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Review of recent advances in the biodegradability of polyhydroxyalkanoate (PHA) bioplastics and their composites

Kjeld W. Meereboer, [ID] [a,b] Manjusri Misra [ID] *[a,b] and Amar K. Mohanty [ID] *[a,b]

The detrimental impact of single-use plastics on the environment is daily news across the globe. Single-use plastic packaging materials and other plastic waste originating from petroleum-based sources are continuously building up in landfills and leaching into the environment. Managing plastic waste remains an urgent crisis in the environment and switching to biodegradable plastics can help mitigate some of these issues. This review will summarize recent advances and opportunities to utilize polyhydroxyalkanoates (PHAs) as a biodegradable substitute in some applications where non-biodegradable and petroleum-based plastics are currently used. PHAs are a well-known family of bacteria-based biodegradable plastics and offer an approach to carbon neutrality and support a more sustainable industry. PHAs such as poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) show biodegradable behaviour in all aerobic and anaerobic environments defined by ASTM standards, and can be used to make completely compostable, and soil and marine biodegradable products – a strong positive compared to the negativity associated with the landfilling of plastics. However, PHAs are relatively expensive compared to petroleum-based alternatives. To reduce the cost, PHAs can be used in biocomposite materials, where bio-based agro-residues are incorporated, while maintaining the performance in certain applications. Organic fillers and fibres composed of cellulosic material can improve the properties of polymers, however, their effect on the marine biodegradable properties of the composite matrix remains an unexplored area. When used in biocomposites with PHAs, they improve biodegradation rates in all environments. In addition to cellulose, other bio-based fillers such as proteins (*i.e.* distillers dried grains with solubles) and starch have been reported to significantly improve soil and marine biodegradability rates compared to other fibres and fillers. Other components that affect biodegradability are additives (*i.e.* chain extenders) and compatibilizers (*i.e.* maleic anhydride *etc.*) that are added to optimize the service life properties, but are reported to inhibit the biodegradation properties by impacting the hydrophilicity of the polymer and enzyme activity. The multitude of possible combinations of polymers and fillers and fibres, and their effect on the biodegradation of PHA-based biocomposites are a largely unexplored frontier. The potential benefits of PHA-based biocomposites make a strong case for further research into this area.

1 Introduction

A seismic shift in economic objectives triggered by the growing and overwhelming evidence from industry suggests that the projected cumulative growth of primary plastic waste produced by 2050 will exceed 25 billion metric tons.[1] Combined with the shift towards sustainability using non-petroleum based plas-tics, the production of bio-based/non-biodegradable and biodegradable plastics projected from 2020 to 2023[2] is expected to grow 13% per annum. Leading plastic packaging producers are moving towards a goal of 100% recycled, biodegradable or reuseable plastics in their products by 2025.[3] This shift towards a sustainable economy has occurred in the recent decade, such that, between 2010 and 2017, bio-based poly(ethylene) (Bio-PE), bio-based poly(ethylene terephthalate) (Bio-PET), poly(lactic acid) (PLA) and poly(hydroxyalkanoate)s (PHAs) have seen production capacity growth of approximately 22%, 10 000%, 300% and 41% respectively.[4] Replacement of petroleum-based plastics with bio-based alternatives is a more sustainable pathway to plastic production due to their lower associated carbon emissions from petroleum extraction and refinement.[5]

[a]*School of Engineering, Thornbrough Building, University of Guelph, 50 Stone Road East, Guelph, Ontario, Canada. E-mail: mohanty@uoguelph.ca, mmisra@uoguelph.ca*
[b]*Bioproducts Discovery and Development Centre, Department of Plant Agriculture, Crop Science Building, University of Guelph, 50 Stone Road East, Guelph, Ontario, Canada*

1.1 Polymer pollution in the environment

Plastic pollution occurs in two fronts, during production (carbon emissions) and their disposal (contaminants and physical hazards) which impacts both the environment and the ecosystem. Replacing petroleum-based polymers with bio-based polymers is a potential solution that produces significantly lower carbon emissions and energy production requirements.[6] Fig. 1 illustrates the reduced impact of bio-based/biodegradable polymers on the environment relative to some commercial petroleum-based polymers. However, regardless of their production method most plastic waste after their service life ends up being incinerated, landfilled, littered or recycled, resulting in carbon or methane emissions over time. It is the disposal after the service life of biodegradable polymers that further benefits the environment compared to non-biodegradable petroleum-based polymers. Non-biodegradable polymers can leak contaminants or additives into soil and waterways, and physically obstruct animal digestive systems.[7] Several industries can thus benefit from this as there are increasing carbon footprint reduction measurements and reporting required due to increased societal pressure on industry to be more environmentally responsible.

1.1.1 Polymer waste disposal streams. Polymer waste disposal can generally be divided into four separate outlets

Kjeld has completed his Master of Applied Science (M. A. Sc) degree under the advice of Professor Manjusri Misra and Professor Amar K. Mohanty in Biological Engineering at the University of Guelph (Guelph, Ontario, Canada). Prior to that he received his bachelor's degree in Biological Engineering from the University of Guelph. Mr Meereboer's research interests include biopolymer processing and characterization, sustainable materials development, biodegradation and compostability. He has published two peer reviewed journal papers and 4 conference presentations from his Master's research.

Kjeld W. Meereboer

Dr Manju Misra is a professor in the School of Engineering and holds a joint appointment in the Dept. of Plant Agriculture at the University of Guelph. Also, she is serving as the Research Program Director for the Ontario Agri-Food Innovation Alliance (Plant – Technology Panel). She is a Fellow of the American Institute of Chemical Engineers (AIChE) and the Royal Society of Chemistry (UK). She has authored more than 700 publications, including 362 peer-reviewed journal papers, 21 book chapters, and 15 granted patents. She was an editor of the CRC Press volume, "Natural Fibers, Biopolymers and Biocomposites", Taylor & Francis Group, Boca Raton, FL (2005); American Scientific Publishers volume "Packaging Nanotechnology", Valencia, California (2009); "Polymer Nanocomposites", Springer (2014) and "Fiber Technology for Fiber-Reinforced Composites", Woodhead Publishing (2017). She was the chief editor of "Biocomposites: Design and Mechanical Performance" Woodhead Publishing (2015). She was the President of the BioEnvironmental Polymer Society (BEPS) in 2009. She serves in the editorial board of "Journal of Applied Polymer Science", "Composites Part A: Science and Manufacturing", "Polymer Testing", and "Composites Part C: Open Access". In 2012, Dr Misra received the prestigious "Jim Hammar Memorial Award" from BEPS and the University of Guelph's "Innovation of the year award' in 2016 for the involvement in developing the "Compostable single-serve coffee pods". In 2017, Professor Misra has also received the "Andrew Chase Division Award in Chemical Engineering" from the Forest Bioproducts Division of AIChE. In 2019, Professor Misra was awarded with the "Woman of Distinction on Science, Technology, Engineering & Math (STEM)" from the Guelph YWCA-YMCA. Total citations: 32 498; h-index: 81; i10-index: 343 (Google Scholar, July 3, 2020). ResearchGate (RG) Score: 46.87 (higher than 97.5% of ResearchGate members') (ResearchGate, July 3, 2020).
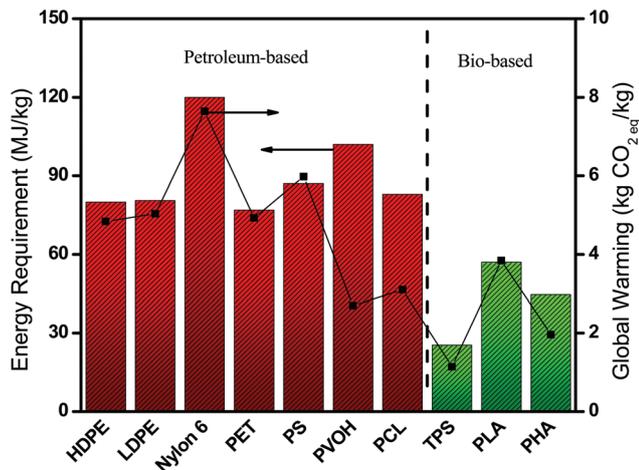
Manjusri Misra

Fig. 1 Production energy and CO_2 emissions of petroleum-based and bio-based polymers.[6,8]

Prof. Amar Mohanty is a Professor and OAC Distinguished Research Chair in Sustainable Biomaterials. He is the Director of the Bioproducts Discovery and Development Centre & University Research Leadership Chair at the University of Guelph, Ontario, Canada. He is the Editor-in-Chief in Sustainable Composites of Composites Part C (An ELSEVIER journal). He is an international leader in the field of bioplastic, biobased materials with a focus on engineering new sustainable materials. He is a highly cited researcher with more than 900 publications to his credit, including 394 peer-reviewed journal papers, and 62 patents awarded/applied (**Google Scholar Citations**: 34 060 with an h-index of 83). Prof. Mohanty received many awards, including the "Lifetime Achievement Award" from the BioEnvironmental Polymer Society (BEPS), USA; the "Andrew Chase Division Award in Chemical Engineering" from the Forest Bioproducts Division of the American Institute of Chemical Engineers (AIChE). He received the top researcher award in 2018 – the "Synergy Award for Innovation" from Natural Sciences and Engineering Research Council of Canada (NSERC) & the prestigious "JL White Innovation Award" from Polymer Processing Society (PPS) in 2020. He has been named with three fellows: Fellow American Institute of Chemical Engineers, Fellow of Royal Society of Chemistry & Fellow of the Society of Plastic Engineers. His constant innovation has catalyzed changes across several manufacturing sectors, including compostable packaging, environmentally friendly consumer products & lightweight biocomposite automotive parts.

Amar K. Mohanty

(Fig. 2): landfill, leakage, incineration and recycling which each have their own drawbacks:[9]

(i) recycling produces some losses and material degradation;

(ii) incineration produces energy at the cost of material and pollution;

(iii) leakage results in environmental hazards which can harm the surrounding environment;

(iv) and landfills result in uncontrolled degradation that can severely harm the environment from methane production.

A new subset of controlled or managed degradation and disintegration is now being developed with the implementation of compostable polymers in industry, following ASTM standards. The end-of-life is value-added usable compost, which could evolve into a multi-billion dollar industry and generate employment for thousands.

1.1.1.1 Recycling. Recycling plastics is an option to reduce the overall plastic waste produced; however, recycled plastics suffer from reduced mechanical performance. This can be overcome by physically treating (annealing) polymers to increase the modulus and strength, use of chemical stabilizers, blending with other recycled plastics or blending recycled plastics with other polymers as a valorisation method.[10] Irrespective of this, recycling generates waste during or at the end of the multi-recycling cycle, with the material being too degraded to use. After many years of implementing recycling, still only a small percentage of plastics in the USA is actually recycled (<10%), compared to non-plastic recycling (25–65%), in 2017.[11] Therefore, an alternative method of plastic waste disposal is required when the service life has ended. Possible alternatives include composting, or diversion to alternative energy production (incineration).

1.1.1.2 Landfill. Landfills are suitable for storage of plastic waste, in comparison to other environments, due to the ease of human intervention. However, landfills go through uncontrolled degradation, releasing greenhouse gases into the environment. Landfills can be subdivided into several types, based on the age and the type of material waste. In consideration of municipal solid wastes, there are old landfills and modern landfills. Old landfills have no control of pollution migration and no gas capturing technology in place. Modern landfills are designed to capture the methane produced, for energy generation.[12] However, in less developed regions, landfills do not have these measures[13] and the off-gases can migrate into the surrounding area.[14] Commodity plastics are not generally landfilled, and, unlike organic materials, do not degrade into methane. But this poses another problem because it remains in the landfill indefinitely under anaerobic conditions. Furthermore, if mismanaged waste results in biodegradable polymers being landfilled, the methane generation can make up to 50% of the total gas release.[15] Another environmental factor is the proper soil coverage after closing the landfill, to prevent plastic waste from being scattered and dispersed into the environment.[16]

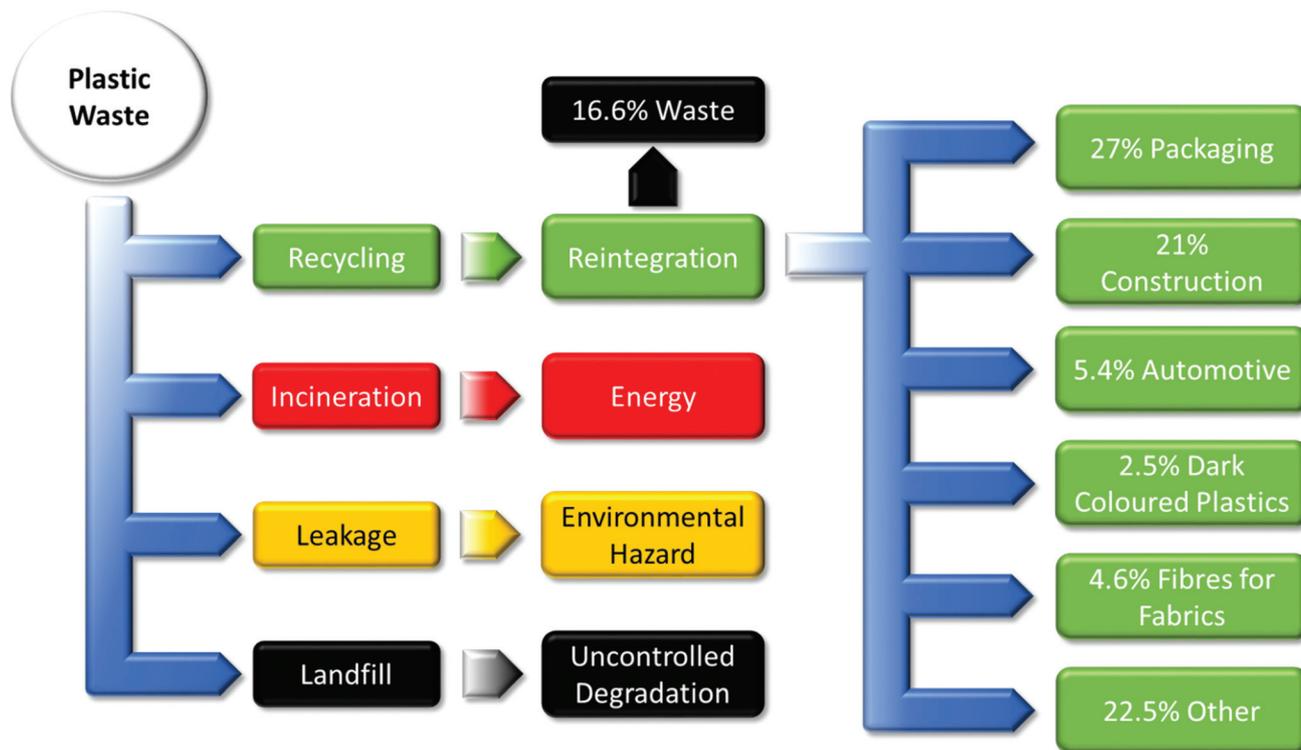*1.1.1.3 Leakage (litter).* Plastic waste in the environment is grouped into two sources, marine-based and land-based,

Fig. 2 Predominant current polymer waste disposal streams.[9]

however the relative quantity remains unclear.[17] Examples of ocean-based waste that likely remains in the ocean today would be lost cargo and plastic pellets from shipping services,[18] or just general shipping waste pollution. Other pathways involve migration of plastics from the land (*i.e.* litter, landfills) to the ocean by environmental elements.[18] Land-based plastic litter can be accidental through environmental elements or intentional, usually by inadequate waste disposal facilities during events or in the public spaces.[19] The plastic litter can also originate from landfills, sewage systems and industrial processes which degrade overtime and accumulate in the soil.[17] Regardless of the sources, plastics containing additives, such as plasticizers and UV stabilizers, contaminate the soil and marine environment over time and impact animal and cellular organ function.[7]

With migration of plastics in the environment it is expected that plastic can be found in a number of oceans (surface and sea floor), shorelines and lakes across the globe.[17] Plastic ending up in the ocean is of concern due to its movement and the difficulty of human intervention. Plastic debris in the ocean can have concentrations up to 580 000 pieces per km^2.[20] This plastic has been found in the Pacific, Atlantic, and Indian oceans in the past two decades, especially in the North Atlantic gyre and the North Pacific subtropical gyre where garbage patches have seen significant growth,[21–23] and it is predicted that 99% of all species of seabirds will ingest plastic by 2050.[20] Furthermore, this plastic lingers in the environment due to its durability if not exposed to microorganisms or UV

radiation.[17] This is exacerbated by the protective coatings applied to polymers to ensure that the properties are not damaged by UV exposure during their service life.[24] This plastic residing in the environment can't be recycled due to contamination or poor residual mechanical and thermal properties. Therefore, biodegradable plastics hold significant importance in combatting mismanaged waste which is expected to double by 2025.[25]

1.1.1.4 Compost. Composting is a subset of biodegradation, such that not all compostable materials are biodegradable in other environments such as marine, soil, landfills *etc.* Furthermore, only a small subset of plastics can be composted, and do not include the commodity recycled ones. Composting can be divided into home composting and industrial composting, with the main difference being the controlled conditions in the industrial composting (~58 °C, 50–55% solids *etc.*). In most climates, home composting is slower than industrial composting, but is suitable for composting organic materials due to their short degradation period.[26] Composting of bioplastics is mainly limited to industrial composting operations and not recommended for home composting.[27] Industrial composting is designed for large amounts of organic waste, has a high turnover and produces compost suitable for soil remediation.[28]

Compostable plastics are predominantly thermoplastic starch (TPS), PLA and poly(butylene adipate terephthalate) (PBAT), making up 83% of the biodegradable plastic produced in 2018. Poly(butylene succinate) (PBS), another biodegradable

plastic, has also experienced growing global production capacity due to the significantly lower production cost compared to other biodegradable polymers (*i.e.* PHAs).[29] The biodegradability of these plastics varies, with some (*i.e.* PLA) being less suitable for home composting due to the long duration,[30] such that in the virgin form only industrial composting conditions are suitable. PLA for example shows effective biodegradable properties in the soil when hybridized with cellulose using appropriate methods, but is not currently implemented on an industrial scale.[31] Furthermore, biosourced plastics utilize renewable carbon, compared to petroleum-based biodegradable polymers (PBAT). Given industrial and commercial composting is not always suitable for compostable plastics, an alternative bio-based biodegradable polymer such as PHAs is well posed for commercial adoption use due to better biodegradable properties in many types of environments.[32]

Therefore, the use of bio-based and biodegradable plastics that can degrade in natural environments (*i.e.* soil, ocean water *etc.*) as a global movement is important because it combats both climate change and plastic pollution – both necessary for sustainable growth and lowering the carbon footprint for the positive environmental effect. An important factor to consider is how bio-based biodegradable plastics differ from other plastics, both in terms of their production and at the end of life. Any modifications in the biodegradation of these plastics, including fibre and filler addition, blends and chemical additions must be considered under a comparable standard under all environmental conditions.

2 Bacterial polyesters

Bio-based polymers can be subdivided into three types, plant-based (*i.e.* starch, cellulose derivatives and natural rubbers), polymerized bio-monomers (*i.e.* PLA, polyimides, polyurethanes, poly(butylene succinate) (PBS), bio-PE *etc.*) and extracted bio-polymers (PHAs).[33,34] Further subdivision of polymerized bio-monomers exists with partially bio-based polymers such as nylon-6,10, poly(trimethylene terephthalate) (PTT) and poly(*p*-phenylene) (PPP).[35,36] Bacterial polyesters are a unique subset of bio-based polymers polymerized by microorganisms. PHAs for example can be synthesized enzymatically *in vivo* by microorganisms as a true natural polyester for intracellular storage,[37] while PLA is produced through fermentation as lactic acid and chemically polymerized.[38] However, despite different production methods, not all bio-based polymers are biodegradable. Among bio-based polymers, only PLA and PHAs are completely bio-based and biodegradable in some form. However, PLA is compostable, but not marine biodegradable like PHAs, making it unsuitable to combat plastic waste leaking into the environment.[39] Moving towards bio-based biodegradable polymers allows for a more sustainable option by implementing a cradle-to-cradle approach,[40] where the output of biodegradation becomes the production input for the same polymer within a reasonable frame of time

(Fig. 3). For example, despite PE and bio-PE being petroleum based and biobased respectively, their main disposal stream is through either recycling or landfill. In addition, petroleum-based polymers such as PBAT are biodegradable,[29] but due to their production method, they do not form a circular cradle-to-cradle approach. PHAs, PLA and cellulosic material are all bio-based and biodegradable, forming a complete carbon cycle; however, PLA is only compostable, not marine biodegradable like PHAs, making it unsuitable to combat the leakage of plastic into the environment.[39] While biodegradable plastic can potentially be recycled in the general waste stream, the resulting properties of the recycled plastic are significantly worse and they are more suited for short life cycles.[41]

2.1 PHAs

PHAs are aliphatic polyesters well known for their biodegradable properties and their bacteria based production methods. Over 91 different polyhydroxyalkanoic acid constituents that make up PHAs have been recorded and the number is continuously growing.[42] Based on the potential combination of monomer units, an uncountable number of PHA copolymers can be formed. PHAs being biodegradable in various environments are an attractive option to replace current single use plastics[11] or plastics that are unsuitable for re-entry into the manufacturing sector due to their poor quality.[43] With their biodegradable properties, PHAs form a closed loop cycle from cradle-to-cradle (Fig. 3) that minimizes the impact on the environment.[44] However, the functionality and production methods are dependent on the type of PHA.

2.1.1 Classes of PHAs. The main subsets of PHAs can be categorized by their chain length: (i) a short chain length of 3–5 carbons; (ii) a medium chain length of 6–14 carbons; and (iii) a long chain length of 15+ carbon atoms. Among short and medium chain length PHAs are unique types with double bonds, when produced from unsaturated fatty acids.[45] The most well-known PHAs are poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate (PHBV), both are short chain PHAs and represent the most basic forms commercially available. Other currently available types of PHAs used in biodegradation studies are poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (PH4B), poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBHx), and poly(3-hydroxybutyrate-*co*-3-hydroxyoctanoate) (PHBO). The functional components are illustrated in Fig. 4. PLA has been previously considered part of the PHA family,[42] however, the polymer production is significantly different compared to PHAs.

2.1.2 Production of PHAs. The synthesis of PHAs is important in biodegradation as the metabolic pathways are related to bio-assimilation. PHAs are usually produced by recombinant *Escherichia coli* for commercial use[46] but can be produced by a number of other microorganisms (*i.e. Aeromonas*, *Azotobacter*, *Cupriavidus*, *Clostridium*, *Methylobacterium*, *Ralstonia*, *Pseudomonas*, *Syntrophomonas etc.*).[15,45,47] *Pseudomonas* is the only reported species to produce long carbon chain PHAs.[48]
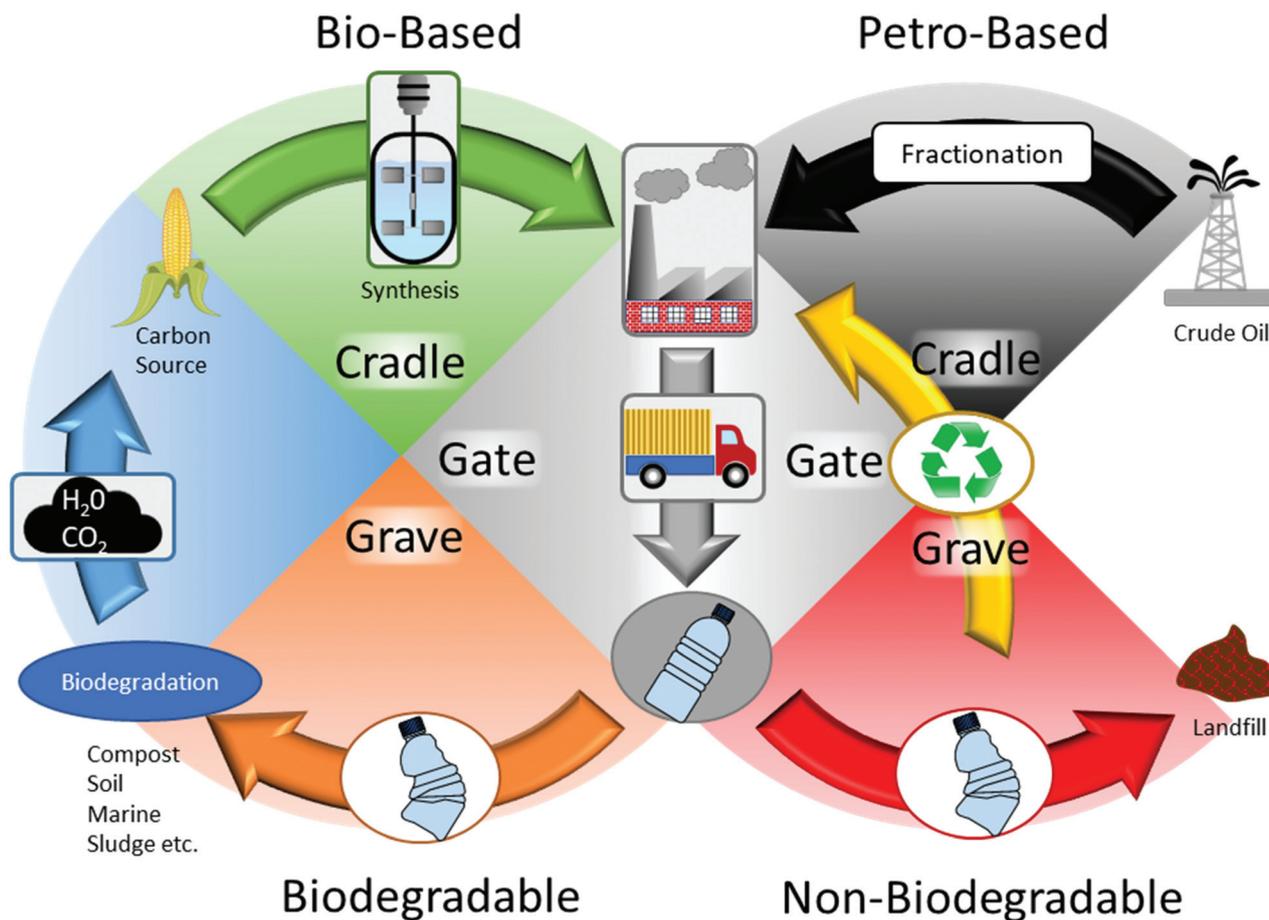
Fig. 3 Cradle-to-cradle and cradle-to-grave approach of biodegradable and non-biodegradable plastics.
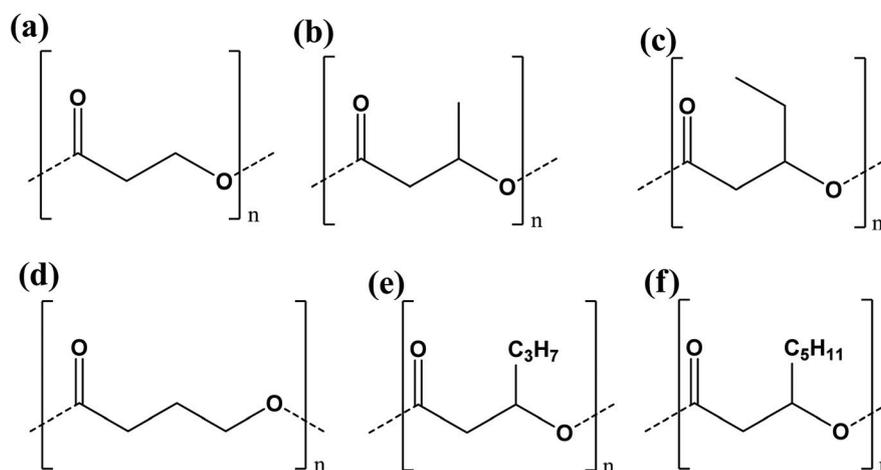

Fig. 4 (a) 3-Hydroxypropionate (HP), (b) 3-hydroxybutyrate (HB), (c) 3-hydroxyvalerate (HV), (d) 4-hydroxybutyrate (4HB), (e) 3-hydroxyhexanoate (Hx), and (f) 3-hydroxyoctonaote (HO) chemical structures.

The bacterial production of PHAs begins under growth limiting conditions because they function as an energy storage molecule for microorganisms,[49] leading to accumulation in the cell walls of bacteria and archaea.[50,51] HB monomers are then bacterially polymerized by PHB synthase of acetoacetyl-CoA,[52] however chemical pathways do exist such as ring opening polymerization (ROP) of β-butyrolactone.[53] PHBV is manufactured under similar conditions but in the presence of

propionic acid. The copolymerization process is initiated enzymatically from acetoacetyl-CoA and 3-ketovaleryl-CoA to form the HB and HV units, respectively.[54,55] However, with additional nutrients and energy costs making up more than 75% of the product cost,[56] PHAs become a relatively expensive product compared to commercial petro-based polymers of comparable properties.[57] For bacterial PHA production, several non-conventional carbon feed sources are used, including mixed carbon sources,[58] organic wastes,[36] methane,[59] peanut oil,[60] soybean oil,[61,62] palmitate oil,[63] waste frying oil,[64] margarine waste,[65] glycerol *etc.*[66] Despite these advantages, the costs associated with the production of PHAs are still very high (comparative to other bio-polymers) and the properties leave much to be desired. Furthermore, while petroleum-based biodegradable plastics can be incorporated into PHAs, to reduce costs and maintain functionality, the sustainability of production becomes lower.

2.1.3 Applications of PHAs. Due to PHAs' biocompatibility and biodegradability, they are considerably attractive for temporary *in vivo* applications, and several have been developed for both PHB and PHBV where a high production cost is less significant. Among these applications are antimicrobial releasing sutures,[67] cellulose support films for gas transfusion,[68] long-term drug release capsules,[69] bone tissue fibres for osteoblast growth,[70] and other tissue scaffold applications such as neural regeneration.[71]

Applications of PHAs outside of the biomedical industry are mainly in the replacement of single use, disposable plastics, such as plastic tableware, food packaging, plant pots and organic waste collection.[72] PHAs also have the potential for sub-

stituting poly(ethylene) (PE) in some film applications, *e.g.* mulch films used to stop weed germination in agricultural farming. PHAs and blends of PHAs with other biodegradable polymers show promise to fulfil the same role as PE with added benefits of degrading during the season to reduce labour costs and farm waste at the end of the season.[73,74] In all cases, biodegradation is a critical factor that makes PHAs marketable and defines their applications outside of the biomedical industry. Currently, PHAs' use in single use plastics for the food industry is limited, despite them showing the potential for bottles, caps, blister packs *etc.*[75] due to odour issues that require additives to remove them.[76] However, the biodegradation of the resulting new blends with any additives remains to be fully explored.

2.2 Challenges of biodegradable plastics

Biodegradable plastics usually suffer from limitations, such as poor mechanical properties (Fig. 5), and the inability to blend them with many other polymers without losing their biodegradable functionality. The market value of biodegradable plastics is in their biodegradable performance when disposed and still requires biodegradation testing to ensure that no chemical interactions hinder the overall biodegradation rate. The two main bio-based and biodegradable polymers, PLA and PHB, are both extremely brittle plastics relative to commodity and engineering plastics. Although some PHAs are more ductile as their chain length increases,[77–79] the cost of PHAs is significantly higher than that of conventional plastics and other bio-based biodegradable plastics.[80] While incorporation of petro-based biodegradable polymers is a potential solution to maintain the biodegradable properties of PHAs, the result-
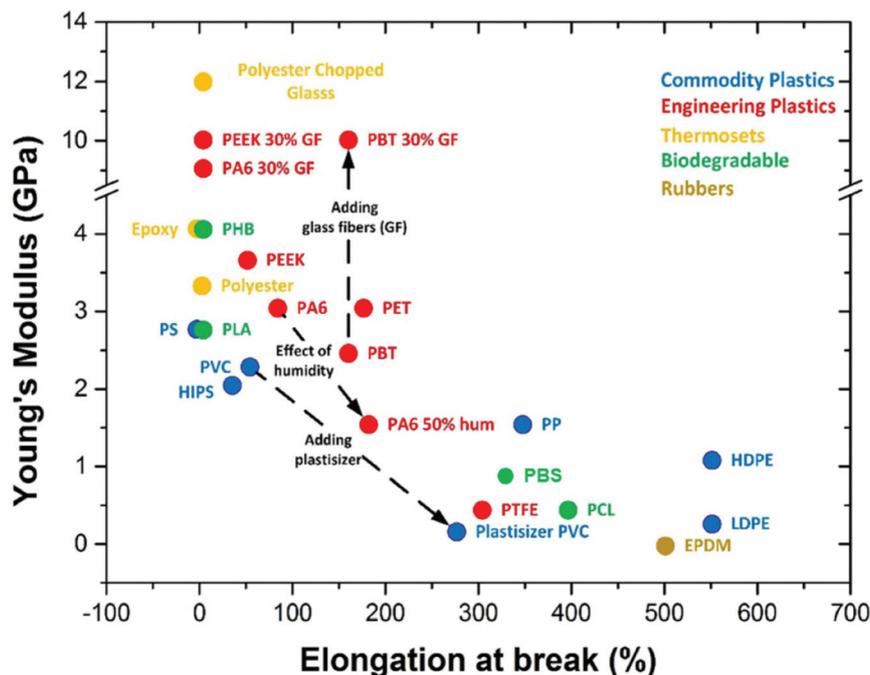

Fig. 5 Young's modulus and elongation at break of plastic types. This figure has been reproduced with permission from ref. 87, Coatings, Creative Commons 2015.

ing blends become less environmentally sustainable due to petroleum usage. For example, to make PLA more flexible, it is blended with PBAT and marketed as Ecovio by BASF. Some studies have investigated blending PHAs with other bio-based or biodegradable polymers. PLA was blended with PHB for improved ductility, toughness and thermal stability,[81] and poly (ε-caprolactone) (PCL) was blended with PHBV for increased ductility.[82] However, the resulting blends suffer from reduced biodegradable performance or costs.

The commercial PHB and PHBV have low impact strength, high brittleness and poor flexibility, making them unsuitable for many industrial and commercial applications compared to other biodegradable or commercial polymers.[83,84] Much success has been found in varying PHA copolymer compositions, such as increasing the Hx content in PHBHx for improved flexibility marketed as Nodax by Makena,[85] or increasing the 4HB content to obtain elastomeric properties.[86] However, with increasing complexity of PHA monomer units, the cost of production increases.

Natural fibres are an option to minimize the cost of PHAs while enhancing their best properties such as modulus and biodegradation. However, the effects of natural fibres, composition and compatibilization techniques on biodegradation remain unclear.

3 What is biodegradation?

Biodegradation is the degradation process involving microorganisms, and is widely accepted as selective, and depends on several factors including the physical and chemical properties of biopolymers. The biodegradation process is defined as polymer degradation by biological microorganisms into CO_2, H_2O, biomass and methane by composting, soil biodegradation, marine biodegradation, or other biodegradation processes.[57] It is also termed biotic degradation and can be enhanced or started after some initial abiotic degradation processes occur such as mechanical, oxidative or hydrolytic degradation (Fig. 6) which can increase the surface area of the organism–polymer interface.[88,89] Thermal or oxidative degradation is non-selective, occurring to all polymers, and introduces thermal or chemical stressors that scission the chains of polymers into smaller units of oligomers, acids, alcohols, esters, and radicals.[90,91] For example, PHAs are less thermally stable than PLA and PCL due to the different thermal degradation mechanisms of random chain scission instead of unzipping depolymerization, and thermal degradation results in a reduced molecular weight along with the esters and alcohol end groups.[92] However, thermal energy can also enhance most if not all other forms of degradation, through disproportionally increasing the high energy collisions between reactants or enzymes and reactants. Catalytic degradation of polyolefins results in gas, oil and waxes by clays, acids, zeolites, aluminium oxides, calcium oxides *etc.* at high temperature.[93] High temperatures are not desired for biodegradation of polymers, due to the inactivity of microorganism enzymes.

The value of biodegradable polymers lies in rapid degradation under natural conditions (*i.e.* soil, ocean water *etc.*), which can become the main marketable attribute, that reduces
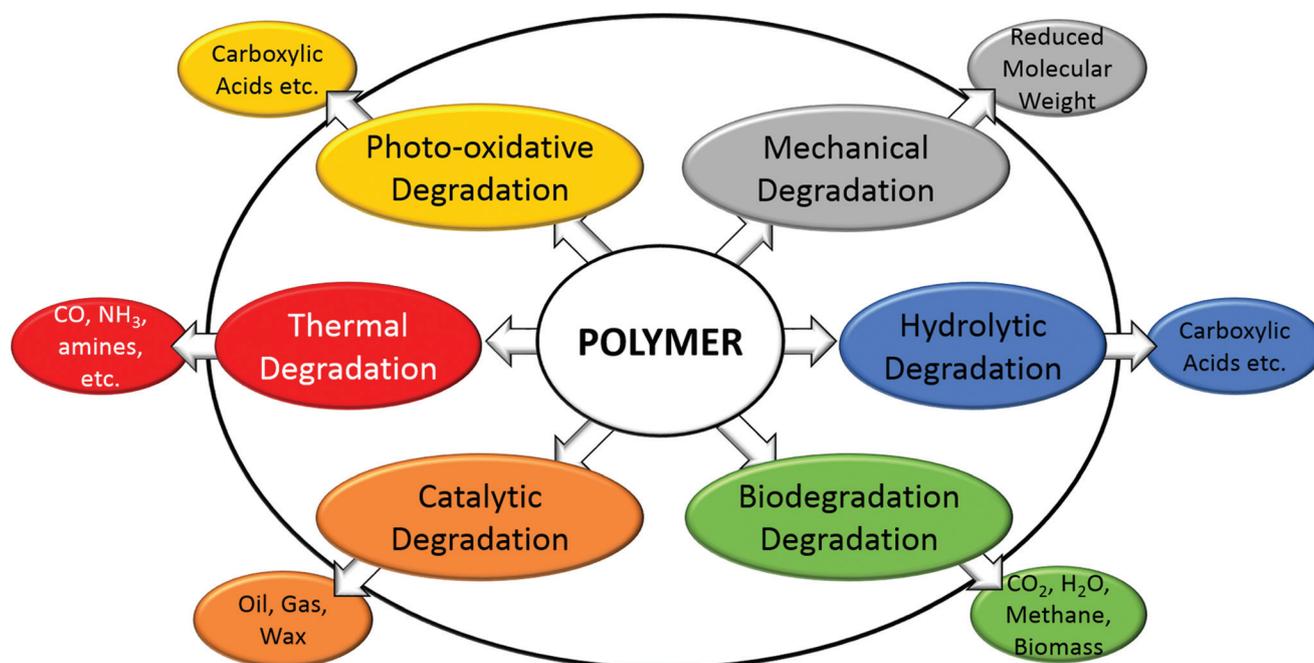

Fig. 6 Basic degradation pathways of polymers.

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potential improperly disposed of plastic waste.[94] Multiple factors affect biodegradation besides the environment conditions (*i.e.* temperature, moisture *etc.*): the polymer composition, molecular weight, crystallinity, composition, chemical structure, reduction potential, hydrophilicity, breakdown products *etc.*[95] but the extent of the effects from some of these factors remains unclear.

When recycling or incineration is unsuitable, biodegradable plastics offer an advantage that has positive effects in environmental waste management. The process can be further enhanced by utilizing bio-based biodegradable polymers, whereby the carbon source input of production biodegrades into CO_2, which is then taken up to produce a carbon source, thereby approaching carbon neutrality.

Biodegradation is influenced by the susceptibility of the polymer carbon backbone to microbial attack.[96] Degradation of any polymer can be divided into two types: surface erosion and bulk erosion. The biotic (enzymatic) degradation is mainly at the surface. The reason is that enzymes are relatively large particles and are unable to permeate the structure of polymers, in comparison to smaller chemicals, free radicals *etc.* Abiotic degradation functions as both bulk and surface degradation and is often used as a pre-treatment to biodegradation. In general, bulk erosion is used for breaking the sample apart into smaller pieces (which enhances the rate of surface erosion) and for molecular weight reduction. For example,

poly(α-hydroxy-esters) samples must exceed a critical minimum thickness (L_c) of 7.4 cm to undergo surface erosion and not bulk erosion (Fig. 7).[97]

Enzymatic hydrolysis of polyesters is completed by lipases and esterases to break ester, carbonate, amide and glycosidic bonds.[99] Due to this nature, warmer temperatures (15–37 °C) and alkali environments increase the rate of PHA and PLA degradation.[100–102] Non-polyesters, considering homochain polymers only, made from entirely carbon backbones are not readily biodegradable. Microbes have difficulty in enzymatically cleaving and degrading aliphatic homochain and heterochain polymers without functional groups (*i.e.* esters, ethers *etc.*) such as PE, polycarbonate (PC), polypropylene (PP), polystyrene (PS) and their derivatives with carbon backbones, and any degradation observed is usually minimal.[103–106] The degradation of polymers follows the schematic of Fig. 8, where biotic or enzyme catalysed reactions are usually a more efficient method. Following polymer degradation by biotic or abiotic methods, the products can be bio-assimilated by microorganisms and used for other needs such as growth and cellular respiration.

Under environmental conditions, biodegradation of PHA following standards is mainly competed extracellularly by extracellular enzymes that cleave the polymer into small enough units to bio-assimilate.[107] During extracellular biodegradation, the PHA is in a highly crystalline form (mainly
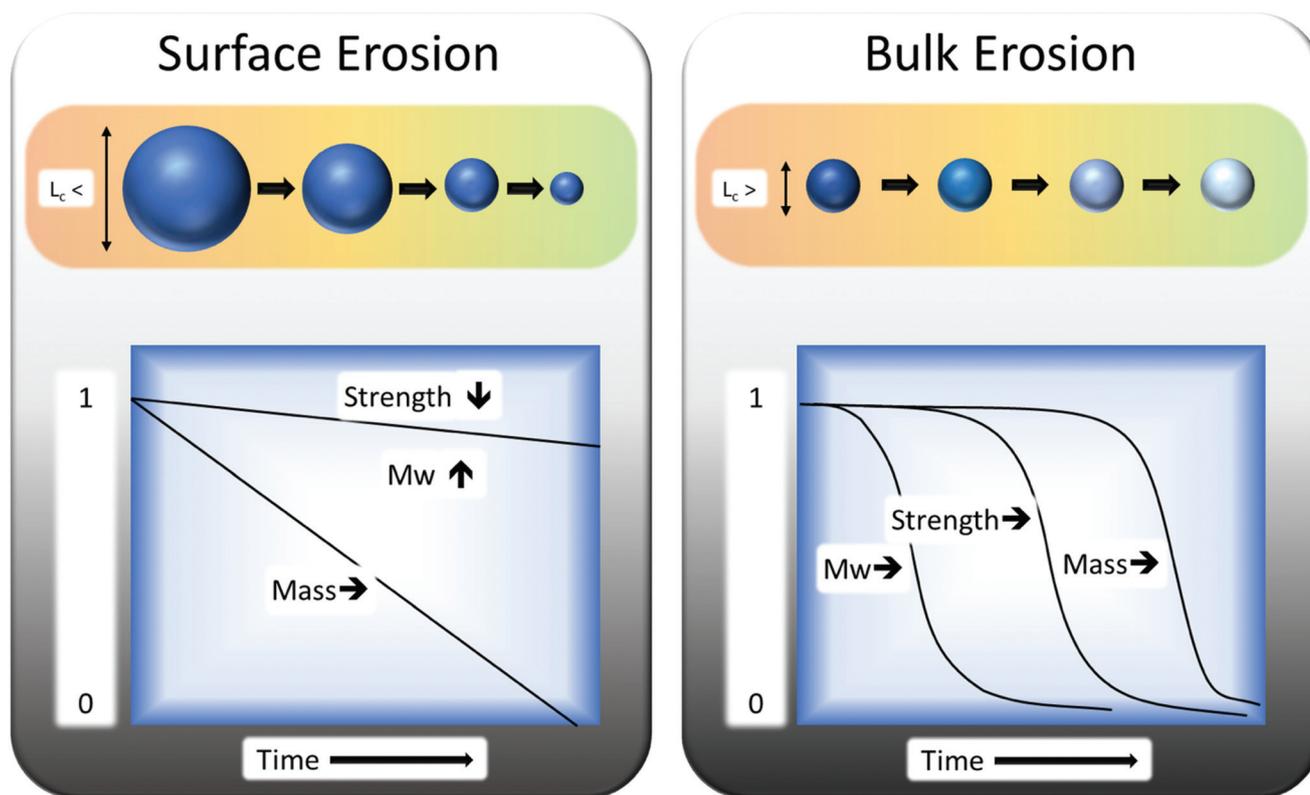

Fig. 7 Bulk erosion and surface erosion effects on polymers as a function of time. This figure has been reproduced with permission from ref. 98, Elsevier, Copyright 2008 (License number: 4800311312040).
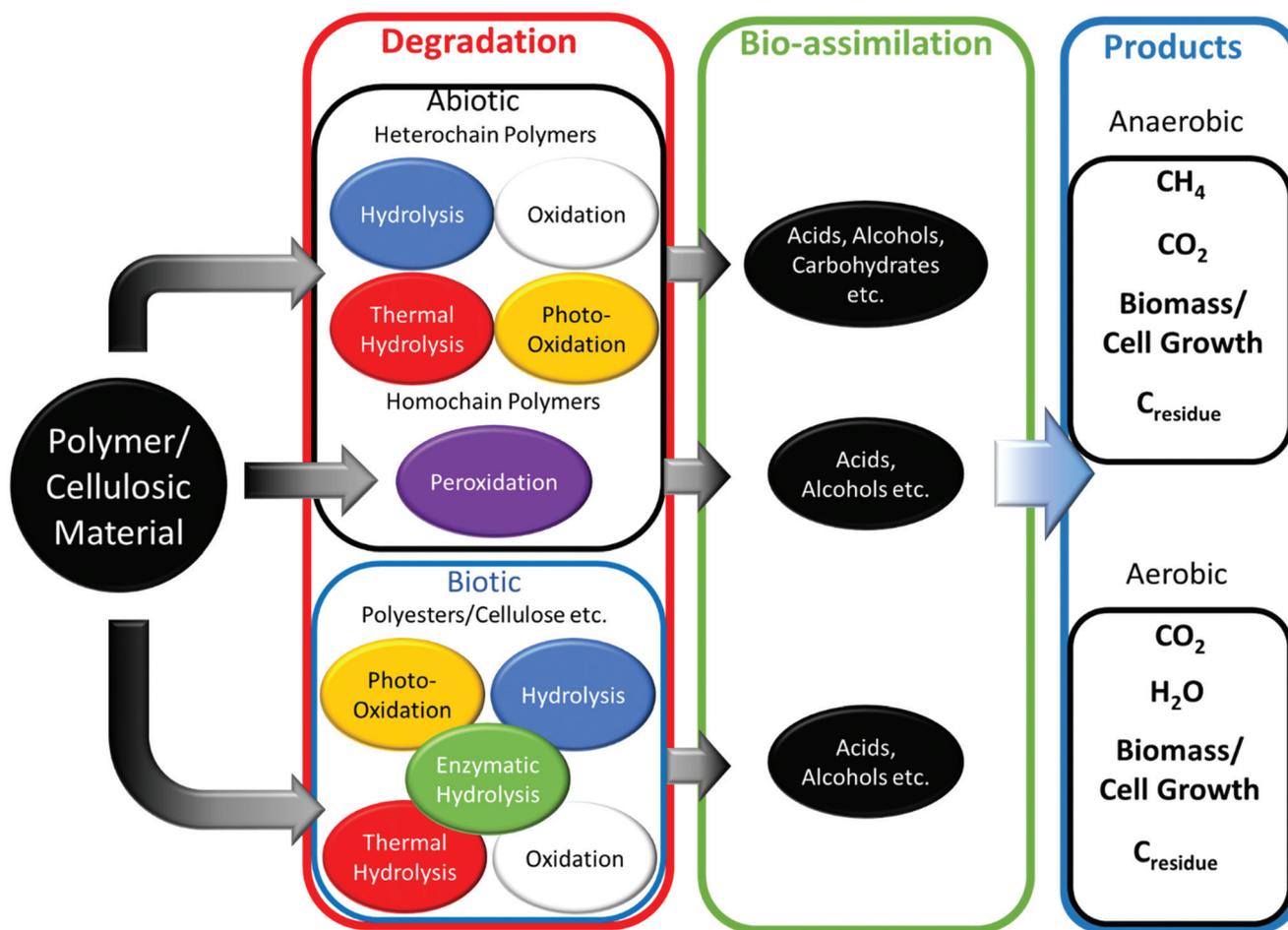
Fig. 8 Theoretical biodegradation pathway of polymer materials.

PHB).[36] However, enzyme-catalysed biodegradation can occur in the cells of microorganisms, and is referred to as intracellular biodegradation.[108] Intracellular PHAs are present in an amorphous state with disordered conformation covered by protein and phospholipids, and are degraded when no alternative carbon source is available.

3.1 Aerobic *vs.* anaerobic biodegradation

Biodegradation of polymers can occur under aerobic or anaerobic conditions, leading to varied products. Aerobic degradation (in the presence of oxygen) mainly utilizes oxygen as a final electron acceptor, while microorganisms that perform anaerobic degradation (in the absence of oxygen) use CO_2, nitrates, sulphates *etc.* as an alternate electron acceptor to generate energy for the cell functions.[109–111]

Most biodegradable polymers show evidence of degradation in both aerobic and anaerobic environments.[112] For enzymatically degraded polymers, it is the temperature that usually affects whether polymer scission occurs. For example, PLA requires a temperature equal to or greater than its T_g (~55 °C) to biodegrade effectively.[113]

The basic chemical equation for aerobic biodegradation is the conversion of organic carbon into CO_2 by microorganisms, in the presence of oxygen (eqn (1)). The carbon atom is generally part of a complex structure, and in some cases oxygen can be derived from the polymer itself, such as from polyesters. During cellular respiration of a carbon source (*i.e.* glucose), the liberated oxygen reacts with free hydrogen ions to produce water (eqn (2)).[114]

$$C + O_2 \rightarrow CO_2 \tag{1}$$

$$H^+ + O_2 \rightarrow H_2O \tag{2}$$

Anaerobic biodegradation on an industrial scale following standards is a significantly less studied area compared to aerobic biodegradation, due to the environment control required for the study, and it does not constitute the majority of natural environments where plastic waste could biodegrade (soil and marine) The stoichiometric mass balance of anaerobic biodegradation of plastics and natural fillers is defined by eqn (1), (3) and (4).

$$C + 2H_2 \rightarrow CH_4 \tag{3}$$

$$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4 \tag{4}$$

Under anaerobic conditions, biodegradation results in methane production and some CO_2 is produced depending on

the residual oxygen in the environment or the type of degraded material. Two types of anaerobic environments exist on a large scale due to commercial actions, biogas facilities and landfills. Biogas facilities deal with anaerobic digestion of organic and plastic materials, capturing the released methane for energy conversion.[115] However, landfills are of particular concern, because any uncontrolled biodegradation of organic and plastic materials can result in methane generation into the environment. In 2007, it was estimated that only 10% of the potential methane generated is captured in the United States,[116] which has only increased to approximately 20% by 2017.[115] Methane is a 25 times more potent greenhouse gas compared to CO_2 over a 100-year period. Furthermore, in the waste sector, the largest contributors of methane in the atmosphere are the landfills and solid waste treatment facilities which do not collect biogas, indicating uncontrolled management of these greenhouse gases.[117]

3.2 Misinterpretation of oxo-degradable plastics

However, another class of biodegradable polymers has been defined as oxo-degradable under ASTM standards. Oxo-degradable plastics undergo abiotic degradation before undergoing either aerobic or anaerobic biodegradation. There is significant concern about defining plastics as oxo-degradable and interpreting them as biodegradable in the plastic community.[118] Oxo-degradation is the physical degradation of plastics into smaller units through oxidation, thermal or ultraviolet actions. Through these processes the molecular weight decreases, and potentially these monomer units could be simpler to bio-assimilate. However, this is not always the case if the final units have little to no functionality or a lack of carbon, nor is it a rapid process.

Oxo-degradable plastics have previously been termed biodegradable plastics by several literature sources, but discrepancies are found in the pre-treatment which applies unrealistically high or accelerated conditions (*i.e.* thermal, UV exposure *etc.*) in temperature and duration respectively. These studies found that 400–600 days are required to obtain at least 30% biodegradation for LDPE films.[119,120] ASTM D6954-18 indicates something like an oxo-degradability standard, but it must be used with other biodegradation standards (*i.e.* soil *etc.*) in the presence of these abiotic actuators.

There are three tiers to the oxidation and biodegradation standard[121] (Fig. 9):

(1) Abiotic degradation to a weight average molecular weight of 5000 or less. Products vary depending on the polymer polarity and the type of initiator. Oxidation of non-polar molecules gives free radicals; UV or heat treatment gives hydrolysed molecules with functional groups.

(2) Biotic degradation in the environment of choice (stated compost, soil or accelerated landfill conditions). The products vary based on the standards followed.

(3) Measure of toxic residue.

There are some claims of oxo-degradable LDPE plastic packaging and UV-degradable plastics, however they only physically degrade, and do not produce CO_2.[39] These types of packaging can't be deemed biodegradable in such cases as they physically degrade into microplastics, and biodegradation is not achieved, which is of significant concern for the potential harm chemicals and polymers can cause on the wildlife both physically and chemically when ingested[122] and of particular concern is the extremely small particles that can find their way into water systems and cause severe harm to ocean-life.

3.3 Aerobic biodegradation standards for polymers and their limits

This review will consider the American Society of Testing Materials (ASTM) standards that define three main areas of aerobic biodegradation standards: soil biodegradation, composting, and marine biodegradation, and three anaerobic biodegradation standards: sewage sludge biodegradation, anaerobic digestion biodegradation, and accelerated landfill biodegradation.

This review does not include standards for unique conditions such as European ISO 14851 and ISO 14852 which are used to certify the OK Biodegradable Water designation in Austria.[123] Furthermore, this study does not include aerobic biodegradation standards withdrawn (not updated within the time limit), including the following: ASTM D5209-92 in 2004, ASTM D5271-02 in 2011, and ASTM D6340-07 in 2016, which represent wastewater and sludge aerobic biodegradation, due to them being uncommon processes compared to the available aerobic biodegradation standards. One major limitation of these withdrawn standards is the static test conditions, whereas the natural environment is more susceptible to changes in conditions from climate, weather *etc.*

3.3.1 Soil biodegradation. Research on soil biodegradation can be divided into two categories, those that indicate the degree of biodegradation, and those that indicate the mass loss over the duration of the study. The latter is more prevalent in the literature due to its ease, however, based on the ASTM standards, it is not enough to determine the degree of biodegradability of polymers by itself. Furthermore, it is the duration of soil biodegradation that makes complete studies following ASTM standards incredibly rare.

Soil biodegradation occurs when biodegradable materials are exposed to soil microbiomes, close enough to the surface to be in an aerobic environment. It does not follow the same level of awareness as composting in society due to there being no implemented collection systems. ASTM D5988-18 and its equivalent ISO 17556 for soil biodegradation only require the initial conditions of the soil to be reported (*i.e.* pH, moisture content, moisture holding capacity, ash content, carbon to nitrogen, *etc.*).[124] Variations in moisture can alter the degradation rate of hydrolytically driven biological processes, and dry conditions slow down hydrolytic reactions, thus the biodegradation studies may not be comparable. This test can also be complemented with mechanical properties and physical degradation identified in ASTM G160-12, but there is no soil biodegradation ASTM labelling standard that defines whether something can be claimed as soil biodegradable.
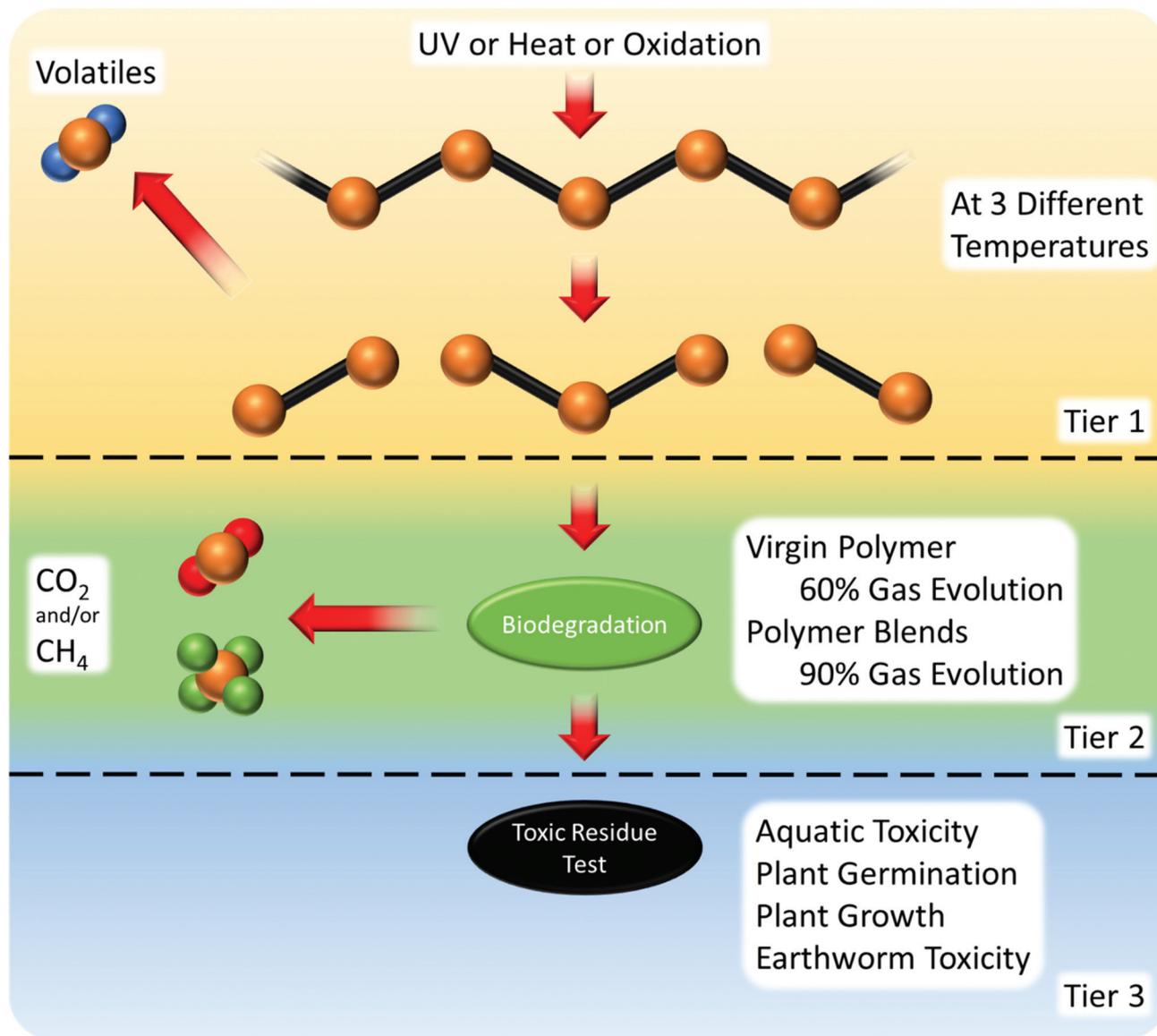
Fig. 9 ASTM D6954-18 oxidation and biodegradation standard test procedure.[121]

The temperature is limited to the range of 20–28 °C, not reflecting a holistic climate approach, however, the soil must be collected from the surface of any natural environment, allowing for some malleability in studies of natural environments. It is also important to note that the efficiency and quantity of cellulose degraders vary based on the type of soil and location,[125] which would be further reflected in the polymer degrader diversity. Therefore, climates, region and the temperatures of the soil and the preceding winters all affect the type and the quantity of microorganisms in and around the soil.

3.3.2 Composting. The most well-known form of biodegradation is composting (ASTM D5338-15 and its equivalent ISO 14855), mainly due to the labelling standard that can be used to define whether something is compostable (ASTM D6400-19), in North America. Biodegradable materials are exposed to a mixture of decomposed materials at higher temperatures than

that found in soil biodegradation. However, the compost standard is for industrial or commercial applications, such that the environment is controlled or optimized in terms of parameters and initial conditions (*i.e.* moisture content, carbon to nitrogen ratio (10–40) *etc.*), which forms a synthetic and stable environment. The inoculum must have less than 70% ash, solids of 50–55% and a temperature fixed at 58 °C, while the cellulose reference sample must be 70% degraded in 45 days less.[126] For marketing purposes, the labelling standard defines that 90% of the polymer must be physically degraded in 90 days, 90% of the polymer must be chemically degraded in 180 days and terrestrial safety (impact upon plant growth) of the final compost must be within certain specifications, to be defined as a compostable polymer.[127] ISO 20200 is a composting disintegration study, which can be used to supplement composting studies, however, it defines physical disintegration ability, and not biodegradation.

Other composting standards available reflect more natural or home composting such as ASTM D5929, which studies the composting of "organic" materials in aerobic conditions under mesophilic conditions (25–45 °C), and has been applied to plastics.[32] However, the standard identifies the conditions to be maintained at 40 °C which is beneficial for biodegradation but not a holistic approach to defining the actual reality of composting (ambient temperatures can vary significantly from one geographical jurisdiction to another) and is not specifically designed for plastic materials.

Three phases of composting exist:[128]

(1) The mesophilic phase: where easily degradable organic matter is broken down. The organic acids produced reduce the pH to 5–5.5. With the rising heat from microorganism activity, the proteins break down and the pH increases to above 8.

(2) The thermophilic phase: where the temperature rises above 40 °C and the degradation of the waste material improves. However, as the temperature continues to increase to 55–65 °C, the microbial activity reduces.

(3) The "maturation phase": where the compost begins to cool with reduced microbial activity after the readily available carbon sources have been consumed, hence "mature compost". At this point the mesophilic microorganisms take over and degrade the residual carbon sources over a long slow process.

The three phases of composting are not well reflected in the testing procedures of ASTM D5338-15, which stipulates a stable temperature. Furthermore, education of the end-consumer is needed to impart a better understanding to avoid the improper disposal of the compostable material (which can be detrimental to the environment to the same extent as disposal of non-biodegradable plastics) and allay misperceptions of what compostable means.

ASTM D6400-19 defines that something can be labelled compostable if it meets 3 tiers:

(i) 1st Tier: 90% physically degraded to a particle size <2 mm in 90 days.

(ii) 2nd Tier: 90% converted to CO_2 within 180 days.

(iii) 3rd Tier: no toxic residue in compost, and at least 90% germination rate and biomass of plants grown in compost compared to blank.

3.3.3 Marine biodegradation. Marine biodegradation is defined by three standards currently in use: (1) ASTM D6691-17, which studies floating plastics at a temperature of 30 °C; (2) ASTM D7473-12, which evaluates plastics buried in the sediment underwater by weight attrition only; and (3) ASTM D7991-15 which requires plastics in a combination of water and sediment, with the option of light imitating day light and a temperature of 15–25 ± 2 °C. Fig. 10 illustrates the limitations of each marine biodegradation standard. ASTM D7473-12 is specifically indicated to be a supplemental assessment to ASTM D6691-17 which identifies their physical degradation and not biodegradability, but it is useful to know if water-logged plastics that have sunk will still physically degrade. ASTM D7991-15 can replicate two locations of plastics, and like the other marine biodegradable standards, the initial conditions need only be reported. However, ASTM D7991-15 only applies in the tidal zone, indicating that plastics are close to the coast and the surface water.[129]

A major limitation of marine degradation is no standard defines testing methods for polymers in deep waters where temperatures are well below the optimal growth conditions for bacteria,[130] and pressure inhibits the rate of biodegradation and bio-assimilation of PHAs by microorganisms.[131] Furthermore, near the ocean floor, water movement is minimal, and anoxic (oxygen absence) conditions can be common near the bottom sediment.[132] Although ocean sediment is reported to have a greater microorganism consortium that can degrade PHAs,[133] it may not be enough to mitigate these unfavourable conditions. Furthermore, water absorption is positively correlated with temperature,[134] which can detrimentally affect biodegradation in cooler climates and conditions. Considering that marine biodegradation will vary across the globe it is understandable that there must be freedom in study. Several limitations have also been identified

ASTM D7991-15
- with or without visible light
- Ground or solid samples

ASTM D6691-17
- floating plastics
- Ground or solid samples

ASTM D7473-12
- weight attrition only
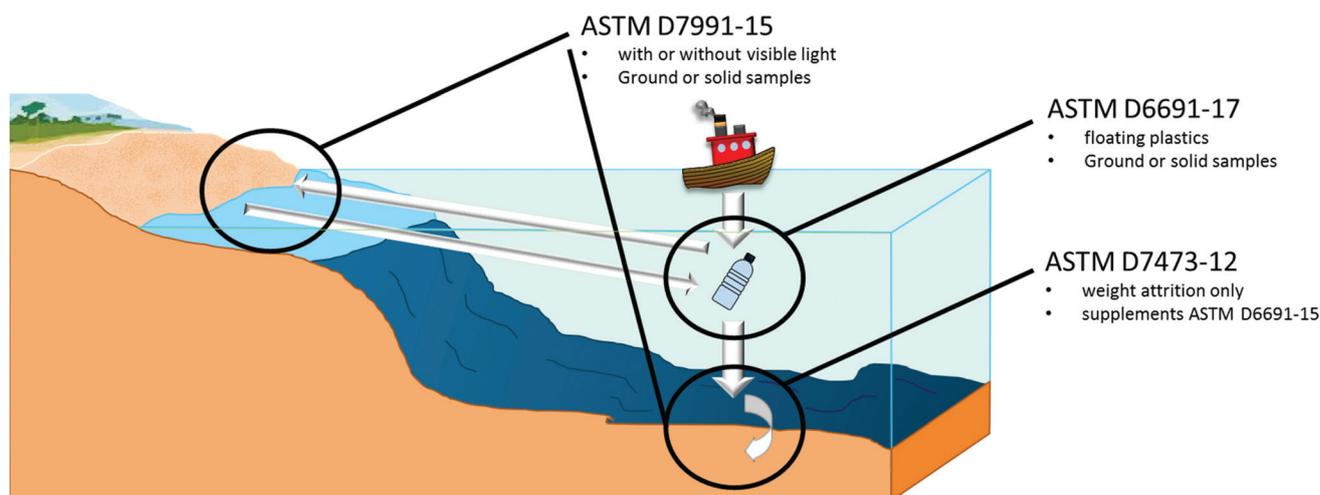- supplements ASTM D6691-15

Fig. 10 Marine biodegradation and degradation ASTM standards.

for biodegradation in aquatic environments, such as differences between lab and real world conditions, benthic environments, toxicity tests *etc*.[135]

To evaluate and claim that marine biodegradability is difficult due to the lack of standards identifying a threshold, ASTM D7081-05 did indicate a threshold for biodegradation to identify if polymers were marine biodegradable (30% CO_2 theoretical evolution and 70% physically degraded below 2 mm in 180 days). However this could only be applied on polymers tested using ASTM D6691-17, and the standard has since been withdrawn.[136] "OK Biodegradable Marine" designation is a valid claim that can be made with 90% biodegradation achieved relative to cellulose or absolute, in addition to physical degradation to a particle size below 2 mm in 180 days, and is supplementary to ASTM D6691.[137]

3.4 Anaerobic biodegradation standards for polymers and their limits

Three main anaerobic studies exist, defining varying ratios of total solids seen in wastewater treatment, landfills and anaerobic digesters. During anaerobic studies, since biogas is measured, dissolved CO_2 in water is not directly measured and must be accounted for afterwards which is not done so in some older studies.[138]

3.4.1 Sewage sludge biodegradation. Sewage sludge biodegradation is a highly active anaerobic study available in many developed areas geographically. Defined by ASTM D5210-92, it is more representative of anaerobic water biodegradation due to inoculum of 1–2 w/v% total organic solids. Such studies are reflected in wastewater treatment plants available in many developed areas geographically. Test samples can be in nearly any form (powder, fragments, pieces *etc.*) representing plastic material that has entered the wastewater treatment facilities and are maintained at a temperature of 35 ± 2 °C under dark conditions. ASTM D5210-92 bears resemblance to ISO 14853.

As of the current date of this review, the standard has since been withdrawn for not being updated in a timely manner but is still used in some studies. ISO 13975 is also similar to ASTM D5210-92, for its inoculum type, however, the temperature can be either 35 or 55 °C.[135]

3.4.2 Anaerobic digestion biodegradation. Anaerobic digestion is a static batch fermentation with 20% solids and can be considered a synthetic method of biodegradation. ASTM D5511-18 and its equivalent ISO 15985 are studied under either thermophilic (52 °C) or mesophilic (37 °C) conditions for a short period of 30 days. Since there is a large variation, the study is mainly to determine if the option is viable for polymers but isn't readily used in industry as a method of plastic disposal for energy recovery. The application is considerably limited due to plastic incineration being faster and more cost-effective as a form of energy production.[139]

3.4.3 Accelerated landfill biodegradation. Landfill biodegradation can be considered the most undesirable form of biodegradation, due to uninhibited and uncontrolled methane production under anaerobic conditions that is freely released into the environment. Landfill biodegradation involves a sludge gestate (high organic content material) solid content of 35% or greater, indicating a reduced amount of available water compared to other biodegradation standards for biological activities. Two types of landfill biodegradation standards exist, ASTM D5526-18, which is completed at 35 ± 2 °C, and ASTM D7475-20 which can be completed under aerobic and anaerobic conditions. ASTM D5526-18 has a sludge gestate total solid content of 35, 45 and 60% and the extent of cellulose biodegradation must be above 70% in 30 days. ASTM D7475-20 has two tiers:

(1) Biodegradation with household solid wastes of 50% total solids at 30 °C for 4 weeks.

(2) Static digestion with a sludge gestate total solid content of 35, 45 or 60% and a temperature of 35 °C.

Landfills are generally packed densely to conserve space; therefore, biodegradation may be limited due to the lack of water or available CO_2 or oxygen to properly degrade over time, in addition to the lack of exposure to other degradation mechanisms due to the depth. The ideal scenario is mismanaged biodegradable plastics end up in the appropriate stream such that it can aerobically degrade instead of anaerobically, resulting in methane release in the environment. Among types of PHA biodegradation, it is the least researched due to the limited studies found at this time.

4 PHA biodegradation

As a biodegradable polymer, PHA has a few significant advantages for applications: (i) slow release of chemicals such as fertilizers or pesticides in agriculture; (ii) photoactivation to induce pollutant oxidation; (iii) leaving no residue behind within a short period of time;[140] and (iv) biodegradation pathways are similar to starch (about 90% of microorganisms that degrade starch can also degrade short chain PHAs[141]). However, starch degraders can usually only bio-assimilate (take in and utilize) biodegradation products and not perform complete biodegradation with large molecular weight polymers or more complex medium to long chain PHAs. Biodegradation has two types of microorganisms, those that physically degrade PHAs, and those that feed off the by-products of the degradation (butyric acid, valeric acid *etc.*).

In this review, biodegradation studies completed *in vitro* where samples are examined for degradation and analysed based on the surface diameter of the degraded polymer are not critically evaluated. Studies following ASTM standards, modified ASTM standards, natural environments and those in controlled environments (*i.e.* laboratory environments), with physical samples (*i.e.* powder, films *etc.*), that show the extent of degradation and/or bio-assimilation are detailed critically.

4.1 PHA biodegrading microorganisms

Holistically, short and medium length PHAs are biodegraded by a number of bacteria, including members of the genera: *Actinomyces*, *Alcaligenes*, *Arthrobacter*, *Aspergillus*, *Bacillus*, *Clostridium*, *Comamonas*, *Corynebacterium*, *Enterobacter*,

Gracilibacillus, *Klebsiella*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Pimelobacter*, *Planococcus*, *Pseudomonas*, *Pseudoalteromonas*, *Staphylococcus*, *Streptomyces*, *Variovorax*.[130,142–150] The dominant PHA destructors in aerobic and anaerobic environments have been reported by the members in the bacterial genera of *Variovorax*, *Stenotrophomonas*, *Acinetobacter*, *Pseudomonas*, *Bacillus*, *Burkholderia*, *Cupriavidus*, *Mycobacterium* and *Streptomyces*.[144,151] Fungi can also degrade PHA and are reported to be even more effective PHA degraders compared to bacteria.[152] Known PHA degrading fungi are in the division *Ascomycota*, *Basidiomycetes*, *Deuteromycetes*, and *Zygomycotina* in aerobic and anaerobic environments.[153,154] Thus, it is reasonable to define PHAs as readily biodegradable in most anaerobic and aerobic environments.[10]

Under favourable conditions, biodegradation follows the ideal bacterial growth curve with a lag, exponential, stationary and death phase, where the biodegradation plateaus between the end of the stationary phase and the start of the death phase.[155] However, if conditions are unfavourable, or fluctuate throughout biodegradation, the rates can vary significantly from the theoretical model due to this complex association between biodegradation and bio-assimilation of the products.

4.2 Extracellular PHA biodegradation

The extracellular biodegradation of PHA is mediated by protein lipases and hydrolases. The process follows the general step-wise approach of polymer breakdown into shorter chain polymers by hydrolytic depolymerases in the presence of water, followed by further conversion of PHAs into trimer and dimer units, which are then processed by lipases and hydrolases.[156] The extracellular PHA depolymerases are most studied; their protein structure consists of three main domains: (i) a binding domain responsible for surface absorption and disruption of the polymer structure; (ii) a linker domain that links the binding domain to the catalytic domain; and (iii) a catalytic domain that cleaves the PHA and any available dimers/trimers in two (Fig. 11).

Enzymatic hydrolysis of PHAs is a two-step process;

(1) Adsorption of enzymes upon the surface of the polymer and active sites.
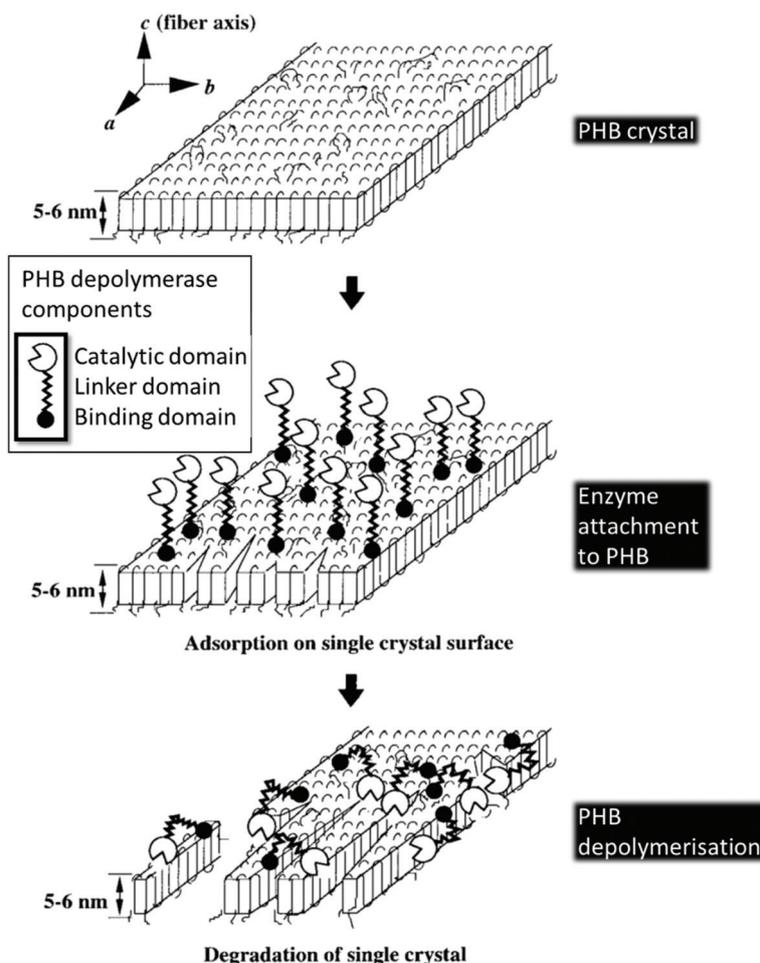
Fig. 11 Single PHB crystal enzymatic degradation by PHB depolymerase. This figure has been reproduced with permission from ref. 163, John Wiley and Sons, Copyright 1999 (License number: 4800311035232).

(2) Enzymatic induced hydrolytic cleavage of PHA bonds which is induced by the hydrophobic domain binding site and the catalytic site respectively.[143,157]

The hydrolysis process is not very specific and releases oligomers of various sizes into the surrounding medium.[158,159] There is observed preference of hydrolytic enzymes toward amorphous surface crystals, with less crystalline polymers and co-polymers being targeted more readily due to their less ordered structure being more specially accessible to the enzymatic action.[157,160] The degradation products vary depending on the type of PHA. In PHB, 3-hydroxybutyric acid is produced, while PHBV products are 3-hydroxybutyric acid and 3-hydroxyvaleric acid.[161] These acids are then taken into the cell to be metabolized into other compounds or more PHAs.[52,162] Furthermore, the type of PHA impacts the way biodegradation proceeds effectively.

4.3 PHA attributes that affect biodegradation

The efficiency of PHA biodegradation is closely coupled with the physical and chemical attributes of the polymer type. Degradation rates depend on the: (i) crystallinity; (ii) copolymers; and (iii) copolymeric structure. In PHBV, the introduction of 3-hydroxyvalerate has a greater amorphous region, which is more susceptible to enzymatic attack,[53,164] due to eased water penetration,[94] absorption,[165] and susceptibility of the isodimorphic crystal region to the enzyme catalytic domain.[166] Thus, it follows that biodegradation varies based on the crystallinity induced by the processing method.[167] Furthermore, based on the copolymer ratio, the amorphous region can be altered and the enzymatic depolymerase activity can be maximized as seen in Fig. 12.

However, other studies indicate that a PHA depolymerase is more effective on PHB than PHBV and most effective on PH4B, although the results are measured in weight loss and the molecular weights vary significantly.[143] This indicates that other enzymes or factors play a part in degrading PHAs. For example, excess side chains may inhibit the rate of interaction with the target location, and other actions coupled with the enzymatic hydrolysis can further enhance biodegradation. Water diffusion, promoted by temperature, can induce some hydrolytic degradation which increases active sites.[169] Ester bonds of polyesters are also sensitive to hydrolysis and can result in a reduction in molecular weight. The lowering of molecular weight invalidates the Arrhenius model for biodegradation,[170] because enzymatic hydrolysis is coupled with ester hydrolysis and overall biodegradation, and not driven by a single actuator. The depolymerisation steps are indicated to be rate limiting steps in PHA biodegradation,[171] thus indicating that any single form can reduce the biodegradable product life expectancy after its service life.

4.3.1 Crystallinity. The effect of PHA monomer units in the polymer structure is inextricably linked to the effect of crystallinity that affects biodegradation. Polymer processing techniques also change the final sample crystallinity, including solvent casting,[172] and quenching,[173] which either increase or reduce the interlamellar phase and affect the long-term bio-
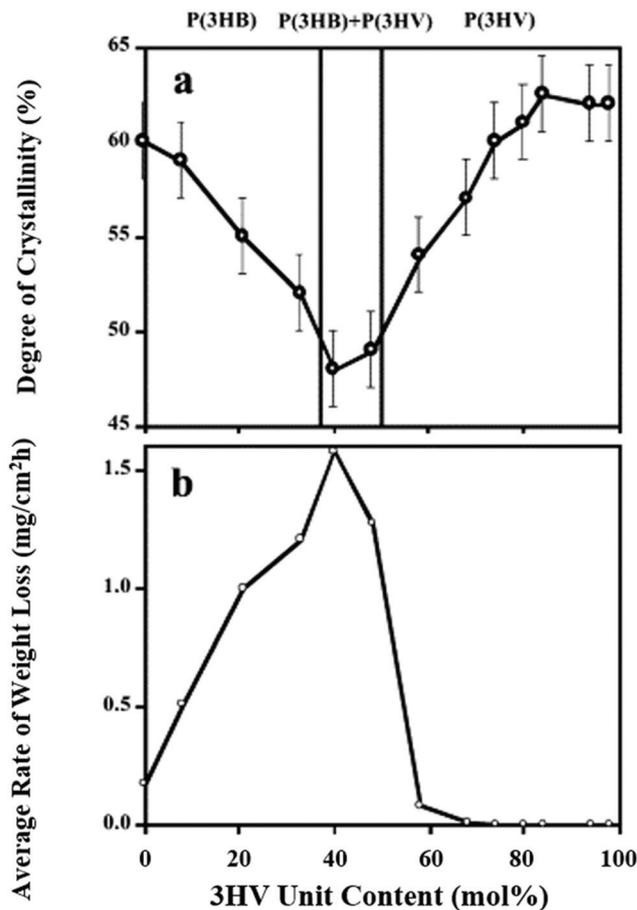

Fig. 12 (a) PHB and PHBV crystallinity from WAXD patterns. (b) Rate of weight loss by 2 µg of Ralstonia pickettii type 1 PHA depolymerase due to 3HV ratio. This figure has been reproduced with permission from ref. 168, Elsevier, Copyright 2004 (License number: 4800311458150).

degradation extent, though quenching is not a concern due to PHAs' low T_g.

As biodegradation proceeds, and mass loss increases, erosion of the interlamellar phase of PHA begins in the initial stages,[174] where the disordered chains are targeted first before samples are eroded in the crystalline regions (Fig. 13). The interlamellar phase or amorphous region gives PHAs their flexibility, and with their degradation it is expected that the crystallinity would increase. These characteristics are seen within 30 days of soil biodegradation[175] and 60 days within marine biodegradation.[176] However, given enough time the crystallinity is expected to be reduced by up to 60%, which occurs in soil biodegradation after 200 days or 30 days in a controlled composting environment due to the intensified conditions.[175] The amorphous region is known to allow permeation of moisture and enzymes, and therefore its degradation would increase the surface area of available crystalline regions. The crystalline regions make up the majority of a crystalline PHA such as PHB and their degradation is expected to reduce the overall polymer crystallinity.
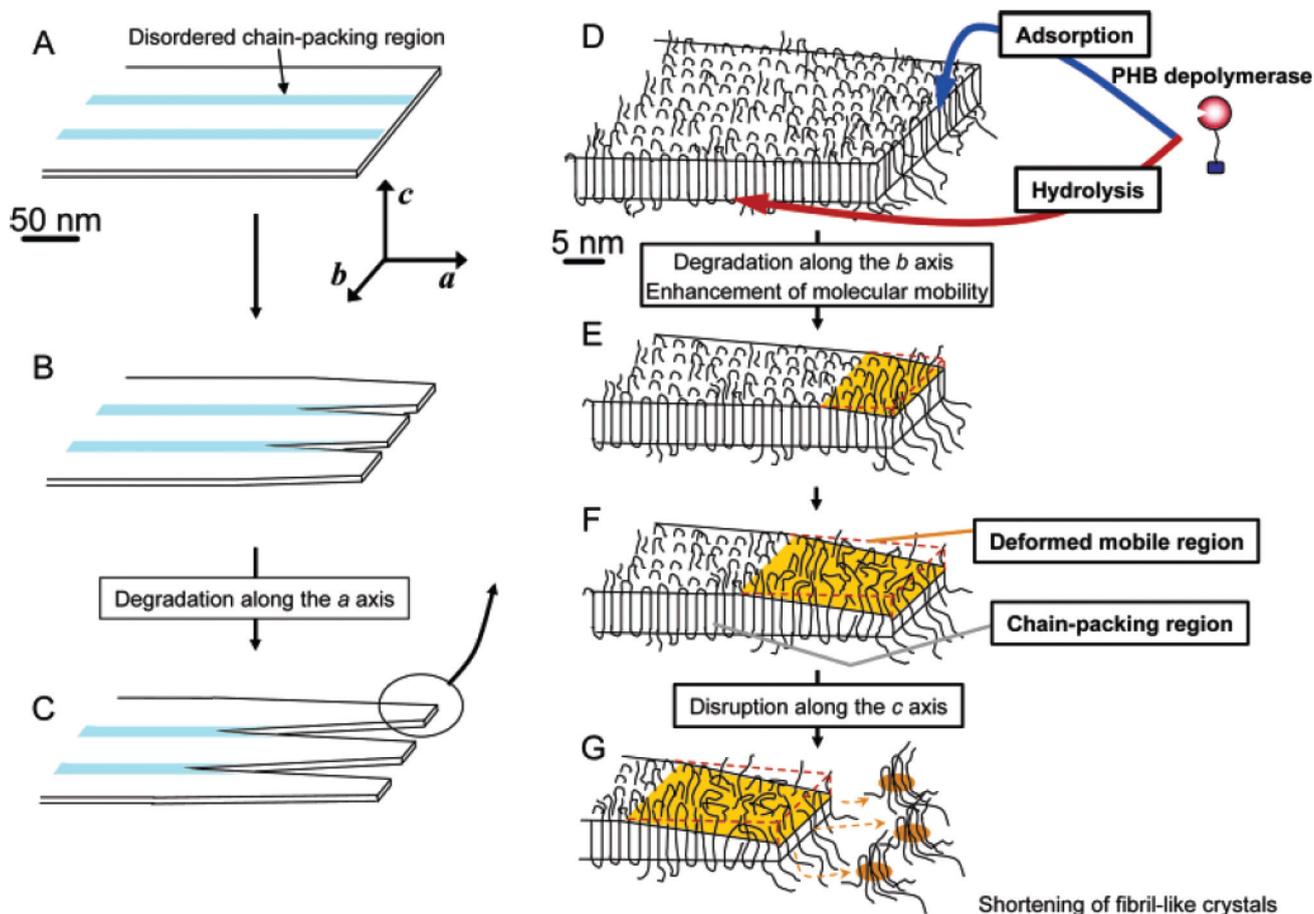
Fig. 13 Schematic model of the enzymatic degradation behaviour of lamellar crystals in P(HB) solution-grown single crystals by PHB depolymerase. Reprinted (adapted) with permission from (Numata *et al.* H. enzymatic degradation processes of poly[(R)-3-hydroxybutyric acid] and poly[(R)-3-hydroxybutyric acid-*co*-(R)-3-hydroxyvaleric acid] single crystals revealed by atomic force microscopy: effects of molecular weight and second-monomer composition on erosion) on April 1, 2020. Copyright (2005) American Chemical Society.[166]

4.3.2 Copolymers. Copolymers are heteropolymers where more than one type of monomer unit is present such as PHBV, PH4B, PHBHx *etc.* that contain HB units and others. Copolymers are known to biodegrade faster than homopolymers due to the presence of the inherited amorphous region. For example, PHBV is formed by addition of HV into PHB, where the relative fractions of HB and HV define modulating crystallinity. This lower crystallinity of the copolymer is directly related to a higher enzymatic activity measured by PHBV weight loss. This correlation is reflected in the degradation extent under both aerobic and anaerobic conditions. In the PHBV example, at 3% HV content, there is no difference between PHB and PHBV degradation, as their crystallinity is similar. As the HV content increases, the crystallinity decreases and the biodegradation rate improves.[177,178] Research indicates that an HV content of approximately 40–50% produces the fastest biodegradation rates in soil,[171] compost,[178] and marine water.[147] The effect is also reflected in anaerobic environments.[15,179] Similarly, in other PHB copolymers, increasing the ratio of 4HB, Hx, and HO relative to PHB correlates with a reduced crystallinity and melt temperature, com-

pared to neat PHB,[180] and improves the biodegradation rate of the copolymers under aerobic (soil,[181] compost,[178] marine[106,182]) and anaerobic (sewage sludge,[106,173] and anaerobic landfill[182]) conditions.

Another aspect to consider is the availability of enzymes to cleave the medium chain length PHAs. Jendrossek identified several microorganisms having short chain length PHA degrading enzymes,[183] and several short chain PHAs and other aliphatic polyesters such as poly (ethylene adipate) (PEA), poly (ethylene succinate) (PES) and poly (2,2,6,6-tetramethyl-piperidenyloxyl-4-yl methacrylate) (PTMA) can be enzymatically depolymerized by similar enzymes, indicating a significant advantage to short chain PHAs. These enzymes attach the ester bond but have low specificity, not being overly discriminative toward the range of side chains.[158] For example, PHB has one carbon long side chain and some PHB depolymerases enzymatically hydrolyze PHBP,[66] which can be the result of PHBPs having no side chain. The enzyme affinity is likely determined by the affinity towards the PHA side chain.[184] However, these enzymes do not function on medium and long chain PHAs effectively. Research availability of medium chain

length depolymerases is minimal with very few species isolated with the particular enzymes,[185] which is further reflected by the few poly (3-hydroxyoctanoate-*co*-3-hydroxydecanoate) degraders seen in *Streptomyces*.[186] This may be why medium chain length polymers such as PHBO with 10% HO content only show between 88–95% biodegradation in anaerobic and aerobic environments.[182] Thus, the use of high carbon PHA monomers such as 3-hydroxydecanoate in medium chain length PHAs can impede biodegradability by reducing the number of chemically reactive binding sites in the side chain structure for enzymes to attack.

The observed effect of crystallinity and copolymer ratio does not apply to all biodegradation studies. Lower molecular weight polymers have enhanced motility and significantly reduced size, which should make enzymatic degradation and bio-assimilation faster, but improvements are not always reflected in solvent casting. The method of sample production (*i.e.* solvent casting) or the sample recovery method can similarly leave behind impurities or toxic residue depending on the solvent[187] that may negatively impact the enzymatic activity or microorganism functionality and produce irregular relationships between PHA copolymer compositions and their biodegradation rates.[171,188] Furthermore, there is evidence that PHB is a more readily used product than more complex PHA monomers with larger side chains such as HV, 4Hb and Hx[148] which is expected to result in faster biodegradation rates, however, this is not always proven, and it has not been determined if its due to crystallinity alone.

4.3.3 Sample morphology. The morphology of PHA samples is directly correlated with the surface area, where the morphologically porous polymers have increased exposed surface area for enzymatic attack at any given time which can increase the total enzyme binding sites. Therefore, the powder form has the largest surface area to volume ratio and should have the fastest biodegradation. Some studies have shown that the PHB films can show a comparable biodegradation rate, for certain film thickness to PHB powder. Gutierrez *et al.*[189] reported that PHB powder had 68% biodegradation in 12 days and 1.2mm PHB films had 67% biodegradation in 19 days. In another study, Sashiwa *et al.*[190] found that PHBHx/PBAT and PHBHx/PLA 440 μm powder and 20 μm film biodegradation had no significant difference in anaerobic sewage sludge. It is not surprising that thin films benefit from enhanced biodegradation rates, due to the ease of moisture permeation and maximized surface area,[191,192] such that their biodegradation rate improves as their thickness is reduced. It is encouraging to see comparable results in both anaerobic[189] and aerobic[32] environments.

Biodegradation of films begins with surface pitting and degradation at the edges (Fig. 14), such that the surface area to volume ratio increases as time goes by. Furthermore, by changing the production method of films from solvent casting to electrospinning, the surface area and water permeability can be increased leading to a significant improvement in biodegradation rate, measured by a mass loss increase from 40% to 100% in a 28 day period.[193] This method also increases the

SEM MAG: 1.00 kx WD: 15.6390 mm DET: SE Detector
View field: 226.20 μm 100 μm Vega ©Tescan
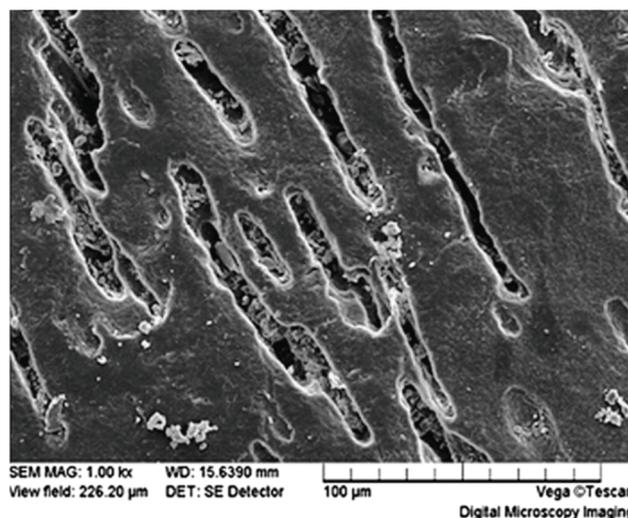Digital Microscopy Imaging

Fig. 14 PHBV film surface at 35 days under composting conditions. This figure has been reproduced with permission from ref. 177, Elsevier, Copyright 2004 (License number: 4800311458150).

surface roughness, further enhancing the surface area of thin films.

Biodegradation of PHA pellets is generally slower than that of films to a certain extent depending on the type of PHA. PHB films benefit more from thin film morphology compared to PHBV.[151] According to ISO 14855 PHB pellets biodegrade by 54% in 45 days and 92% in 78 days,[194] which is comparable to PHB plates of 1.2mm thickness.[32] Therefore, when comparing biodegradations studies, it is important to consider the morphology of the samples. However, the comparisons become challenging as only a few completed studies followed ASTM standards.

4.4 Chemical additives and blending effect on PHA biodegradation

The addition of other chemical additives or blends all affects PHA biodegradation on both a chemical and physical scale by impeding enzymatic degradation. Non-biodegradable co-polyesters and chemicals minimize the interactable area between enzymes and biodegradable polymers. This can be further exacerbated if the non-biodegradable polymer makes up the continuous phase with PHA dispersed within,[195] or the biodegradable polymer is unsuited for the particular environment (*i.e.* PLA in marine water). This is mainly reflected in the rate of enzymatic hydrolysis.

4.4.1 Chain extenders and anti-fouling agents. Chain extenders and anti-fouling agents are some of the most commonly applied chemical modifications on polymers. As such, they are indicated to impact biodegradation at various degrees, by inhibiting the extracellular enzyme activity or by inhibiting the microorganisms' ability to produce the enzymes. For example, 4,5-dichloro-2-*n*-octyl-4-isothiazolin-3-one (DCOI) is an anti-fouling agent, which is known to inhibit the onset of PHB and PHBV biodegradation according to ASTM D5988.[196]

The chain extender Joncryl ADR-4368-CS had a similar inhibitory effect on the onset of PHA biodegradation, despite it being reported to reduce the crystallinity of PHAs, which should improve the biodegradation[197] – at loadings of 5%, the Joncryl chain extender reduced the extent of soil biodegradation from 70 to 22% in 340 days following the ASTM D5988-03 protocol. The inhibitory effect is also seen under composting conditions and marine conditions at 0.2 and 5% loadings, negatively impacting the biodegradation start time, rate and extent. Joncryl is expected to have a scavenging effect which may sequester available electron acceptors, acting as an antifungal/antibacterial agent.[150] Specific to chain extenders, the chain extension increases the molecular weight and make PHAs more difficult to enzymatically hydrolyse. However, the extent of chain extenders' effects or different types have not been evaluated in other ratios with PHAs, such as 0.5%, which is suitable for food packaging applications. PLA/PBAT blends with 0.5% Joncryl-ADR-4368C have shown a 50% reduction in compost biodegradation extent in 126 days,[198] and 0.5% Joncryl ADR-4370S and 1,6-hexanediol diglycidyl ether, similarly reduced the hydrolytic degradation extent by 23 and 10% respectively.[199] Therefore, there is strong evidence that chain extenders will reduce the biodegradation of PHAs.

4.4.2 PHA blends. Biodegradation studies of PHA blends are not regularly completed in most natural environments (*i.e.* soil and marine environments) due to the few other bio-based polymers showing evidence of biodegradation such as PLA. Two main avenues of PHA blends have been explored, plasticized PHA and PHA/PLA blends, due to their bio-based origins, as well as potential applications.

Plasticization of PHAs with tributyl citrate (TBC) or oxypropylated glycerol reduces the crystallinity, but also slows down the biodegradation rate in all blend ratios in soil, compost and anaerobic sewage sludge.[32,189,200] However, this may be due to the preservative effects (inhibits microbial growth) of glycerols as a food additive, its reduced bio-assimilation due to oxypropylation, and the synthetic production method and hydrophobicity of TBC.[201,202] PLA has been plasticized with acetyl tributyl citrate which inhibited hydrolysis action due to increased hydrophobicity whereas triethyl citrate based plasticizers are more hydrophilic and can increase PLA degradation,[203,204] and these effects likely play a part in reducing PHB degradation. Furthermore, the way plasticizers are closely associated with polymer chains may inhibit the enzyme interaction with binding groups.

PHA/PLA blends similarly reduce the crystallinity and should result in improved biodegradation of PHA components. However, virgin PLA is not readily biodegradable in soil[205] and marine water,[190] due to the suboptimal temperatures.[206] Some instances of 25–30% PLA in PHB, PHBV and PH4B have shown evidence of similar or slower physical degradation or biodegradation rates in soil conditions but no complete biodegradation is reported.[175,207,208] However, according to ASTM D5988-12, a PHA/PLA blend was seen to degrade to 99% in 176 days, but the ratio and presence of additives were not identified.[209] The addition of PLA, PBAT or PBS into PHBHx has also resulted in a reduced biodegradation rate (3–23%, 10–90% and 20–90% respectively) compared to PHBHx in marine water. The level of reported biodegradation is the result of PLA, PBAT and PBS biodegradation in the blends, despite the virgin polymers not degrading in marine water.[190] There is no reported reason behind the unexpected biodegradation of PLA, PBAT and PBS in marine water but it may be the presence of the highly amorphous hydroxyhexanoate,[210] which interferes with the crystallization of the other blend components or promoting biofilm development. Some success has been found in PHBV/PBAT/flax composites where soil degradation rate of PHBV and flax in the composite was enhanced with PBAT addition, however this was attributed to phase separation that improved microbial ingress into the samples.[211]

In higher temperature environments (∼58 °C) such as controlled composting and anaerobic digestion, the biodegradation of PLA is common, and it is expected that there would be benefits of incorporating PHAs into PLA to improve the compostability. However, PLA/PHBV 70/30 blends have shown no biodegradation improvement compared to PLA and PHBV alone (all 90–92% biodegradation in 200 days) under ASTM D5338-15,[175] or have even a reduced biodegradation rate in compost,[212] and under anaerobic digestion.[213] The effects are similarly reflected in PLA/PHB/ATBC and PLA/PHB/PEG based blends where disintegration properties were the same as virgin PLA or the PHB is reported to slightly inhibit PLA degradation under ISO 20200.[214] There is also evidence that PHAs biodegrade slower than PLA in anaerobic digestion at 52 °C than PLA alone.[213] Considering the cost of PHA production being about double compared to PLA,[80] it is not economical to seek compostability or anaerobic digestion improvements with the addition of PHAs. However, for mismanaged waste, industrial composting is not a suitable representation for the natural environment and virgin PLA is not suitable for biodegradation in natural environments.

Overall, it has been established that PHAs fully and rapidly (time frame) biodegrade in the natural environment. Of the available studies few illustrate the biodegradability of PHAs in a comparable manner.

4.5 PHA soil biodegradation

Recent PHA soil biodegradation studies are indicated in Table 1. Studies defined by mass loss did not indicate (%) biodegradation based on CO_2 evolution. Soil biodegradation is known to be an incredibly dynamic environment that can vary across the globe. The process is considered relatively slow compared to other aerobic and anaerobic processes, due to the mild conditions. Cellulose for example can take nearly 200 days to degrade by 90%,[194] however, if the conditions are favourable, the reference material can take significantly less.[215] Therefore, biodegradation of PHAs will vary between studies.

PHAs are considered the most promising soil biodegradable materials, with 100% biodegradation in 90 days, whereas PCL would take up to 270 days according to ASTM D5988.[215] In non-ASTM studies, PHBV is degraded by 40% in 120 days

Table 1 PHA soil biodegradation studies from 2010–2020

	PHA (co-monomer %)	Protocol and source	Conditions	Form	Days	Results	Ref.
1	PHBV (12% HV)	ASTM D5988-12, agriculture field soil	23–25 °C, 20% moisture(w.b.)	Film	200	35% biodegradation	175
2	PHBV (2% HV)	ASTM D5988-03, forest soil	25 °C	Powder	350	70% biodegradation	150
3	PHB	Non-ASTM, fertile garden soil	30 °C, 80% relative humidity, 10 cm depth	Nano-fibre Film	28	100% mass loss	193
4	PHB	Non-ASTM, field soil	21 and 28 °C, 50% moisture(w.b.)	Film	35	60 and 95%, mass loss	181
5	PHBV (12% HV)					90 and 100% mass loss	
6	PHBHx (12% Hx)					92 and 100% Mass Loss	
7	PH4B (10% 4HB)				35 and 28	100 & 100% Mass Loss	
8	PHB	ASTM D5988, natural mature soil	11–30 °C, 17–23% moisture(w.b.)	Film	112	60% mass loss	196
9	PHBV (8% HV)					60% mass loss	
10	PHB	ASTM D5988-03, commercial soil	23 °C, 33% moisture(w.b.)	Film	80	82% mass loss	60
11	PHA					76% mass loss	
12	PHA	ASTM D5988-03, a mixture of topsoil, farm soil and sand	20 °C, 60% of water holding capacity	Film	660	70% biodegradation	113
13	PHA	Non-ASTM, farmland topsoil	35% moisture(w.b.)	Film	140	32% mass loss	191
14	PHA	Non-ASTM, farmland topsoil	35% moisture(w.b.)	Film	60	33% mass loss	218
15	PHA	Non-ASTM, farmland topsoil	25 °C, 35% moisture(w.b.)	Plate	120	35% mass loss	219
16	PHA	Non-ASTM, farmland topsoil	30–40% moisture(w.b.)	Film	60	22% mass loss	192

(25–30 °C) while no degradation was observed for PBS and PLA.[216] However, only 25–30% of microorganisms are PHA degraders, the rest only bioassimilate the products of enzymatic hydrolysis.[142] Furthermore, soil biodegradation rates have been reported with several different conditions: (i) soil source; (ii) soil moisture content and pH; and (iii) temperature of soil biodegradation studies. Based on the literature, there is no standard that defines a (%) biodegradation limit for polymers, to indicate whether it is biodegradable or only shows biodegradable behaviour. The existing OK Soil degradable certification states that it must be 90% biodegraded absolute or relative to the reference sample in 2 years under ASTM 5988,[217] of which few studies have continued for that long.

4.5.1 Soil source. According to the ASTM standards, the soil source needs only be reported, and no specific location or soil type is required. It has long since been known that the soil type significantly impacts the degradation of the most simple, short chain PHA polymers.[220] However, no internal reference point has been applied to give some form of comparability besides the cellulose sample.

Measurements most often reference the location and depth of the sample in the soil, and in rare cases identify the microorganism community. However, the characteristics of the soil not only affect the conditions, but also how the conditions will change over the duration of the experiment. For example, PHBV (8% HV) powder tested in sand (dry silica with low organic material) achieved 80% biodegradation after 600 days,[176] which is significantly longer than most soil biodegradation tests. Zaidi et al.[211] reported no degradation of PHBV (3% HV) in botany sand in a natural environment within 110 days. Sand has relatively poor moisture holding

capacity, substantiating these slow biodegradation rates that take months to years for any evidence and by mixing in organic soil and farm soil into sand, the biodegradation rate of PHAs is not seen to improve[113] (Table 1, entry 12). The degradation of PHBV (9.8% HV) film samples indicates minimal physical degradation after 5 months in a mixture of beach sand, horse manure and fertile soil.[221] The effect of moisture holding capacity is also reflected in Clarion loam soil 6% PHA biodegradation in 5 months after watering once a week,[222] even though clarion loam soil is rated to have very good lytic activity among many other types of soil.[125]

The limitations of sandy soil can be improved in natural environments near the coast, where frequent water permeation happens continually such that the biodegradation of starch can be 4 times faster than garden soil.[215] Other natural soil biodegradation studies include soil sourced from South African agricultural fields, black soil and leaf mould, oil palm cultivation soil, soil from Russia and Vietnam, and soil from California landfills. Field soil and landfill soil appear to have the highest rate of PHA biodegradation activity, being the only samples approaching 100% PHA degradation[142,144,151,175,181,205,223,224] (Table 1, entries 1 and 4–7). This may be due to the microbial diversity found in moist soil with consistent environments that maximize microbial population growth and diversity.

The location of soil in a specific area is of significant impact on the potential biodegradation rate. Between subsoil and topsoil, the fungi community is richer in topsoil[225] and therefore the biodegradation rate with increased microorganisms will be significantly higher. Variations in the natural environments such as humidity under larch and birch trees,

and in harsh winters over a yearly basis were reflected in lower microorganism counts and slower mass loss due to lower humidity.[144] The environment fosters the microorganisms; comparing PHA degradation in greenhouse soil and farmland soil it was found that PHA degradation is significantly greater in farm land soil for similar sized films/plates under similar conditions[192,218,219] (Table 1, entries 14–16). Celestina *et al.*[225] reported that greenhouse soil treated with a fertilizer has a lower bacterial and fungal community count and diversity compared to untreated soil. Therefore, it is important to note the soil source and conditions being used during the biodegradation study of PHAs.

4.5.2   Soil moisture content and pH. The enzymatic hydrolysis of PHAs is coupled with the moisture content to properly hydrolyse the ester bonds, and therefore high moisture content maximizes PHA enzymatic and biodegradation performance. For example, PHBV can degrade in soil by 30–40% in 120 days provided there is enough moisture,[216] but in arid "dry" environments, PHBV degradation can extend as biotic and abiotic processes slow down. In the literature, it's referred to as either water holding capacity (%) or soil moisture/humidity (%). According to ASTM 5988-96, at 90% water holding capacity, PHB powder can degrade by 100% in 90 days.[215] Sand has incredibly poor moisture holding capacity as it is almost entirely based on silica oxide. Soil biodegradation studies without a measure of moisture content are considerably older since the standard has been updated to include soil moisture. Comparing similar soil studies using field soil at 28 °C and 50% moisture content to fertile garden soil at 30 °C and 80% moisture content, PHB films take 28 and 35+ days to achieve 100% mass loss under optimal microbial conditions, respectively[181,193] (Table 1, entries 3 and 4). PHBV (12% HV) only biodegrades by 35% in 200 days at 23–25 °C, which can also be attributed to 20% moisture content[175] (Table 1, entry 1).

Soil pH is closely related to moisture and the degradation of PHAs, by maximizing enzyme function. Optimal pH for the most common enzymes used in PHB degradation is between 6–9 with some variation between different types.[226] Furthermore, PHB and PHBV ester hydrolysis and physical degradation naturally occur in alkali environments without microorganism presence.[101] Few studies consider these points, however, it has been indicated that the optimal soil for PHB degradation is saline soil with a pH of 7–8 compared to clay, sandy, tarine, and laterite soil with a pH lower than 7.[227] The effect of soil pH on PHAs is similarly caused by alkali treatment of the PHB to improve the hydrophilicity and initiation time of mass loss at the cost of slightly reduced mechanical properties.[228] PHA degradation is maximized with high moisture and optimal pH range, however, this does not capture the soil pH and moisture relationship around the world. 50% of the tested regions in North and South America have an acidic surface soil pH (<6.5) and evidence of a positive correlation between moisture content and acidic pH indicates that ideal conditions for natural PHA degradation are very limited.[229]

4.5.3   Temperature effect on soil. Enzyme kinetics is directly correlated with temperature, with the best rates observed at optimal temperatures for the microorganisms and the set of enzymes. Several studies have since shown that soil biodegradation at higher temperature ranges proved more favourable. In hardwood soil, at temperatures of 15, 28 and 40 °C, PHB degradation increased to 8%, 23% and 26% respectively.[179] In other studies PHB degradation at 30 °C had a 100% improvement over degradation at 20 °C.[227]

For standard purposes, soil biodegradation should be completed at 20–28 °C, but does perform better at higher temperatures. PHA copolymers have greater thermal sensitivity during biodegradation; PHBV (10% HV) degradation at 15, 28 and 40 °C was 11%, 32% and 100% respectively over approximately 200 days.[179] The degradation rates of PH4B (10% 4HB), PHBHx (12% Hx), PHBV (12% HV) and PHB have all seen degradation improvements as the study temperature is increased from 21 to 28 °C, with all copolymers benefitting the greatest with increased temperatures[181] (Table 1, entries 4–7). The PHA molecular mass reduces faster at higher temperatures in soil, suggesting that it would benefit more from composting conditions. In most cases <40 °C gives the best improvement in PHA degradation rate (due to microbial action or enzyme activity) while temperatures approaching 60 °C were significantly worse for PHA degradation.[230] This can be related to the type of microbial species and proteins present and their respective functions as there is a shift from mesophilic to thermophilic microorganisms at 40 °C,[128] in addition to PHB depolymerase activity reducing as temperatures increase from 30 to 70 °C.[226] In soil biodegradation low temperature (<20 °C) studies are not regularly completed, limiting the available literature on biodegradation in cooler climates and environments.

4.5.4   Applications of soil biodegradable PHA. The desirable soil biodegradation properties of PHAs make them an excellent candidate for single use plastic applications that are common in plastic waste, including biodegradable plastic applications in the agricultural industry. For example, PHA based mulch films can be supplemented with nutrients and other additives that can be slowly released, timed by the degradation of the mulch films. Furthermore, some mulch films can assist in moisture retention by protecting the soil from moisture evaporation. Redondo *et al.*[209] studied a PHA/PLA blend in commercial soil under ASTM D5988-12 where mulch films degraded completely after 176 days, covering a considerable amount of the growth season. However, the environment of surface soil is not generally hospitable to biodegradation action. UV degradation does not assist PHA mulch film degradation and the low moisture with high temperatures makes it unfavourable for microbial degradation.[231] However, there is evidence of enhanced PHB soil biodegradation when exposed to long periods of UV irradiation.[232]

Considering that there is no standard that defined whether a polymer can be claimed soil biodegradable after achieving a certain extent of degradation, it is difficult to define whether PHAs undergo soil biodegradation. However, there is enough

evidence to claim in the literature that PHAs are soil biodegradable provided the conditions and sample morphology are appropriate, but the duration may vary anywhere from 20 days to several hundred days or more.

4.6 PHA composting

Industrial composting is characterized by the controlled temperature of 58 °C, and can be completed industrially. Most studies are completed under industrial compost conditions, as home composting doesn't consistently achieve this high temperature level. Under ISO 14855 (equivalent to ASTM D5338) composting conditions, cellulose degrades by 92% in 45 days[194] (Table 2, entry 15). Comparatively under ASTM 5338, a PHA bag has 94% biodegradation in 180 days[39] (Table 2, entry 1), and exceeds the degradation of petroleum based biodegradable polymers like PCL,[233] and passed the 2nd tier of the ASTM 6400-19 compost labelling standard which is 90% biodegraded in 180 days. PHA composting studies within the last decade are outlined in Table 2.

Although industrial composting is considered very controlled, there is still some variability between samples; PHBV films have been reported to take between 45–200 days (Table 2, entries 2 and 3). This variability may have to do with the inoculum source.

4.6.1 Inoculum source in composting. The inoculum source for composting is the material used to introduce the microorganism into the compost that will be degrading the materials. ASTM D5338 indicates that the inoculum can be from municipal solid waste, plant waste, yard waste or a mixture of green waste and municipal solid waste. In such cases some variability can be introduced. PHA biodegradation has been studied using inoculum sources from activated sludge,[235] mushroom farm compost[175] (Table 2, entry 2), organic waste from the composting factory[178] (Table 2, entries 7–11) or mixtures of chicken manure with wood chip dust.[236] Having no defined ASTM standards, the compost initial conditions (pH, carbon/nitrogen ratio *etc.*) have significantly more

variability between studies. Comparing activated sludge, and chicken manure mixed with wood chip dust as an inoculum source, activated sludge inoculated compost had a significantly higher biodegradation rate.[235,236] Activated sludge is sourced from wastewater treatment and inherently contains a variety of bacteria and protozoa that can vary across the globe in terms of represented microbial populations and more locally, depending on the treatment methods.

Following ASTM D5338-15 or its equivalent ISO 14855-1, a comparison of the organic waste inoculum and mushroom compost inoculum indicate that organic waste is more effective[175,178] (Table 2, entries 2 and 11). Organic compost is characterized by a high moisture content from the organic material improving the conditions for microbial growth and its starting microbial load is significantly higher than the inoculum sourced from the mushroom compost.

4.6.2 Non-commercial compost (home). Home or non-commercial composting is a more natural method where the temperature and moisture content are not controlled, allowing for more variation in the initial compost composition and temperature profile. As such, home composting performs slower than commercial or industrial composting.

Biodegradation of PHAs under home composting or industrial composting follows the pattern of other biodegradable plastics studied under the same conditions, influenced by the microbial profile, temperature and pH during the process. PHAs show minimal if any biodegradation in home composting conditions where the temperature is low, or when the pH becomes low.[233] Higher temperatures of industrial composting benefit biodegradation by enhancing non-enzymatic and enzymatic catalysed hydrolysis.[237] Because of these variable conditions, PHA biodegradation varies between each study. Mergaert *et al.*[179] reported the biodegradation of PHB, PHBV (10% HV) and PHBV (20% HV) to be 4%, 6–17% and 67% in 152 days where the temperature varied from 8–30 °C. Gilmore *et al.*[145] reports that PHBV (26% HV) had 59% mass loss in 186 days, indicating a slower biodegradation which is slightly

Table 2 PHA composting biodegradation studies from 2010–2020

	PHA (co-monomer %)	Protocol and source	Form	Days	Results	Ref.
1	PHA	ASTM D5338	Bag	180	94% biodegradation	39
2	PHBV (12% HV)	ASTM D5338-15, mushroom compost	Film	200	90% biodegradation	175
3	PHBV (2% HV)	ASTM D5338	Film	45	95% biodegradation	150
4	PHB	ASTM D5338-98	0.24 mm plate	112–140	99–100% mass loss	32
5			1.2 mm plate	84–112	98–100% mass loss	
6			5 mm plate	210	45% mass loss	
7	PHB	ISO 14855-1, compost factory organic waste	Film	110	80% biodegradation	178
8	PHBV (3% HV)				80% biodegradation	
9	PHBV (20% HV)				89% biodegradation	
10	PHBV (40% HV)				90% biodegradation	
11	PH4B	ISO 14855-1, compost factory organic waste	Film	110	90% biodegradation	178
12	PHB	ISO 14855-1, mature compost	Film	45	80% biodegradation	177
13	PHBV (3% HV)				81% biodegradation	
14	PHB	Non-ASTM, home composting	Tensile sample	84	50% mass loss	234
15	PHB	ISO 14855, mature organic municipal solid waste	Pellets	78	92% mass loss	194
16	PHB	ASTM D5929-96	0.5 mm plate	182	100% mass loss	32
17			1.2 mm plate	182	100% mass loss	
18			3.5 mm plate	350	94% mass loss	

slower despite the temperature between 40–63 °C throughout the study. The difference is more likely due to the differences in inoculum and temperature profile which is reflected in PHB which degrades by 50% in 84 days in organic waste home compost with 74–89% humidity and a temperature of 34–66 °C[234] (Table 2, entry 14). The unpredictability of PHA home composting makes it difficult to study the effects of the temperature profile throughout the composting process.

Several composting studies (Table 2, entries 1, 3–5, 7–11, 15–17) following ASTM D5338 or ISO 14855 have identified PHB and PHBV to pass the 1st and 2nd tier of ASTM D6400. Samples are 90% physically degraded in 90 days, defined by (%) mass loss, and 90% biodegraded in 180 days, defined by (%) biodegradation.

4.7 PHA marine biodegradation

PHAs are the only class of bio-based polymers that exhibit efficient marine biodegradation following ASTM standards, compared to other polymers such as PLA which does not degrade.[238] Among other polymers showing biodegradable behaviour in marine water (*i.e.* PCL, PES, PEA *etc.*), PHAs outperform most in all water environments,[188] but the research in PHA marine biodegradation following repeatable ASTM standards is still very limited. Recent PHA marine biodegradation studies are outlined in Table 3. Over 70% of the world is covered in water and presents a variety of differing conditions in the natural environments which must be investigated to ensure that PHA waste is biodegraded if improperly disposed.

4.7.1 Marine water source. Marine water can come from various sources (*i.e.* oceans, lakes, rivers *etc.*) but saltwater from ocean sources is usually classified as marine water and

will be considered as such in this study. However, some studies have been completed in fresh water, static water, river water or a combination thereof. Marine water has a higher level of sulphates (compared to fresh water) which can act as a secondary terminal electron acceptor for some microorganisms. The pH of fresh water is usually lower than that of marine water,[182] which does not benefit PHA biodegradation. Furthermore, the majority of aquatic microorganisms are bacteria while fungi are found considerably less often in large water bodies across the globe.[105] Knowing that fungi are considered more efficient PHA degraders, the marine biodegradation performance of microorganisms will be slightly slower.

In certain studies, a combination of marine water and sand has been used to study the effect of the different microorganism's biodegradation performance. PHBV shows biodegradation in both water and sand alone as well as a combination. The presence of marine water is seen to promote biodegradation, which suggests a wide distribution of PHA degraders in water or more effective enzymatic hydrolysis. PHBV is reported to take 210 days to degrade by 90% in a sand and seawater medium, but over 600 days to degrade by 80% in only sand[176] (Table 3, entries 11 and 12). During non-ASTM studies under non-laboratory conditions, Sridewi *et al.*[133] reported that the degradation of PHAs is high where tide inundation is higher due to the presence of water and the introduced microbial populations are not found in the soil alone. However, the improvement may be attributed to the presence of water and not the microorganisms in it, because sand based environments are reported to have a greater Shannon-weaver diversity index and overall diversity of phylum compared to water environments.[240] Therefore, sediment provides a great

Table 3 Recent PHA marine biodegradation studies

	PHA (co-monomer %)	Protocol and source	Conditions	Form	Days	Result	Ref.
1	PHB	Non-ASTM, Eutrophic reservoir	18–25 °C	Film	42	43.5% mass loss	100
2	PHB	Non-ASTM, South China sea	27–30 °C	Solid	160	62% mass loss	146
3				Film		58% mass loss	
4	PHBV (11% HV)	Non-ASTM, South China sea	27–30 °C	Solid	160	87% mass loss	146
5				Film		54% mass loss	
6	PHB	ASTM D6691, Woods Hole Harbor water	30 °C	Film	100	90% mass loss	164
7	PH4B (44% 4HB)	ASTM D6691, Woods Hole Harbor water	30 °C	Film	100	80% mineralization	164
8	PH4B (47% 4HB)					82% mineralization	
9	PHBV (8% HV)	ASTM D6691, Woods Hole Harbor water	30 °C	Film	100	85% mineralization	164
10	PHBV (12% HV)					100% mineralization	
11	PHBV (8% HV)	Non-ASTM, Lorient Harbour	25 °C	Film	180	36% mass loss	176
12	PHBV (8% HV)	Non-ASTM, Lorient Harbour water + Foreshore sand	25 °C	Powder	210	90% biodegradation	176
13	PHA 2200	ASTM D6691-09	30 °C	Film	365	52% biodegradation	238
14	PHA 4100					82% biodegradation	
15	PHBV (12% HV)	Non-ASTM, Baltic sea water	17–20 °C	Film	42	60% mass loss	235
16	PHBHx (6.5% HV)	Non-ASTM, coastal sea water	23 °C	Film	148	89% biodegradation	106
17	PHBHx (7.1% HV)				195	55% (77%)[a] biodegradation	
18	PHA	Non-ASTM, tropical river water	28 °C	Film	86	71% mass loss	239
19	PHBHx (11% HV)	Non-ASTM, sea water	27 °C	Film	28	35% biodegradation	190
20	PHBV	OECD 301, river water	25 °C	Film	90	90% biodegradation	150

[a] Biodegradation level after removal of the outlier in data for the sample.

microbial diversity while marine water may significantly enhance the enzymatic function.

Other inoculum sources for non-natural marine bio-degradation include sewage sludge and anaerobic digester sludge. Anaerobic digester sludge outperforms sewage sludge as an inoculum source for marine aerobic biodegradation of PHAs.[241]

4.7.2 Temperature effect on marine biodegradation. The effect of temperature on marine biodegradation reflects temperature-dependent findings from soil biodegradation studies.[147] In cold water (at approximately 5 °C) the bio-degradation of PHAs is negligible,[242] indicating that cooler water bodies located in the far northern or southern hemispheres are less conducive to supporting biodegradation. At milder temperatures (between 10.9–19.8 °C), PHA bio-degradation in water was slower at lower temperatures, during the initial phase of the study.[176] The initial phases of bio-degradation affect the microbial growth characteristics, which may require biofilm development before more rapid bio-degradation proceeds. The optimized degradation temperature for PHB in fresh water (no data for other PHAs) is indicated to be 30 °C, doubling the degradation rate when compared to 20 °C. The water temperature of 40 °C did not further improve PHB degradation rates.[227] Based on these data, PHA plastics would be a clear benefit to curb the plastic pollution in tropical environments, including the garbage patches floating in the North Pacific and Atlantic Ocean.[21–23]

4.7.3 Natural marine environments. Natural marine environments are mainly characterized by their dynamic nature, integrating a continuous ecosystem, opposed to a batch or static process in the laboratory. There are strong indications that dynamic sea water assists in the degradation of biodegradable polymers relative to static sea water in the lab environment.[243] Closed experimental systems also limit the potential biodegradation rate by having accumulation of by-products as well as limited nutrients available, which may result in lowering the pH and slowing down the PHA break-down into acids *etc*. A study of a PHA sample in tropical marine water under static conditions observed the reduction in the pH of the system from 7.5 to 4.7 and degradation only up to 71%, after which degradation stopped due to the acidic pH[239] (Table 3, entry 18). The acidification limited the enzymatic/hydrolytic degradation of the long chain PHA polymers. Furthermore, static environments promote the development of biofilms more readily compared to dynamic environments. Deroiné *et al.*[176] reported the effect of 0, 5% and 50% biofilm (fish breeding tank wall biofilm concentration in marine water) on the biodegradation of PHBV (8% HV) and found that no biofilm had 36% mass loss in 180 days (Table 3, entry 11), 5% biofilm had 97% mass loss in 200 days and 50% biofilm had 88% mass loss in 300 days. Excessive biofilm can produce an overabundance of enzymes which can cover the active sites for enzyme binding to perform hydrolytic degradation. Current marine biodegradation standards do not capture the full advantages of the observed natural marine biodegradation rate.

Natural marine environments can also include fresh water sourced from rivers and lakes, where biodegradation of plastics may occur. Compared to other biodegradable polymers, PHBV biodegrades most efficiently in many aquatic environments. PHB and PHBV biodegrade at a different rate in the aquatic environment, having a 6 times faster rate in seawater than fresh water ponds/canals.[179] Under favourable conditions, PHA can be degraded in its entirety, within 28 days in both fresh and saltwater rivers/lakes/oceans. PHB and PH4B degrade slightly slower within the 28-day period. Overall, chemically synthesized polymers tend to degrade at a slower rate and to a lower extent and are more dependent on the origin of water: PCL and PEA have comparable biodegradable behaviour in fresh and salt water to PHAs; PES biodegrades well in fresh water only; and PBS and poly(butylene adipate) (PBA) show some biodegradable behaviour in fresh water and minimal in salt water.[188] However, bio-based PCL is still not widely accepted as marine biodegradable and does not always show marine biodegradable behaviour.[244] In terms of the scope of ocean saltwater, PHAs are well placed to replace plastics that may end up in the ocean. However, at considerably greater ocean depths, PHAs degrade slower.[141]

While marine biodegradation does not contain an equivalent labelling standard to ASTM D6400, under ASTM D6691 PHB, PHBV (8–12% HV) and PH4B (47% 4HB) biodegrade beyond 90% in 100 days relative to the glucose reference[164] (Table 3, entries 6, 8–10), fulfilling part of the OK marine biodegradable standards.[137] Therefore, PHAs are marine bio-degradable under ASTM D6691 in appropriate environments but physical degradation must still be assessed.

4.8 PHA sewage sludge biodegradation

Sewage sludge biodegradation is the industrial biodegradation of sewage sludge in municipal waste facilities, and sewage sludge biodegradation studies use sewage sludge as an inoculum. However, there is little rationale why PHAs would be found in sewage sludge. Biodegradation in sewage sludge is usually completed at a mesophilic temperature of 35 °C in a non-industrial scale to stabilize sewage sludge for land application, allowing many types of biodegradable polymers to degrade rapidly. Sewage sludge biodegradation is classified by low total solids required in the inoculum, and is more representative of anaerobic water biodegradation. Under these conditions PHAs are still reported to degrade into 50% CO_2 and 50% methane,[245] and at least 2 times faster than other biodegradable polymers (PBS, PLA and PCL).[182,246] Standards of this kind have been withdrawn as anaerobic digestion with higher solids is a more applicable and representative form of anaerobic biodegradation used in sewage sludge processing on an industrial scale. PHA anaerobic sewage sludge bio-degradation studies are outlined in Table 4.

Sewage sludge biodegradation varies significantly but approximately 40 days are required for PHB films to biode-grade to 90% under ASTM D5210 (Table 4, entries 5 and 9). Following Non-ASTM standards under laboratory conditions, PHA biodegradation can occur in as little as 16 days.

Table 4 PHA anaerobic sewage sludge biodegradation studies

	PHA (co-monomer %)	Protocol and source	Form	Days	Results	Ref.
1	PHB	ISO 13975, anaerobic sludge	Powder	10	90% biodegradation	149
2	PHBHx (6.5% Hx)	Non-ASTM, wastewater	Film	85	55% biodegradation	106
3	PHBHx (7.1% Hx)				77% biodegradation	
4	PHB	ASTM D5210-92, diluted sewage sludge	Powder	12	67% biodegradation	189
5			Plate	19	68% biodegradation	
6	3 PHBs (commercial or research)	Non-ASTM, synthetic sludge with anaerobic biomass	Powder	40	50–79% biodegradation	247
7	PHBO(10% HO)	Non-ASTM, wastewater and septic sludge	Powder	61	88% biodegradation	182
8				141	95% biodegradation	
9	PHBV (8% HV)	ASTM D5210-91, wastewater	Film	40	89% biodegradation	245
10	PHB	Non-ASTM, domestic sewage sludge	Powder	21	90% biodegradation	138
11	PHB	Non-ASTM, sewage sludge	Powder	16	87% biodegradation	15
12	PHBV (13% HV)				96% biodegradation	
13	PHBV (20% HV)				83% biodegradation	

4.8.1 Inoculum source of anaerobic sewage sludge. The inoculum of anaerobic sewage sludge largely defines the microorganism community that develops in the digestor and ultimately determines the biodegradation pathways developed and the final products. Under anaerobic conditions, sewage sludge microorganism communities are able to produce a greater amount of CO_2 relative to methane, compared to fresh water and marine water.[182] Therefore, the inoculum from anaerobic sewage sludge digesters has been applied for waste-water treatment plants and biomass biodigesters. Of the potential sources, the inoculum from mesophilic (<40 °C) microorganism consortia is more effective in breaking down and converting PHAs, being best suited to the environment in a sewage sludge anaerobic biodegradation. Conditions from waste water treatment plants can be thermophilic (55 °C) and the thermophilic microorganisms and their enzymes may not be suited to function under mesophilic conditions.[245–247] Comparing the inoculum sources of fresh water sediment and marine water, there is no significant difference between the biodegradation extent.[182]

4.8.2 Products of anaerobic sewage sludge. The products of anaerobic sewage sludge have been evaluated for biomethane production to maximize the methane production for industry. The anaerobic biodegradation of PHB produces biogas with 80% methane, reaching a theoretical conversion yield of 60% of the total carbon available in the polymer[138] (Table 4, entry 10). PHAs with higher HV content lead to more rapid methane yield, however, there is no significant difference in final % methane produced between PHBVs of different HV contents.[15] Shin *et al.*[245] reported that from PHBV (8% HV) 50% of the biogas produced is methane (Table 4, entry 9). Production of methane can occur depending on the inoculum and the substrates. It has been found that marine water produces higher methane content as biogas compared to fresh water sediment as an inoculum[182] (Table 4, entries 7 and 8). Methane production is increased in the co-digestion of PHAs with waste organic matter[247] (Table 4, entry 6). Modifying the methane production during anaerobic biodegradation has the potential to improve the value of anaerobic biodegradation compared to aerobic biodegradation because methane can be captured and utilized instead of being released in the environment as a greenhouse gas. However, not all anaerobic processes are controlled and managed by industry. Therefore, anaerobic sewage sludge conditions may vary significantly, and are expected to impact the PHA biodegradation.

4.9 PHA anaerobic digestion biodegradation

Anaerobic digestion is a part of the wastewater treatment utilized to reduce the organic matter content present in its effluent. The process can be either thermophilic (55 °C) or mesophilic (35 °C) and can be used to manage waste or produce biomethane. For the disposal of polymers, it is a suitable method to produce methane from food contaminated materials that can't be recycled. Under ASTM D5511 PHA biodegrades entirely in 20 days under mesophilic conditions.[39] PLA and PCL both require temperatures above 37 °C to show any form of biodegradation under anaerobic conditions.[149,246] While thermophilic anaerobic digestion is faster and more beneficial in methane production, mesophilic anaerobic digestion is more common in industry due to the energy input.[248] Under anaerobic conditions, PHAs degrade faster under mesophilic conditions, while at thermophilic temperatures, PLA degradation is faster.[213] Exploring mesophilic anaerobic biodegradation for PHAs has significant potential as a waste disposal method if plastic ends up in the wastewater treatment systems.

4.9.1 Product ratio modification in anaerobic digestion. The products of anaerobic digestion are significantly important for industrial bio-methane production through the biodegradation of organic matter. PHA biodegradation normally yields 50–60% methane from the total theoretical carbon yield, or approximately 80% methane from the total biogas yield.[246] This is comparatively lower than PLA and PBS yields. Co-digestion has also been studied under conditions like anaerobic digestion, where food waste and PHA film were co-added. The methane production can be maximized in 45 days, however, during this period, no or limited PHA biodegradation occurs due to glucose repression.[249] Despite the increased methane yield, the lag in the onset of biodegradation defeats the point of using PHAs for that feature.

4.9.2 Temperature effect on anaerobic digestion. The effect of temperature on anaerobic digestion is correlated with the enzyme activity. It is well established that mesophilic enzymes function best at 30 °C, and their activity linearly reduces until 70 °C, where no activity is reported.[226] According to ISO 13975, PHB powder degrades by 90% in 10 days at 37 °C, while PLA, PBS and PCL had <43% biodegradation in 277 days. At 55 °C PHB had 83–98% biodegradation in 22 days, while PCL and PLA had 82–84% biodegradation in 96 days and PBS did not degrade. There is evidence that PHB degrades slower under thermophilic conditions,[149,246] however, this characteristic is not evident in all studies. Hegde *et al.*[213] reported that PHA/PLA blends degraded 40% slower than virgin PLA at 52 °C in 60 days, and PHA only helped initiate the degradation of PLA. Furthermore, there is evidence that PHB degrades faster in 30 °C over 40 °C anaerobic sewage sludge,[227] and even faster under aerobic digester conditions.[241] Therefore, it is reasonable to conclude that PHAs are suited for applications where the disposal is under natural temperature range conditions where PLA, PCL and PBS show minimal, if any, degradation.

4.10 PHA accelerated landfill biodegradation

Accelerated anaerobic landfill conditions are at mesophilic temperatures (35 °C) with a high ratio of solids, reflecting an environment much like a landfill. Landfill conditions are usually uncontrolled due to the quantity of material flowing into landfills continuously. Under optimal conditions and a high enough moisture content, PHB will degrade completely within 9 days, while the more complex PHBV may take longer to bio-assimilate (29% biodegradation in 42 days) after being degraded.[148] The bio-assimilation is mainly based on the microorganism consortium.

4.10.1 Inoculum source of anaerobic landfill. The inoculum sources greatly affect the biodegradation rate under landfill conditions. Weaver[250] characterized the effects of different inoculum sources for anaerobic landfill digestion conditions and found that among a landfill reactor, waste water treatment plant, landfill leachate and anaerobically digested organic waste, the digestate and municipal solid waste provided the most reproducible results and also found that the inoculum sources significantly affect the rate and extent of biodegradation. Both the digestate and waste from a municipal solid waste plant have the advantage of being from a continuous system where the microorganism turnover is continuously occurring, and the organic material promotes growth conditions.

Moisture content is another factor. Under a high enough moisture content (50–90%), PHAs have been reported to completely degrade within 14 days.[148,173] However, when the moisture content is below 50%, the biodegradation extent is halved and biodegradation can exceed 180 days.[182]

The main limitation of PHA anaerobic landfill biodegradation analysis is the few available studies being completed following either ASTM D5526-18 or ASTM D7475-20. Furthermore, while there is consistent evidence that PHAs show favourable anaerobic biodegradation behaviour, there is no labelling standard to define whether it can be claimed as such and it is not desirable to landfill biodegradable plastics unless the off-gases can be captured.

4.11 Conclusions of biodegradation

Several factors affect the biodegradation of PHAs in the natural environment (non-laboratory) including location, temperature, nutrients, microorganisms present, UV light exposure, dissolved oxygen and salinity. In lab scale studies, optimal temperature, moisture content, pH and higher amorphous content consistently promote enzymatic depolymerization of PHAs in all types of aerobic and anaerobic biodegradation. Combined with abiotic factors, the optimal conditions for PHA degradation can be derived (Fig. 15). Other polymers may include factors such as their T_g (*i.e.* PLA), due to the transition from a glassy to rubbery state resulting in an increased free volume[251] around biodegradation temperatures that may impact hydrolytic and enzymatic degradation. Small chain PHAs also do not suffer from thermal degradation under 60 °C.[252] Under composting conditions, PHAs would undergo some thermal degradation; however, the conditions become thermophilic and the thermophilic enzymes used are more stable in this environment, becoming the major driving force for PHA degradation.

The bio-assimilation of PHAs mainly depends on the complexity of the polymer, where less complex polymers are more easily assimilated, but at the same time are limited by their high crystallinity (that limits the degradation rate). Furthermore, the presence of other organic matter can either promote the bio-assimilation of PHA to produce specific products (methane) or can repress the bio-assimilation of PHA in favour of more easily accessible carbon sources (*e.g.* cellulose, starch, glucose *etc.*). Mesophilic temperatures are reported to be most favourable for PHAs compared to other biodegradable polymers (*i.e.* PLA), in both aerobic and anaerobic biodegradation.

The effect of additives and blends of PHAs are important to consider since virgin PHAs are not usually used in industry. Chain extenders, antifouling agents and the synthetically produced plasticizers TBC and glycerol inhibit the biodegradation of PHAs, despite reducing the crystallinity, either by inhibiting the enzymatic action, reducing hydrophilicity or inhibiting the microbial growth. In such cases these can delay the onset of biodegradation for tuneable attributes if so desired.

Inoculum sources provide a significant added diversity of microorganism consortia that is responsible for the variability of PHA biodegradation between studies. In high solid content studies, such as soil biodegradation, marine biodegradation and landfill biodegradation, a high moisture content allows for the microorganisms to have an increased diversity and population that can enhance the biodegradation rate. Therefore, to ensure that biodegradation studies are applicable in research, they should follow ASTM/ISO standards, and also have inoculum sources that are comparable to either a natural environment or comparable inoculum sources that are readily available such as those from waste water treatment plants.
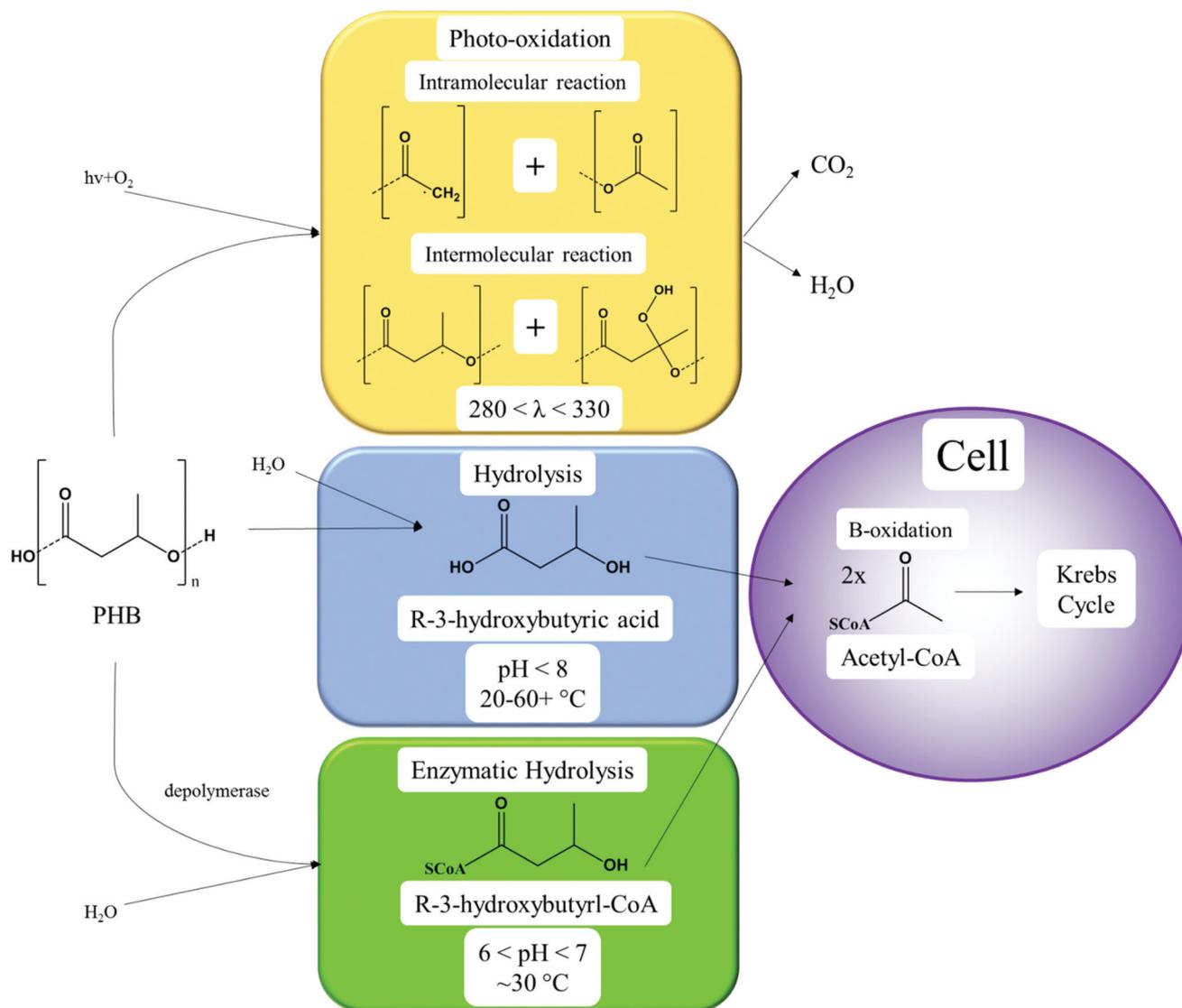
Fig. 15 Simplified main pathways and optimal conditions for PHB degradation and bioassimilation in natural environments.[91,226,253–255]

5 PHA-based biocomposites

Biocomposite applications exist mainly in the automotive, packaging, and consumer product industries to reduce material costs and weight. Biocomposites can have improved impact and mechanical modulus compared to the virgin polymer. However, the effects are dependent on the fibre modulus, aspect ratio, morphology and interfacial adhesion of the fibre to the polymer, in addition to the properties of the polymer itself that are being reinforced. The impact and/or modulus are not always improved if the properties of the fibre are lower than the virgin polymer.[256] The use of biocomposites introduces a sustainable application for agricultural fibres when their service lives end aside from disposal.[10] Cotton for example is still the most produced fibre today,[257] and is the most basic natural fibre consisting of predominantly cellulose. Other types of fibres contain cellulosic material such as hemi-

cellulose of lignin depending on the source. In fibres, lignin acts as the cement and cellulose is the rigid structure that gives the biocomposite the increased moduli.[258] PHA biocomposites are composites using natural fillers instead of inorganic fillers; it's important to consider the composition of the natural fillers and fibres for biodegradation purposes because they need to be degraded during the process.

Natural fibres and fillers, composed of organic materials, are essentially made up of cellulose, hemicellulose and lignin, with some other components depending on the type such as protein, starch, silica and other impurities. Natural fibres are usually composed of the first three, which results in a structure seen in Fig. 16. Variation of these fibre ratios results in different types of fibres (bast, leaf, grass, straw and seeds). Several PHA-based biocomposites have been developed using kenaf,[223] abaca,[259] flax,[260] starch,[141] wheat straw,[174] sisal,[261] hemp,[262] cellulose,[263] lignin,[150] seagrass,[264] wood flour,[165]
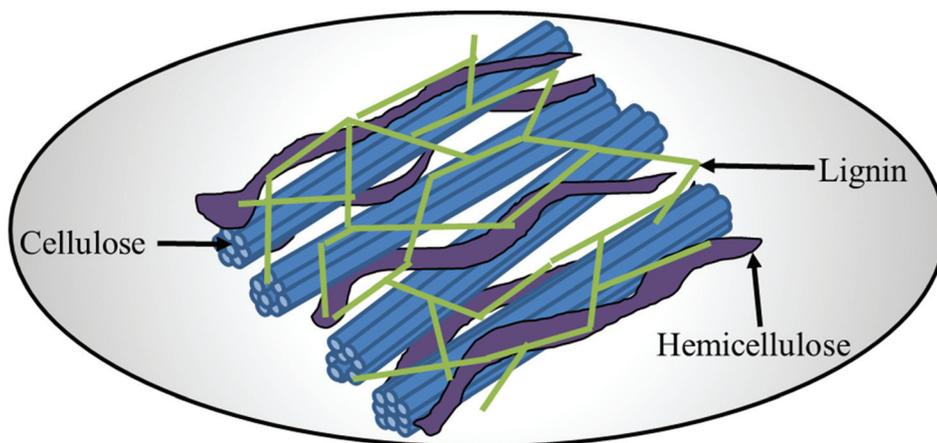
Fig. 16 Basic natural fibre structure.

etc.[192,264–267] However, in many cases it is always assumed that the addition of fibre improves the biodegradation of PHAs.

5.1 PHA-based composite biodegradation

Research on the biodegradation of biocomposites following any ASTM standard is commonly compared to virgin polymers. Several studies of PHA biodegradation have been completed with non-bio-based fillers such as TiO_2,[193,268] and carbon nanotubes, and organically modified clay claim to slow down the biodegradation of PHAs either by increasing the composite carbon content,[269] limiting segmental motion of high molecular chains or limiting diffusion of water molecules into the bulk sample.[270] Natural based fillers provide a benefit from a sustainable point of view and the majority are biodegradable in the appropriate environment. Natural rubber and other rubber constituents do show some biodegradation behaviour but unfortunately slow down the biodegradation of PHAs,[271,272] as a result of rubber biodegradation being a slow process requiring oxidation.[273] PHA-based biocomposites with natural fillers have the potential to reduce the cost and promote the biodegradation of PHAs improving the water diffusion rate and maximizing water absorption.[134]

With the establishment that polysaccharide-based fillers can degrade when in composites, more complex fillers have varying effects that can be considered beneficial for biodegradation. Fibres such as bast when aged in water cause swelling of the cell walls, separating layers which can improve the availability of enzymatically active sites.[274] Long sisal fibres 5 mm in length are reported to increase water absorption in PHBV/sisal composites, compared to 0.25 mm fibres. The fibre loading is also positively correlated with water absorption.[275] The absorbed moisture not only separates layers, the fibre/matrix can debond, reducing the overall mechanical properties,[266] but in turn increase the capacity to absorb water. These factors are attributed to the improved water absorptivity and hydrophilicity of composites working in a similar way that hydrophilic plasticizers improve PHA biodegradation.[276]

Furthermore, the physical structure of the fibre is important as a larger morphology negatively impacts the biodegradation rate by reducing the surface-to-volume ratio.[277]

Biodegradation of biocomposites mainly begins around the interface of the matrix and the fibre;[205] thus, any fibre treatment may affect the biodegradation rate. While MA grafting is reported to have a minimal effect,[134] acetic anhydride and pyradine treatment can slightly inhibit biodegradation but it remains unknown if the end % biodegradation will be affected, because no such study was found at this time that was completed until a plateau.[205]

The final attribute affecting the biodegradation of composites is the fibre composition. Natural fibres contain a variety of glucose monomer units which can be broken up by cellulases, amylases and cutinases, which are readily produced by microorganisms.[99] Dewaxing of jute fibres before composite fabrication can improve the compostability of PHBV/jute composites, by removing the non-polar elements.[278] As these natural fibres degrade, they also provide channels that allow water and enzymes into the internal structure of the polymer matrix, thereby enhancing biodegradation through an increased surface area.[279] Furthermore, polymers containing proteins and lipids can biodegrade in soil, as the lipid and proteins provide a number of useful nutrients for microorganism growth.[280]

A major consideration of natural fibres is the limiting effects they have on conventional biodegradation. Lignin is reported to slow down microbial biodegradation and bioassimilation due to its complex structure and chemical formula, more suited towards fermentation processes.[281] Therefore, claiming the biodegradability of biocomposites without an assessment of the individual components as a bare minimum is a fallacy. Furthermore, natural fibres provide the more desirable glucose as a carbon source which can slow down biodegradation by glucose repression. It is still well documented that PHA degradation is repressed in the presence of glucose and cellobiose,[282] However, once these specific carbon sources are degraded in a relative area, the PHA availability will be maximized and its biodegradation will resume.

A second misinterpretation is the idea that cellulose derivatives will biodegrade, which is not always correct. Cellulose acetate (CA), a derivative of cellulose, acts like a fibre when not plasticized and can improve the biodegradation of PHA,[283] provided its degree of substitution is not high. Increased degree of substitution inhibits the biodegradation of CA, and beyond 2.5, it shows no degradation at all under aerobic composting conditions.[284] Cellulose acetate butyrate (CAB) improved PHA elongation at break but reduced degradability[285] and lignin-based PHB composites reduced biodegradation performance.[286] Thus, research of PHA-based natural fillers and fibre biocomposites can prove to be invaluable in accelerating biodegradation and providing a more sustainable approach in plastic research.

5.1.1 Compatibilizers/coupling agents. Despite the hydrophilic favourable properties of natural fibres, making them relatively more compatible with polar biopolymers, compatibilizers are usually required to enhance the shear stress between fibre and matrix.

Use of maleic anhydride (MA) is one of the most common fibre-biopolymer grafting methods, although others such as silane treated fibres have been successfully used as well.[287] MA grafting does hold other benefits such as reducing odour release during processing.[288] However, MA can target the oxygen species in polymers, thus enabling it to functionalize the carboxyl and hydroxyl groups in biopolymers and the hydroxyl groups in cellulose, lignocellulose and hemicellulose.[289] This is of particular concern for biodegradation because of the hydrophilicity associated with polar carboxyl and hydroxyl groups.

In PHBV, MA grafting occurs predominantly on the PHB molecule, due to the relative number and chemical/statistical/steric effects. The ethyl group on PHV hinders reaction and reduces acidity, decreasing the availability of hydrogen atoms. Fig. 17 illustrates the reaction scheme between PHBV and MA,[290] where the enzyme availability of HB may be inhibited.

5.1.1.1 Grafting effect in biodegradation. However, some chemical additives or polymers interfere with enzymatic actions upon the fibre. Of greatest concern is grafting compounds into PHAs, which fundamentally changes the chemical structure and may limit the enzyme function. Several studies of the biodegradation of PHA films grafted to 10 wt(%) acrylic anhydride (AA) or MA have been completed in field soils and greenhouse soils. An increase in the mass loss (%) by 3–5% was observed with the addition of maleic anhydride or acrylic anhydride. The indicated location of grafting for both anhydrides is on the butyrate side chain of PHB, which is expected to reduce the availability of the primary PHA degradation target and inhibit enzyme action. However, the crystallinity of all PHAs is slightly reduced with the grafting and the water absorption increased, as a result of either the increased amorphous region or unreacted anhydride improving the hydrophilicity due to the presence of oxygen species, or a combination thereof and improved the biodegradation rate. With the addition of fibres, the water absorption and mass loss of grafted PHAs perform poorly compared to non-grafted
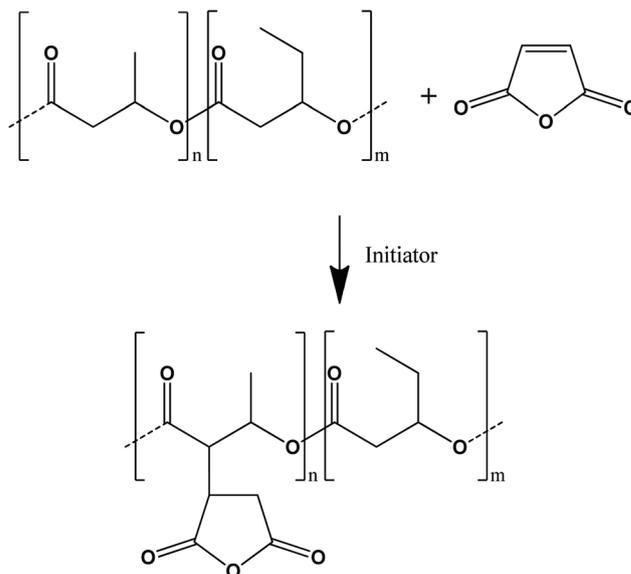
Fig. 17 Reaction scheme between PHBV and MA in the presence of an initiator. This figure has been reproduced with permission from ref. 290, Elsevier, Copyright 2007 (License number: 4818250316231).

samples,[165,191,192,218,219,291] likely due to the available anhydride oxygen species being bonded to fibres.

5.1.1.2 Surface treatment. Surface treatment is an alternative method to improve fibre–matrix interfacial adhesion and the overall properties by targeting the fibre/fillers themselves. In this regard, the polymer does not undergo chain scission. Physical methods include treatments by plasma, electron irradiation and surface roughening which can affect the hydrophilicity by removing hydroxide groups.[292,293] Chemical methods such as water washing or NaOH treatment can either wash away undesirable chemicals,[294] or functionalize the fibre surface.[292] NaOH treatment of fibres is more effective in improving the biocomposite properties when the matrix is more hydrophilic, such as in PHBV compared to PE,[84] mainly by substituting the hydrogen for Na$^+$ to increase the polarity.[295] Furthermore, NaOH treatment can remove fibre lignin and hemicellulose, roughening the fibre to maximize the surface area of fibres during biodegradation.[292]

Silane treatment is a form of chemical modification of the fibre surface, which consumes hydroxyl groups on cellulose as new chemical bonds are formed, and is reported to reduce the water absorption in Sisal/PHBV composites.[275] The removal of hydroxyl groups can also increase the surface roughness,[296] but the method is more commonly used for non-polar polymers due to the reduced fibre hydrophilicity.[297]

5.1.2 Composite soil biodegradation. Due to the relative ease with which soil biodegradation can be completed to obtain a general idea of the effects of fibres, literature is well populated with the effects of fibres on PHA biodegradation. The most basic composites are made from starch or cellulose, both easily biodegradable by various microorganisms. Increasing the content of starch improves the biodegradation

of PHA.[298] Studies of more complex fibres such as wood have also been conducted in PHB; however, the improvement is marginal.[194] The complexity of fibre increases with the content of lignin; therefore, the fibre type can have a significant effect on the PHA biodegradation improvement. Recent PHA composite soil biodegradation studies are outlined in Table 5. With greater natural fibre and filler addition, the soil biodegradation of every composite is reported to improve.

5.1.2.1 Effect of natural fibre/filler type. Soil biodegradation of PHA biocomposites has been completed with several fibres and fillers including starch, soy, lignin, flours, wheat straw *etc*. Starch based granules are a separate subset among natural fillers due to the alpha repeating units of glucose, providing a direct source of functional carbon in an optimal form with minimal degradation. Increasing the starch content from 10 to 30% increased the % degradation of PHA by 34%, indicating that there are synergistic benefits in the presence of fibres.[298] Wei *et al.*[299] reported PHB/potato peel waste composite films where the potato peel waste component degraded in the early stages through analysis of the melt temperature, which is the result of potato peel waste being composed mainly of starch (66%) despite containing some cellulose, hemicellulose and lignin.[300]

The complex structure of lignin is not easily degraded in soil biodegradation and showed evidence of no biodegradation compared to other natural fillers when in a PHA matrix in an aerobic environment.[298] Although lignin is reported to act as a channel for degradation of the polymer matrix in anaerobic environments,[301] it severely retarded degradation in anaerobic environments.[182] Wheat straw contains 16–25% lignin,[302] which is expected to inhibit the degradation of PHAs. Avella *et al.*[174] reported PHBV/wheat straw 70/30 samples degraded

by 23% in 180 days in garden soil, but no improvement in the rate of biodegradation was found compared to PHBV. PHA/wood flour 50/50 composites under natural and laboratory conditions are reported to show wood flour degradation improvements although the two studies vary from 12.5% in 365 days to 50% in 84 days respectively, which can be attributed to the higher surface area and moisture in laboratory studies.[165,224] Furthermore, depending on the type of wood flour the lignin content can vary from 21–34%.[303]

The presence of lignin is still not enough to determine if the biodegradation rate will be impacted and to what extent. PHA/rice husk 80/20 biocomposites improved the water absorption of PHA and increased the % mass loss in farmland topsoil from 33 to 75% in 60 days,[218] despite rice husk containing 25–30% lignin. However, rice husk also contains 50% cellulose and 20% silica,[304] the former being easily degradable and contains only glucose unlike hemicellulose and lignin. Hemicellulose does provide benefits; being the amorphous fraction of the fibre, it is more easily hydrolysed. Peach palm fibre contains a significant amount of hemicellulose which is composed of a number of other hexose and pentose monomers besides glucose,[305] and 0–25% weight ratios in PHBV showed a slight improvement in physical disintegration.[221] Abaca fibre contains 66% cellulose, 24% hemicellulose and 12% lignin, giving a balance between the cellulose and hemicellulose, with little lignin,[306] and 10% abaca fibre improves the degradation of PHBV from 30% to 50% in 180 days in regularly watered gardening soil at 25–30 °C.[205] Kenaf fibre has a comparable cellulose and lignin content to that of abaca fibre with 13% hemicellulose,[257] and PHBHx/kenaf 70/30 composites improve the degradation from 5.5 to 13% in 42 days[223] (Table 5, entry 2). Therefore, it is not only the ratio of cellulose,

Table 5 PHA composite soil biodegradation studies from 2010–2020

	PHA (co-monomer %)/composite	Protocol and source	Conditions	Form	Days	Results	Ref.
1	PHBV (1% HV)/wood flour (i) 80/20, (ii) 50/50	ASTM G160-12, subtropical field	~20 °C, 7cm depth	1.6 mm plate	365	(i) 6.5%, (ii) 12.5% mass loss	224
2	PHBHx (3% Hx)/untreated kenaf 70/30	Non-ASTM, oil palm cultivation soil	30 °C, 81% relative humidity	3 mm plate	42	13% mass loss	223
3	PHBHx (3% Hx)/alkali and acid kenaf 70/30	Non-ASTM, oil palm cultivation soil	30 °C, 81% relative humidity	3 mm plate	42	7.5% mass loss	223
4	PHA/palm fibre (i) 80/20, (ii) 60/40	Non-ASTM, farmland topsoil	30–40% moisture(w.b.)	Film	60	(i) 72%, (ii) 90% mass loss	192
5	PHA-g-MA/silane treated palm fibre (i) 80/20, (ii) 60/40	Non-ASTM, farmland topsoil	30–40% moisture(w.b.)	Film	60	(i) 65%, (ii) 82% mass loss	192
6	PHA/marine algae powder (i) 90/10, (ii) 80/20	Non-ASTM, greenhouse	25 °C, 35% moisture(w.b.)	Powder	120	(i) 70%, (ii) 88% mass loss	219
7	PHA-g-AA/silane marine algae powder (i) 90/10, (ii) 80/20	Non-ASTM, greenhouse	25 °C, 35% moisture(w.b.)	Powder	120	(i) 61%, (ii) 78% mass loss	219
8	PHB/wood fibre 80/20	ISO 17556, forest soil with garden soil	20 °C, 40–60% moisture(w.b.)	Pellet	195	60% biodegradation	194
9	PHA/tea plant fibre (i) 80/20, (ii) 60/40	Non-ASTM, farmland topsoil	35% moisture(w.b.)	Film	140	(i) 74%, (ii) 89% mass loss	191
10	PHA-g-MA/silane treated tea plant fibre (i) 80/20, (ii) 60/40	Non-ASTM, farmland topsoil	35% moisture(w.b.)	Film	140	(i) 67%, (ii) 81% mass loss	191
11	PHA/rice husk (i) 80/20, (ii) 60/40	Non-ASTM, farmland topsoil	35% moisture(w.b.)	Plate	60	(i) 82%, (ii) 95% mass loss	218
12	PHA-g-AA/rice husk (i) 80/20, (ii) 60/40	Non-ASTM, farmland topsoil	35% moisture(w.b.)	Plate	60	(i) 75%, (ii) 90% mass loss	218

hemicellulose and lignin that plays a role in the biodegradation improvement of PHA, but also the composition of the hemicellulose monomers (*i.e.* hexose, pentose glucose *etc.*).

Natural fillers such as grains include large amounts of protein besides cellulose, hemicellulose and lignin. Protein provides a nitrogen source, which after processing can be more readily available after protein degradation. PHA/soy 67/33 composites degraded by 89% in 168 days, exceeding similar ratios of PHA/starch composites.[298] The nitrogen can be utilized by microorganisms for proteins and cell growth. The proteins (with a variety of essential amino acids), oils and cellulosic fibre found in DDGS[307] can also provide suitable nutrients to allow growth of microorganisms and are significantly cheaper. The composition of DDGS in PHA/DDGS composites has allowed for comparable degradation to that of PHA/starch composites of similar weight ratios.[298] Additionally, Madbouly *et al.*[222] reported that 10% DDGS increased the biodegradation rate of PHA by 6 times. However the study was not completed until a plateau, and also utilized clarion loam soil which is known to have very good cellulolytic activity.[125]

Other natural PHA-based composites include PHA/marine algae powder, which performed poorly compared to PHA/rice husk under similar weight ratios and study conditions[218,219] (Table 5, entries 6 and 11). Marine algae powder can have 15–66% polysaccharides (starch based or similar), making it unknown what was affecting the biodegradation rate.[308]

5.1.2.2 Effect of fibre treatment. Fibre treatment can be used to improve the surface characteristics for not only the interfacial adhesion, but also the interaction between water/enzymes and the fibres. The most basic fibre treatment studied in soil biodegradation is alkali or acid treatment. Acetic acid and pyradine treated abaca fibre slightly improved the water absorption but failed to improve the biodegradation any further than PHBV/abaca fibre composites.[205] Joyyi *et al.*[223] reported that alkali and acid treated kenaf fibres in PHBHx/kenaf 70/30 biocomposites had no effect on water absorption and reduced the degradation (Table 5, entry 3). The alkali treatment is indicated to remove the hemicellulose and lignin,[309] which would result in a reduced amorphous fraction that may limit its susceptibility to enzymatic and hydrolytic attack. Hybridization of solubilized PLA and cellulose using *N,N*-dimethylformamide and 1-butyl-3-methylimidazolium acetate successfully improved the soil biodegradable performance of PLA/cellulose composite films and is attributed to the reduced crystallinity of PLA and cellulose,[31] however the effectiveness of this approach has not been explored in PHA composite biodegradation.

Silane treatment of fibre surfaces has been studied on several types of fibre by Wu *et al.* 20% loading of marine algae powder,[219] palm fibre[192] or tea plant fibre[191] increased the soil biodegradation rate of PHA by 100% (Table 5, entries 5, 7 and 10). However, with treatment of the fibres with tetraethyl orthosilicate or tetraethoxysilane, the biodegradation rate of the composites decreased by approximately 10%. The crosslinking effect upon the fibre can interfere with the enzyme activity, in addition to increasing the molecular weight. Furthermore, the ethyl groups on the silane agent are hydrophobic which can reduce the water absorptivity of the fibres. Acetylated or silane treated fibres similarly reduced the physical degradation in PBS-based composites in soil[310] and composting.[311]

Other forms of fibre treatment completed in composite soil biodegradation are regenerated cellulose (lyocell) and peat (decayed matter) which represent special attributes that make them suitable for low release of fertilizers. PHBV/lyocell 60/40 biocomposites were studied in soil biodegradation with 80% humidity, but showed no improvement in biodegradation compared to PHBV. Shibata *et al.*[216] reported PHBV matrix coated lyocell and did not allow the advantages of the filler to enhance the degradation of the composite. These characteristics are not seen in PBS/lyocell and PLA/lyocell composites and can be attributed to the hydrophobicity of PHAs. In such cases the fertilizer encapsulated by PHA can be released only once degradation of PHA proceeds to a certain extent. Peat is the decayed form of organic matter, and compared to wood flour it has a lower moisture absorption but still improves the biodegradation rate of the composite to a greater degree.[312] Peat also has the added benefit of providing beneficial nutrients to plant growth.

The main application of soil biodegradation of PHA biocomposites is modulation of encapsulated nutrients, fertilizers and other compounds. PHA based composites with oil palm fibres have already been studied to create slow release fertilizers in the agricultural industry.[313] This solution can be applied to several PHA biocomposites if PHA biodegradation needs to be accelerated and low-cost natural fillers.

5.1.3 Composite composting. PHA composite composting is important due to the societal focus on implementing composting services. PHA composites hold the potential to fulfil a rapid degradative role that PLA is unable to achieve. Recent PHA composite composting studies are outlined in Table 6. Composting studies on three forms of PHA/cellulose composites have been completed. Under industrial composting conditions (no standard), PHB is reported to degrade by 50% in 84 days, and with 10–30% lyocell loadings the biodegradation slightly improves to 55–68% in 84 days[234] (Table 6, entry 7). Sanchez-Safont *et al.*[314] reported similar findings on PHBV/cellulose composites where there was no significant improvement in the degradation rate or extent with cellulose incorporation under ISO 20200 in 54 days (Table 6, entry 9). 5% Cellulose nanocrystals in PLA/PHB 75/25 blends halved the degradation period from 21 days to 10 days which can be attributed to the increased surface area nanosized particles have. However, 5% surfactant was utilized to modify the nanocrystal but it caused delayed onset of degradation by 4 days.[315] Given that cellulose has a minimal effect on the compostability of PHAs, it can be attributed to the cellulose crystalline structure. Furthermore, cellulose may situate itself inside the PHA matrix, minimizing microorganism exposure in the initial stages of composting.

Under natural composting conditions, PHBV/starch composites showed a remarkable improvement in degradation rate,

Table 6 PHA composite composting studies from 2010–2020

	PHA (co-monomer %)/composite	Protocol and source	Form	Days	Results	Ref.
1	PHBV (20% HV)/lignin 80/20	ASTM D5338, mature compost	Film	60	85% biodegradation	150
2	PHA/soy 67/33	Non-ASTM, home composting	Small pot	84	100% mass loss	317
3	PHA/DDGS 80/20	(21–61 °C)			100% mass loss	
4	PHA/starch 90/10				57% mass loss	
5	PHA/lignin 80/20				21% mass loss	
6	PHB/jute (i) 90/10, (ii) 80/20, (iii) 70/30	Non-ASTM, home composting (34–66 °C)	Tensile sample	84	(i) 65%, (ii) 68%, (iii) 85% mass loss	234
7	PHB/lyocell (i) 90/10, (ii) 80/20, (iii) 70/30	Non-ASTM, home composting (34–66 °C)	Tensile sample	84	(i) 55%, (ii) 58%, (iii) 68% mass loss	234
8	PHB/hemp (i) 90/10, (ii) 80/20, (iii) 70/30	Non-ASTM, home composting (34–66 °C)	Tensile sample	84	(i) 58%, (ii) 62%, (iii) 68% mass loss	234
9	PHBV (3% HV)/cellulose 97/3, 90/10, 75/25, 55/45	ISO 20200, mature compost	Plate	47	100% mass loss	314
10	PHB/wood fibre 80/20	ISO 14855, organic municipal solid waste	Pellet	78	95% biodegradation	194
11	PHBV (5% HV)/wheat straw (i) 90/10, (ii) 80/20, (iii) 70/30	Modified ASTM D5338, mature compost	Film	48	(i) 55%, (ii) 60%, (iii) 65% biodegradation	174

increasing PHBV (12% HV) degradation from 7–25% and 49%, with 30 and 50% starch loadings respectively. The increased starch content is also reported to increase the PHBV degradation rate in the composite.[316] Starch is well known to have an amylose and an amylopectin fraction, the former being amorphous in nature which can reduce the crystallinity of the composite. Furthermore, starch is hydrophilic in nature, benefitting enzymatic catalysed hydrolytic degradation and is further reflected by its faster rate of soil biodegradation compared to cellulose.[171] These attributes are also seen in hemicellulose, but lignin is reported to reduce the crystallinity; however, it does reduce the degradation extent.[150] Lignin composites perform poorly compared to starch under composting conditions with both a PHA and PLA matrix.[317] Therefore, a crystallinity reduction is not adequate to improve the biodegradation rate and some hydrophilic functions as well as a relative ease of degradation is required. This is further illustrated by PHBV/wheat straw composites which reduced the crystallinity but had a negative impact on the degradation rate in compost.[174] The main cause is attributed to the high lignin (16–25%) and low cellulose.[302]

Aside from starch, neither cellulose nor lignin benefits PHA composting in high ratios. Gunning *et al.* studied PHB based jute, hemp and lyocell composites. With 30% loadings of the natural filler, jute increased % degradation from 50% to 85% while hemp and lyocell increased it to 68%[234] (Table 6, entries 6–8). The mass rate improvement can be attributed to the slightly lower cellulose and higher hemicellulose content in jute fibre.

Another consideration found in composting is the presence of protein which reflects the effect seen in soil biodegradation. PHA Soy 67/33 and PHA/DDGS 80/20 were both reported to have 100% degradation in 84 days, unlike PHA/starch which was at 57%[317] (Table 6, entries 2–4). Both natural fillers contain nitrogen sources for microbial growth. The effect of DDGS is also seen in PBAT, which made the composite degradation initial stages comparable to cellulose.[318] The DDGS composition is approximately 19% cellulose, 17% hemicellulose, 5% starch and 30% protein,[294] making it an amorphous, hydrophilic, nitrogen containing source suitable for biodegradable composites.

5.1.4 Composite marine biodegradation. Although PHAs are well known for their marine biodegradable properties, the research into the effect of natural fillers in the PHA matrix remains relatively unexplored compared to soil biodegradation and composting (Table 7). The ideal representation of marine degradation of PHA composites is PHBV/corn starch which showed rare biphasic degradation which also follows the ideal

Table 7 PHA composite marine biodegradation studies

	PHA (co-monomer %)/composite	Protocol and source	Conditions	Form	Days	Results	Ref.
1	PHBV (12% HV)/starch (i) 70/30, (ii) 50/50	Non-ASTM, tropical coastal water	26–32 °C	Tensile sample	365	(i) 80%, (ii) 100% mass loss	141
2	PHBV (19% HV)/starch (i) 75/25, (ii) 50/50	Non-ASTM, anaerobic digester water	30 °C	Film 150 μm	(i) 21, (ii) 8	100% mass loss	241
3	PHBV (19% HV)/starch (i) 75/25, (ii) 50/50	Non-ASTM, anaerobic digester water	30 °C	Film 800 μm	(i) 32, (ii) 21	(i) 85%, (ii) 100% mass loss	241
4	PHBV (5% HV) composite/seagrass (i) 90/10, (ii) 80/20	Modified ASTM D6691, seawater and sediment	30 °C	Pellets	216	(i) 20%, (ii) 27% biodegradation	264
5	PHBV (5% HV) composite/seagrass 80/20	Non-ASTM, mariculture centre	12–27 °C	Tensile sample	365	23% mass loss	264

bacterial growth curve.[155] Imam *et al.* reported that the presence of starch also improved the degradation rate of PHBV as the composite biodegradation exceeded the combination of the two constituents. There is evidence that enzymes may penetrate the PHA wall to degrade the filler and adherence of the filler to the polymer matrix is critical to whether the filler can be degraded effectively[141] (Table 7, entry 1). Biphasic degradation is disadvantageous because the overall composite degradation rate may remain unchanged. Ramsay *et al.*[241] reported that 25 and 50% wheat starch reduced the PHBV degradation time from 32 days to 21 and 8 days respectively. The significant improvement in PHA biodegradation is the result of improved water uptake which caused fibre swelling and some mechanical strain to assist in the degradation action[319] (Table 7, entry 3). A second advantage of fibre incorporation into PHA composites for marine biodegradation is the enhancement of biofilm growth,[264] which is more common in stable or static environments in soil or compost. Therefore, natural fillers' addition into PHAs improves the marine biodegradation by allowing enzyme permeation into the amorphous fractions. However, the literature available on composite marine biodegradation is limited, and it remains unclear whether the improved enzyme motility is a characteristic of starch or the increased water content.

5.1.5 Conclusions on composite biodegradation. Most factors that positively impact PHA biodegradation are clearly carried over to PHA-based composite biodegradation. The addition of most fibres, no matter the type, improves the biodegradation. Starch is the most beneficial fibre to improve PHA biodegradation due to its hydrophilicity, amorphousness and ease of biodegradation in soil and marine environments. Natural fibres benefit PHA biodegradation when cellulose and hemicellulose are in balanced ratios as hemicellulose reduces crystallinity, while cellulose and hemicellulose to a lesser extent are both readily biodegradable. PHA/jute composites degrade faster than PHA/lyocell composites. Lignin inhibits biodegradation, despite reducing crystallinity due to its complex structure making degradation difficult in all aerobic and anaerobic biodegradation environments (excluding fermentation). Fillers such as DDGS and Soy which contain protein provide a nitrogen source which benefits microbial growth and further enhances biodegradation rates under both soil and composting conditions.

Treatment of fibres with acids, alkalines, or silanes as per the literature found in this review caused no improvement in the water absorption and reduced the degradation in PHAs. The processes either removed the hemicellulose fraction, increasing crystallinity, or crosslinked the fibres, inhibiting the enzymatic functions. Grafting of the PHA with anhydrides did improve the extent of degradation – however, with the addition of fibres, the grafted PHAs performed poorly as crosslinked fibres inhibited enzymatic activity. Other forms of structural treatment such as cellulose regeneration (lyocell) gave no improvement, and the use of decaying organic matter improved the biodegradation of PHAs.

6 Conclusion

For a sustainable circular economy that mitigates the negative effect of conventional plastics on the environment and the eco-system, research and development into bio-based biodegradable polymers is imperative. Not only can bio-based biodegradable polymers reduce greenhouse gas emissions, but they can combat mismanaged waste that leaks into the environment, where human intervention is ineffective. And depending on the end-of-life, there is significant employment generation potential for value-added compost. To implement biodegradable polymers a deep understanding of the environments and their corresponding standards is necessary. Soil and marine environments are representative of a large pool where mismanaged waste ends up, such that studies in natural biodegradation environments can supplement development in the improvement of biodegradable polymers.

PHAs are considered the most readily biodegradable polymer among many (*i.e.* PLA, PBS, PBAT *etc.*), supported by the literature, in aerobic environments (soil, compost and marine), and show promising biodegradable behaviour in anaerobic (sewage sludge, digesters and landfills) environments. PHAs benefit from biotic degradation by several types of bacterial and fungal enzymes. Furthermore, the surface area can be maximized and the crystallinity lowered based on different processing techniques or longer chain PHAs to optimize the biodegradation performance. The characteristics all improve the permeation of moisture and the enzyme interaction that accelerate the enzymatic catalysed hydrolysis. Furthermore, PHA degradation products are easily assimilated into usable products for microbial growth.

However, the addition of certain additives (chain extenders) and other biodegradable/non-biodegradable polymers that are used to improve its properties can be detrimental to its biodegradable performance. The negative effects of Joncryl, antifouling agents and non-degradable or hydrophobic plasticizers inhibit the onset and/or extent of PHA biodegradation in aerobic and anaerobic environments. Although these attributes are beneficial for biodegradation modulation in the agricultural industry, it is not desirable for single use plastic consumer waste. Blending PHAs with PLA, PBAT *etc.* all inhibit the biodegradation of PHAs in most environments, except for compost, where PLA does outperform PHAs.

To further improve the biodegradation of PHAs, incorporation of natural fibres and fillers can accelerate the rate of biodegradation based on the composition of fibres, while inorganic fillers either inhibit biodegradation or do not degrade at all. Natural fibres high in hemicellulose and cellulose benefit from improved hydrophilicity and eased biodegradation respectively. Fillers with high lignin and cellulose with low hemicellulose can inhibit the biodegradation of PHAs due to their complexity and low hydrophilicity respectively. Starch based fillers provide high hydrophilicity and eased biodegradation, allowing PHA biodegradation to significantly improve. Furthermore, by incorporating fillers with proteinaceous materials such as DDGS and soy, the biodegradation of

PHAs can improve beyond starch and natural fillers without. However, the research in the area of PHA biocomposite biodegradation is severely limited in marine environments, where a significant mismanaged plastic waste is found.

Conflicts of interest

The authors have no conflict of interest to declare.

Acknowledgements

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