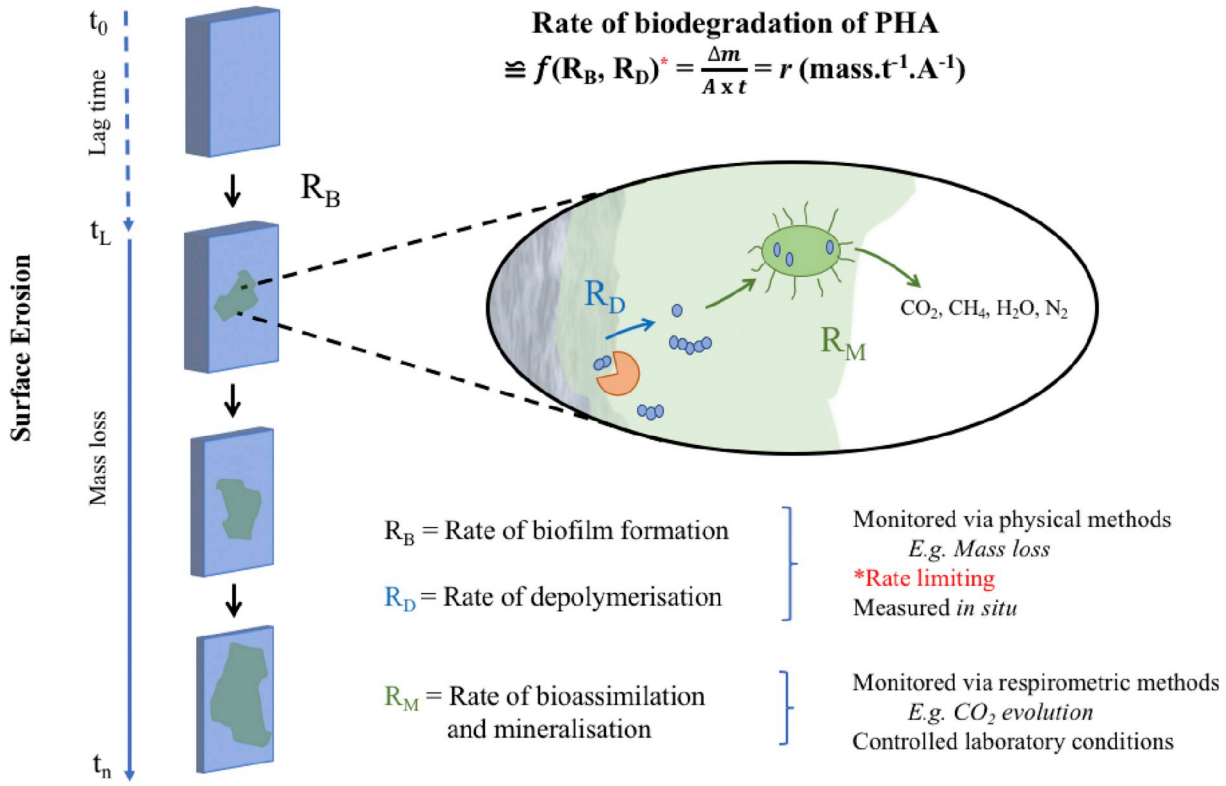

Fig. 1. Rate of biodegradation of PHA. R_B = Rate of biofilm formation; R_D = Rate of depolymerisation, R_M = Rate of bioassimilation and mineralisation; t_0, t_L, t_n = initial time point, lag time and final time point respectively; A = surface area, m = mass. It should be noted that whilst the steps of biodegradation are shown as occurring sequentially for means of communication, all processes are concurrently taking place, one process does not occur in totality before the next commences.

studies. It should be noted that this review does not cover work that only analyses the biodegradation of PHA by specific bacteria/enzymes, or that only assesses the microbial communities that are present during biodegradation but not the associated rates of biodegradation.

2. Methodology

Scopus and Google scholar were searched (final search December 2018) using a combination of search terms (Table 1) relating to polymer type and degradation environment. Only papers that focused on the biodegradation of PHA in a natural setting, or in a laboratory setting using a natural inoculum, were included. Any papers that focussed only on inoculation with specific bacteria were excluded. A final selection of 20 papers relating to biodegradation in the marine or aquatic environment was identified, and a list of these papers is detailed in Table 2. A list of the papers identified for soil, compost and anaerobic digestion environments is included in the supplementary information (Table S1).

Table 1
Search terms. One term from each category was included and all combinations were tested.

Polymer type	Environment	Biodegradability
PHA	Marine	Biodegrad*
PHB	Seawater	
PHBV	Aquatic	
Biopoly*	Soil	
	Compost	
	Anaerobic	

The asterisk is used as a wildcard symbol when searching databases to widen the search (finding words that start with the same letters but have different endings).

For the selected marine/aquatic papers, information relating to the following points was recorded if mentioned (presented in Table 2):

- The material and method (polymer composition, location of the study, method of monitoring biodegradation (mass loss, CO_2 evolution, loss of mechanical properties), shape of sample, length of study);
- The controlling variables (temperature, dissolved oxygen, salinity, UV exposure if near the surface, nutrients, pH, bacterial concentration and identification);
- The outcomes (final extent of biodegradation (based on mass loss, CO_2 evolution or other property changes as recorded in the study considered), molecular mass changes (M_w or M_n), crystallinity changes).

Of the literature identified in Table 2, that focusing on the biodegradation of PHB or PHBV in the natural marine environment was reviewed. The studies that included sufficient information on the biodegradation rate and material characteristics (starting mass, sample shape and surface area) were selected. A brief summary of these papers is presented in the supplementary information (Table S2). Data was normalised to a rate of polymer biodegradation based on initial surface area ($mg{\cdot}day^{-1}{\cdot}cm^{-2}$) (Eq. (1)). This enabled a comparison between studies in the marine environment as well as between environments (i.e. marine, soil, compost, anaerobic digestion).

$$r = \frac{\Delta m}{A \times t} \quad (1)$$

where r represents the specific rate of mass loss ($mg{\cdot}day^{-1}{\cdot}cm^{-2}$) and is a function of R_B and R_D as presented in Section 1; Δm is the change in mass (mg); A is the initial surface area (cm^2) and t represents time (days). The area of the face of a film was taken as the length multiplied by the width, and does not account for surface topography, pores and

Table 2
Detailed description of all factors considered in marine biodegradation of PHA studies.

	Material		Location			Test method		Shape		
	PHB	PHBV	Lab	Field	Sediment considered?	Weight loss	CO_2 evolution	Film	Thickness (μm)	Solid
Brandl and Puchner (1992)		✓ (8%)		✓[a]	✓	✓		✓	Not stated	✓
Deroiné et al. (2014a)		✓ (8%)	✓	✓		✓				✓
Deroiné et al. (2015)		✓ (8%)	✓	✓	✓	✓	✓	✓	200	
Doi et al. (1992)		✓ (0–61%)		✓		✓		✓	50–150	✓
Greene (2012)	✓		✓		✓		✓	✓	not stated	
Ho et al. (2002)	✓		✓			✓	✓	✓	Not stated	
Imam et al. (1999)		✓ (12%)		✓		✓		✓	510	
Kasuya et al. (1998)		✓ (14%)	✓			✓		✓	100	
Mayer (1990)		✓ (8, 24%)	✓		✓	✓		✓	Not stated	
Mergaert et al. (1995)	✓	✓ (10, 20%)		✓		✓				✓
Muhamad et al. (2006)	✓	✓ (20%)	✓			✓		✓	Not stated	
Rutkowska et al. (2008)		✓ (12%)		✓		✓		✓	115	
Sashiwa et al. (2018)		Other	✓				BOD	✓	20	
Sridewi et al. (2006)	✓	✓ (5%)		✓	✓	✓		✓	Not stated	
Thellen et al. (2008)	✓	✓ (5–12%)	✓	✓	✓		✓	✓	190	
Tsuji and Suzuyoshi (2002a)	✓			✓		✓		✓	50	
Tsuji and Suzuyoshi (2002b)	✓		✓			✓		✓	50	
Tsuji and Suzuyoshi (2003)	✓		✓			✓		✓	50–200	
Volova et al. (2010)	✓	✓ (11%)		✓		✓		✓	100	✓
Wang et al. (2018)		Other	✓				✓			✓

	Shape		Molecular mass	Crystallinity	Temp (°C)		UV	Nutrients	Bacteria and fungi		Mechanical property changes considered?	Length of study (days)
	Thickness (cm)	Powder	Changes considered?	Changes considered?	Range (°C)	Investigated as key point?	Considered?	Measured or added?	Counted?	Identified?		
Brandl and Puchner (1992)	Bottle				< 6 6–8							245
Deroiné et al. (2014a)	0.4		✓	✓	4, 13, 25, 40	✓			✓		✓	360
Deroiné et al. (2015)		✓	✓	✓	11–20				✓			180–600
Doi et al. (1992)	0.2		✓		13–26					✓	✓	365
Greene (2012)					30							365
Ho et al. (2002)					28			✓ (added)	✓			86

(*continued on next page*)

Table 2 (*continued*)

	Shape		Molecular mass	Crystallinity	Temp (°C)		UV	Nutrients	Bacteria and fungi		Mechanical property changes considered?	Length of study (days)
	Thickness (cm)	Powder	Changes considered?	Changes considered?	Range (°C)	Investigated as key point?	Considered?	Measured or added?	Counted?	Identified?		
Imam et al. (1999)					Various 25–29 32–36				✓		✓	365
Kasuya et al. (1998)					25			✓ (added)				28
Mayer (1990)					30							84
Mergaert et al. (1995)	Dog bone		✓		Marine 6–14					✓	✓	180
Muhamad et al. (2006)					37							Not clear
Rutkowska et al. (2008)			✓		17–20						✓	42
Sashiwa et al. (2018)		✓	✓		27							28
Sridewi et al. (2006)					32							56
Thellen et al. (2008)					Field 12–22			✓ (both)				49–100
Tsuji and Suzuyoshi (2002a)			✓		19–26						✓	150
Tsuji and Suzuyoshi (2002b)			✓		25						✓	300
Tsuji and Suzuyoshi (2003)					25						✓	35
Volova et al. (2010)	0.5		✓	✓	27–30				✓	✓		160
Wang et al. (2018)	0.1				Ambient			✓ (added)		✓		195

[a] Only a freshwater environment was considered.

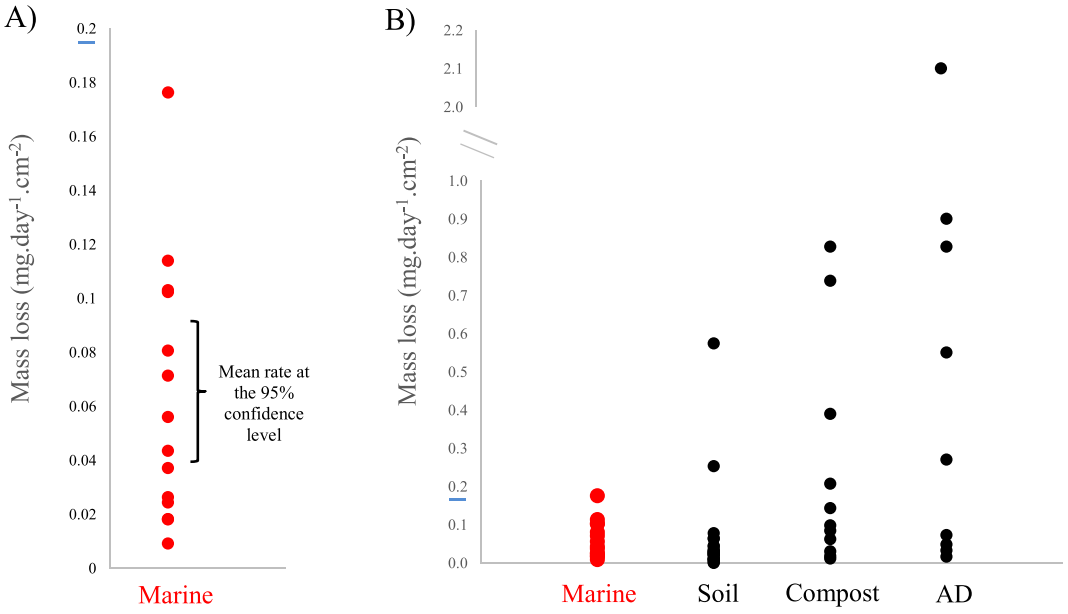

Fig. 2. Normalised biodegradation rate of PHA in different environments; A) Marine data in detail including the 95% confidence interval of the mean. B) Degradation rate in different environments (marine data is the same as in A). Note the break in the y-axis for B.

voids. Change in mass was calculated from the start of the experiment to the final time point presented and the rate was assumed to be linear, with no adjustments made for lag time or biodegradation plateaus. Furthermore, no adjustments were made for the acceleration of biodegradation that can occur as a result of autocatalysed hydrolysis or increase in surface area (from increased surface roughness or fragmentation). This gives an average rate of biodegradation, rather than a maximum rate, leading to more accurate lifetime predictions. Where a starting mass was not reported, the density (assumed to be 1.24 g·cm^{-3} (TianAn PHA)) and dimensions of PHA were used to estimate the initial mass.

The 95% confidence interval of the mean for the specific rate of mass loss was calculated and then converted into a rate of surface erosion per day (mm·day^{-1}) as shown in Eq. (2).

$$\lambda = \frac{r}{\rho} \tag{2}$$

where λ represents the rate of surface erosion (mm) and density (ρ) was taken as 1.24 g·cm^{-3}. An estimation of the likely polymer lifetime can then be made using Eq. (3).

$$t_d = \frac{h_0}{2\,\lambda} \tag{3}$$

where h_0 is the starting thickness of the film (mm) and t_d is time to complete biodegradation (days). Dividing by two accounts for surface erosion on both faces of the film.

3. Results and discussion

The rate of biodegradation of PHA in a marine environment was calculated through collating the results from eight identified papers that contained sufficient information to allow for normalisation of biodegradation rate on a mass per surface area per time basis (Fig. 2A). Given that biodegradation of PHA occurs via a surface erosion mechanism (Guerin et al., 2010; Laycock et al., 2017) and it has been shown that surface area is an important factor influencing biodegradation rate (Chinaglia et al., 2018), normalising to surface area is important in order to allow comparison between the different studies. The rate of biodegradation is also influenced by a variety of factors which differ across studies and cannot be controlled for. This is a limitation that will exist for any collation of rates measured in a natural and continuously fluctuating environment and is why the 95%

Table 3
Factors that are a property of the material.

Properties of the material	
Polymer type	Within the polyhydroxyalkanoate family, most marine biodegradation studies have focussed on PHB and PHBV (most commonly within the range of 5–20 mol% HV content). When directly comparing the biodegradation rates of PHB and PHBV (11% HV) films, Volova et al. (2010) noted no difference between the biodegradation rates. However, Thellen et al. (2008) noted faster biodegradation rates for PHBV (12%) when compared to PHB, as did Mergaert et al. (1995) for PHBV (10% and 20%). Doi et al. (1992) had inconsistent results with the 21% HV content sample degrading faster than all of the other samples (4% HV content sample, PHB, and 61% HV content sample which all had similar rates to each other). In a review on polymer lifetime prediction, Laycock et al. (2017) reports that copolymers consistently degrade faster than homopolymers.
Shape and surface morphology	There has not been a great deal of work into understanding the influence of shape on biodegradation rates. Most of the studies of PHA biodegradation in the marine environment focus on the biodegradation of films, in the range from 20 to 510 μm. Two papers analysed dog-bone specimens (Deroiné et al., 2014a; Mergaert et al., 1995) whilst only three papers experiment with both films and 3D forms (Brandl and Puchner, 1992; Doi et al., 1992; Volova et al., 2010). Volova et al. (2010) found that films (100 μm) degraded faster than compacted pellets. However, no other study specifically comments on this. In regard to consideration of surface morphology, Tsuji and Suzuyoshi (2003) produced PHA films with pores on the surface and found that this significantly enhanced the biodegradability.
Crystallinity	None of the papers interrogate crystallinity as a controlling factor. In regard to changes in crystallinity during biodegradation, Volova et al. (2010) and Deroiné et al. (2014a) found that the crystallinity index does not change with biodegradation of PHA which is consistent with a surface erosion mechanism.

Table 4
Factors that are a property of the environment.

Properties of the environment	
Location in the water column	Although only considered in a few studies, it appears that contact with sediment plays a significant role in influencing rates of biodegradation. Mayer (1990), found that biodegradation with sediment contact is faster than just in water and Sridewi et al. (2006) found that objects on a sediment surface degraded slower than those that were completely buried. Deroiné et al., 2015 found the biodegradation kinetics to be slower in just sand compared to a saturated sand and seawater combination, proposing that this is due to degree of surface contact. Thellen et al. (2008) also considered sediment addition and concluded that sediment and the associated microbes play a role in influencing rates of weight loss but could not establish a simple relationship.
Temperature	A range of different temperatures have been considered in the studies reviewed. All of the studies support the idea that biodegradation is faster when water temperature is higher. Mergaert et al. (1995) observed that biodegradation of a sample monitored in the environment was faster over summer and Brandl and Puchner (1992) found that biodegradation of a PHA bottle was faster closer to the surface of a lake. Both related these results to the temperature of the water, with higher water temperature leading to faster biodegradation rates. Doi et al. (1992) also observed that the rate of surface erosion was markedly dependent on the temperature of the sea-water. Thellen et al. (2008) suggests that the effect of colder water temperatures (and limiting nutrient supply) is what slowed the rate of weight loss in a natural environment compared to standard laboratory methods. Deroiné et al. (2014a) is the only study to consider controlled trials of different water temperatures as part of accelerated ageing experiments designed to assess the validity of lifetime estimation based on the Arrhenius relationship. The temperatures used were 4, 25 and 40 °C. They found that the increase in temperature did not have a substantial impact on weight loss. This needs further investigation.
Nutrients	None of the studies performed in the natural environment report the nutrient levels of the water or discuss it as a controlling factor. This is an oversight given that nutrient levels have been known to influence bacterial populations for many years (Zobell and Grant, 1943) and are likely an important and variable factor across different field settings.
Microbes	Seven of the studies quantify the concentration of bacteria in their study (Deroiné et al., 2014a, 2015; Ho et al., 2002; Imam et al., 1999; Mayer, 1990; Volova et al., 2010; Wang et al., 2018) whilst a few take this a step further and perform sequencing to identify the organisms present (Doi et al., 1992; Mergaert et al., 1995; Volova et al., 2010; Wang et al., 2018). Some of the studies measure concentrations before addition of the PHA whilst others measured the concentration after. None looked at a change in the microbial community over time.
UV light exposure	The only paper that mentions UV in a marine setting when considering PHA biodegradation (Tsuji and Suzuyoshi, 2002a) doesn't measure UV radiation, but states that they believe it is having no influence given that no changes in molecular mass are observed. PHA is denser than water, so it is not expected to be found on the surface in a marine environment and therefor UV exposure will probably be minimal.
Dissolved oxygen and Salinity	Dissolved oxygen and salinity are recorded but not discussed as a controlling factor in any of the studies.
pH	No studies discuss the influence of the pH of the natural environment on PHA biodegradation. The only study that focuses on pH considers hydrolysis in the absence of enzymes under controlled conditions (Muhamad et al., 2006). The mass loss of PHB and PHBV samples in pH 7.4, 10.0 and 13.0 at 37 °C, were monitored and it was found that degradation proceeded faster in an alkaline medium. However, the mechanism was not delved into further.

confidence interval of the mean should be focused on rather than the sample mean. The calculated 95% confidence interval of the mean is 0.04–0.09 mg·day^{-1}·cm^{-2} (see Supplementary information, Table S2 for individual data points) – i.e. there is 95% confidence that this interval contains the true mean of the rate of biodegradation of PHA in the marine environment. The factors that may then influence the biodegradation rate of a PHA product in a specific environment (within this range) are discussed in Table 3 and Table 4. It is highlighted that this result is based on the best information available to date, and should be updated once the controlling factors are more thoroughly understood.

To add context to these results, the 95% confidence interval was used to calculate the potential lifetimes of different PHA items in the marine environment (Fig. 3). As would be expected, the time required for complete biodegradation of a product and the range of uncertainty increases with thickness of the material. A 35 µm PHA bag, for example, could be expected to last between 25 days and two months before it has completely biodegraded. On the other hand, a PHA bottle, with a wall

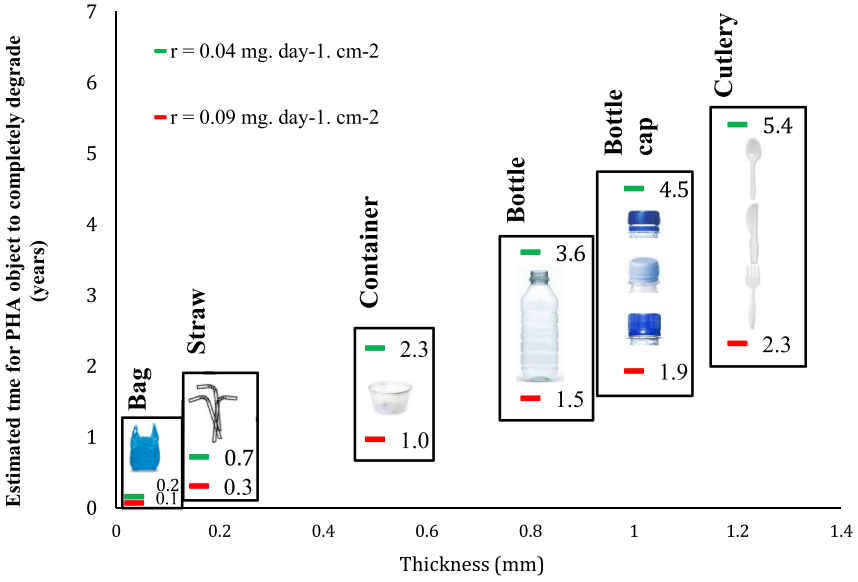

Fig. 3. How long will it take a PHA item to degrade? Lifetime values estimated using the 95% confidence interval for the mean of the rate of biodegradation of PHA in the marine environment.

thickness of 800 μm, is expected to take much longer, with the shortest lifetime being one and a half years, but the upper limit being approximately three and a half years. These are currently the best predictions that can be made. However, there is clearly more research required to reduce the level of uncertainty, as well as to tailor the predictions to the many different ocean environments that exist. Fig. 3 is useful in that it defines upper and lower bounds for what could be expected for the mean of lifetime estimation of different PHA objects. However, factors such as temperature, nutrient availability, and location in the water column (explored in Tables 3 and 4) will influence what the mean lifetime for a specific material in a specific environment could be expected to be.

A key limitation with this value, which is unavoidable given the available data, is that it heavily draws on work performed on thin films. It could be expected that thin films would degrade faster than thicker objects due to the propensity for the formation of pores and cracks, increasing surface area (Tsuji and Suzuyoshi, 2002a, 2002b) and enabling more rapid fouling and fragmentation (Volova et al., 2010; Wang et al., 2018). This means that the biodegradation rates calculated in the majority of the reviewed papers have the potential to over-estimate biodegradation rates when extrapolated to solid objects. This said, thicker objects (that have a longer lifetime) will be less influenced by the uncertainty surrounding lag time than thinner objects (with shorter lifetimes). Thus, there is also a margin of error on the degradation times for the thinner objects such as plastic bags and this is related to the time taken for biofilm formation.

It is known that biodegradation kinetics can vary depending on the environment. In order to understand how the biodegradation rates of PHA in the marine environment compare to those in other environments, some key papers relating to the biodegradation of PHA in soil, compost or anaerobic digestion were identified and, where possible, results normalised in the same way as described for the marine studies to allow for comparison (Fig. 2B). The ranges for biodegradation rate in both compost and anaerobic digestion (AD) were much larger than for the soil and marine environments, indicating the capacity for a much higher rate of biodegradation in these systems. This would be expected given that both compost and AD are controlled systems, with higher concentrations of microbes and higher operating temperatures, designed to provide optimal rates of breakdown (Haider et al., 2018). On a within-study basis, Rutkowska et al. (2008) found that the weight loss of PHBV was different between different environments and decreased in the expected order with anaerobic sludge > aerobic sludge > river sediment > seawater. Manna and Paul (2000) also found the biodegradation rate of anaerobic digestion to be faster than soil, which in turn was faster than in compost or marine settings. However, more targeted research with controlled samples across the different environments will be required to confirm these results.

In an attempt to develop a more nuanced understanding of the biodegradation rate of PHA in a marine environment, the factors that have the potential to influence the rate of biodegradation were identified and evidence relating to their influence were collated from all of the PHA marine biodegradation studies. The results of this detailed analysis are recorded in Table 2. The benefit of this collation is that it allows a rapid assessment of which factors have received considerable exploration and which factors have not.

Each of the data points for biodegradation rate of PHA in the marine environment presented in Fig. 2 were then coded for the four factors that all of the studies reported on. Namely, material type (PHB or PHBV), environment (freshwater or marine), thickness of the sample (> 2 mm or film) and temperature of the test environment (< 0 °C, 3–10 °C, 11–25 °C and > 25 °C). The analysis (not shown) did not reveal an identifiable relationship between biodegradation rate and any of these four factors. However, this finding is obviously limited in its significance due to the limited data available.

Given that no clear relationship emerged, the information available on the effect of each of the controlling factors presented in Table 2 was qualitatively reviewed. Both structure and property of the polymer as well as location, weather and climatic conditions substantially influence biodegradation rates (Volova et al., 2006), so the factors have been divided into two groups - those that are a property of the material being studied (presented in Table 3) and those that are a property of the environment being studied (presented in Table 4).

Ultimately, it is hard to isolate the influence of any of these factors in the natural environment and it is the influence and interplay of each of these identified (and potentially other unidentified) factors that need to be understood.

In regard to the influence of environmental factors, it is important to consider the ultimate location of PHA in the marine environment. PHA contains heteroatoms in its backbone and is denser than water, meaning it is more likely to sink than a conventional polymer. This suggests that PHA is likely to be in contact with sediment rather than be free-floating, and that its dispersal via ocean currents will be different to a conventional polymer (potentially remaining closer to its point of entrance to the ocean versus being distributed to the open ocean). Any sort of light exposure and UV degradation will probably be minimised if it settles in the sediment, removing one of the most important factors initiating the abiotic photodegradation of conventional polymers (Gewert et al., 2015). In addition, sediment, and in particular deeper sediment layers, are suggested to host a larger consortium of microorganisms and will have low dissolved oxygen concentrations (Andrady, 1994). Furthermore, if PHA remains close to shore it would likely be exposed to higher temperatures and more active bacterial populations (Deroiné et al., 2014a; Rutkowska et al., 2008) given that the bacterial population in deeper, colder water can be at least one order of magnitude lower than in shallower testing environments (Deroiné et al., 2014a; Imam et al., 1999). This suggests that tests conducted in sediment with nutrient and temperature profiles similar to a shoreline are more likely to be reflective of the biodegradation of PHA than those conducted as suspended samples in the open ocean.

In regards to the influence of the material characteristics, surface phenomena, particularly roughness, can influence bacterial attachment and enzymatic action (Woolnough et al., 2013), as can porosity (Chan et al., 2019; Tsuji and Suzuyoshi, 2003) and crystallinity (Spyros et al., 1997). The type of polymer processing (solvent cast, melt pressed, extruded), post processing treatment, and surface chemistries (e.g. orientation effects) can also influence biodegradation rate. For example, Sridewi et al. (2006) suggested that the increased surface porosity of a poly(3-hydroxybutyrate-co-5 mol% 3-hydroxyhexanoate) film compared to other films contributed to its increased biodegradation rate whilst Boyandin et al. (2013) suggested that due to the presence of micropores at inter-particle boundaries in pellets formed through a pressing mechanism, they degraded faster than samples produced through casting.

A deeper understanding of these factors influencing biodegradation rates will require studies to investigate targeted comparisons between samples as opposed to just considering biodegradation in general. In particular, studies are required that look at the influence of shape, surface morphology, porosity, additives and processing techniques as they have the potential to be used as a controlling factors (Chan et al., 2019; Sridewi et al., 2006; Tsuji and Suzuyoshi, 2003). There has also been no targeted consideration of the method of production of the PHA objects tested or the post-processing methodology, although this influences the material properties and potentially the biodegradation rate (Cherpinski et al., 2017; Follain et al., 2014; Laycock et al., 2017). Most of the literature to date has focused on thin films (< 200 μm) which may behave differently than thicker objects and this affects lifetime estimations.

It is also important that more studies be conducted in the natural environment, as there are issues with transferability of results from laboratory studies (Deroiné et al., 2014a) and in the natural environment the polymer may not be a preferred substrate relative to other available materials (Haider et al., 2018). As discussed, the likely sinks

(e.g. sediment) must also be considered and should be the target locations for biodegradation studies (Nauendorf et al., 2016).

Of the literature reviewed, many papers failed to report the critical information that is required to make a comparison between the different pieces of research, limiting the utility of the body of research to date. Initial mass and surface area or sample dimensions should always be reported to allow for standardisation between studies.

4. Conclusion

The aim of this paper was to determine the mean biodegradation rate and lifetime estimation of PHA in the marine environment, dependent on the information available to date. The key result is the determination of the mean rate of biodegradation of PHA in the marine environment as 0.04–0.09 $mg \cdot day^{-1} \cdot cm^{-2}$ ($p = 0.05$). This was used to estimate the average lifetime of various PHA products in the marine environment. For example, using the calculated biodegradation rate a PHA bottle could be expected to take approximately one and a half to three and a half years to completely biodegrade. No single environmental or morphological factor emerges as the key factor influencing biodegradation rate and there is not enough information to understand their individual effects, or develop a robust understanding of how an individual factor would effect lifetime estimation. This in itself is an important contribution, guiding future research and demonstrating that more targeted studies are required that directly compare the influence of different factors (particularly properties of the test sample) as well as ones that consider the ultimate location of the PHA. The calculated rate can be updated once these controlling factors are more thoroughly understood.

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Appendix A. Supplementary data

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