

Review article

Factors affecting polyhydroxyalkanoates biodegradation in soil

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ABSTRACT

Polyhydroxyalkanoates (PHAs) are polymers with widespread applications, from medical devices to packaging. PHAs can be biodegradable in natural environments, such as soil, but the blend of PHA with other materials can change the polymer properties and consequently affect the biodegradation process. The composition of the microbial communities in soil also significantly affects the biodegradation, but other factors such as temperature, pH, and soil moisture, can also be determinant. These ecological and physicochemical factors change in different seasons and in different soil layers. It is essential to know how these factors influence the PHAs' biodegradation to understand the impact of PHAs in nature. This review compiles the results on PHA polymers and PHA blends biodegradation, with focus on laboratory tests. The main factors affecting PHA's biodegradation in soil, both in laboratory tests and in the environment are also discussed.

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1. Introduction

The so-called "conventional" plastics are synthetic and semi-synthetic polymeric compounds that are produced mainly from fossil carbon sources such as crude oil and natural gas. Specific characteristics of plastics, including durability, processability, and low production price, have led to their widespread use in extensive and varied applications worldwide since the 20th century. Packages are the major market sector in which plastics are used, and are mostly conceived for immediate disposal [1]. This fact represents an environmental problem, as conventional plastics are very resistant to biological degradation. Packages usually consist of conventional plastics, such as polyethylene (PE) or polypropylene (PP), which are recalcitrant and thus accumulate in landfills and marine environments [2]. They cause the contamination of drinking water and the ingestion of disintegrated waste plastics by marine wildlife, introduce them in the food chain, which has inestimable consequences in human health, threaten global biodiversity and impact the environment [3]. These adverse effects and the excessive use of plastics led to significant challenges for waste treatment processes. Extensive efforts have been developed to create alternative plastic materials that can be competitive in economic terms, made of renewable feedstocks, and that can, preferentially, undergo biodegradation, without causing harmful effects in the envi-

ronment [4]. Nowadays, existing plastics can be classified into four groups, as indicated in Fig. 1, regarding whether they are considered biodegradable and the source of the feedstock used for their production [5].

Conventional plastics, also known as petro-based or synthetic plastic, are generally derived from non-renewable resources and are non-biodegradable [5]. These fossil-based plastics may be biodegradable, and in that case, they are called biodegradable plastics. The group of bio-based plastics refers to those plastics synthesized from natural resources or biomass. These plastics can either be biodegradable or not, and are designated as biodegradable bio-based plastics (e.g., Polylactic acid (PLA), polyhydroxyalkanoates (PHAs)) or non-biodegradable bio-based plastics (e.g., bio-PE), respectively [5].

The PHA family of polymers is considered biodegradable, non-toxic, environmentally friendly, and can be produced from renewable resources [6,7]. Nowadays, PHA polymers have the potential to compete with conventional plastics due to their characteristics that can include a high degree of polymerization crystallinity and insolubility in water. [6,7]. PHAs are biopolyesters that naturally accumulate intracellularly in a wide range of microorganisms, such as bacterial and archaeal cells. They are produced through the fermentation of sugars and lipids and function mainly as energy and carbon storage compounds [8]. These polyesters are produced when bacterial growth is limited by depletion of nitrogen or phosphorous, or when an excess of carbon source is available [9]. For example, *Sphingopyxis chilensis* survived during carbon starvation or frozen conditions by consuming the accumulated PHAs [10]. PHAs are composed of 3-hydroxy fatty acid monomers, which

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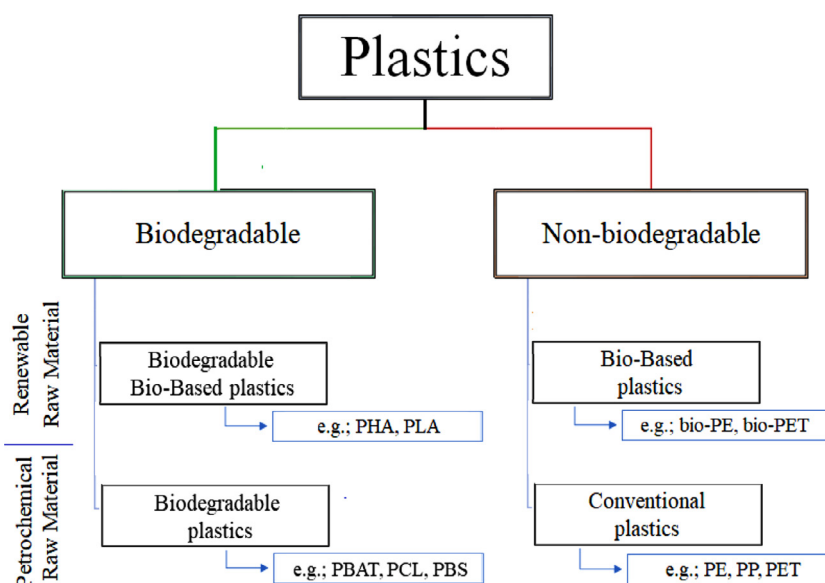


Fig. 1. Classification of plastics according to the source of feedstock (petrochemical raw or renewable raw material) and whether they are considered biodegradable or not. Abbreviations: PHA - polyhydroxyalkanoate, PLA - polylactic acid, PBAT - polybutylene adipate terephthalate, PCL - polycaprolactone, PBS - polybutylene succinate, PET - polyethylene terephthalate, PE - polyethylene, PP - polypropylene, bio-PE - bio based Polyethylene, bio-PET - bio based Polyethylene terephthalate.

form linear, head-to-tail polyester molecules. They are typically polymers of 10^3 to 10^4 monomers, which accumulate as inclusions of 0.2–0.5 μm in diameter [11]. These inclusions or granules are synthesized and stored by microorganisms and cause no harmful effects to the hosts. Depending on the number of carbons in their monomeric constituents (3-hydroxyalkanoate units), PHAs can be classified as short-chain-length (scl-PHA), containing monomers of 3–5 carbon atoms, and medium-chain-length (mcl-PHA), with monomers containing more than 6 carbon atoms [12]. The scl-PHAs are considered thermoplastic due to their relatively high crystallinity and their properties that resemble those of some petrochemical-based polymers, whereas mcl-PHAs present minimal crystallinity and exhibit elastomeric and/or free-flowing properties [12].

The existence of PHAs in bacteria has been known since 1926 when Lemoigne reported the formation of poly(3-polyhydroxybutyrate) (PHB) in the cytoplasm of the bacteria *Bacillus megaterium* [13]. The PHA bioaccumulation is common in the domains *Bacteria* and *Archaea* with PHA producing organisms belonging to more than 70 genera [14,15]. Most species of bacteria that produce PHA are Gram-negative from genera *Azohydromonas*, *Burkholderia*, *Pseudomonas*, and *Cupriavidus* [14] and *Cupriavidus necator* (formerly *Wautersia eutropha*) is the most widely studied [16]. PHA production in Gram-positive bacteria has been described in genera *Bacillus*, *Caryophanon*, *Clostridium*, *Corynebacterium*, *Micrococcus*, *Micrococcus*, *Micrococcus*, *Nocardia*, *Rhodococcus*, *Staphylococcus*, and *Streptomyces* [14]. PHA is also found in *Archaea* but is limited to *Haloarchaea* and include the genera *Haloferax*, *Halalkalicoccus*, *Haloarcula*, *Halobacterium*, *Halobiforma*, *Halococcus*, *Halopiger*, *Haloquadratum*, *Halorhabdus*, *Halorubrum*, *Halostagnicola*, *Haloterrigena*, *Natrialba*, *Natrinema*, *Natronobacterium*, *Natronococcus*, *Natronomonas*, and *Natronorubrum* [17].

Around 150 different PHA monomers have been identified so far [18]. The tendency is that this number will rapidly increase due to the synthesis of the novel PHAs as a result of chemical or physical modifications of naturally-occurring PHA [19]. PHAs are produced biologically from several carbon sources, including gases such as methane, n-alcohols such as ethanol, n-alkanes such as octanes, n-alkanoic acids such as oleic acid, and saccharides such as

fructose or glucose [20–22]. Waste streams as for example, plant oil mills effluents, frying oil waste, vinegar waste, waste fats, food waste, and agricultural waste, have also been reported as alternative carbon sources for PHAs biosynthesis [23]. Indeed, exhaustive research has been conducted to expand PHA production from lower-cost carbon sources and waste in order to reduce production costs [24]. In this context, the co-culturing of different microbial strains has been applied as a strategy to reduce production costs. In these cases, the first microorganism transforms the carbon substrate into a metabolite that can be later consumed by the second microorganism for PHA production. For example, *Cupriavidus necator* cannot efficiently metabolize sugars, whey or starchy waste, but when cultivated together with lactic acid-producing bacteria, those substrates can be transformed into lactate, that can be then used by *C. necator* to produce PHAs [25].

The intracellular PHA granules produced by the bacteria need to be extracted, and for that purpose, the bacterial cells are usually separated from the medium by centrifugation. Organic solvents such as acetone, chloroform, methylene chloride, or dichloroethane are utilized to lyse the cells and recover the intracellular PHA [26,27]. Digestion methods with sodium hypochlorite [28] or enzymatic digestion procedures using, for example, EDTA or SDS [29,30] are alternatives to organic solvents.

Polyhydroxybutyrate (PHB) is the most common and well-studied polymer within the PHA family [31]. Other polymers belonging to the PHA class include poly(4-hydroxybutyrate) (P4HB), poly(3-hydroxyvalerate) (PHV), poly(3-hydroxyhexanoate) (PHH), poly(3-hydroxyoctanoate) (PHO), and their copolymers.

Several microorganisms are able to decompose PHAs, by using intracellular depolymerase enzymes and use it as an energy and carbon source [32,33]. In the environment, various microorganisms produce and release extracellular enzymes that can degrade PHAs [34], such as PHA hydrolases and PHA depolymerases [35]. Mergaert and Swings [36] identified 695 microbial species capable of degrading PHB. Usually, depolymerases catalyze the hydrolysis originating free D(-)-3-hydroxybutyrate that is then oxidized to acetoacetate by a NAD-specific dehydrogenase. NADH, pyruvate, and 2-oxoglutarate are known to inhibit this enzyme. The acetoacetate is finally converted to acetoacetyl-CoA by an acetoacetate/succinate CoA transferase. Thus, acetoacetyl-CoA is simulta-

neously a precursor of PHB synthesis and a product of PHB degradation [37].

PHA polymers may present different monomers composition, physicochemical properties, size, and structure because they can be produced by different microorganisms and from diverse substrates [38]. The lower environmental impact of PHAs turns them an ideal alternative for petrochemical polymers, particularly in packaging and coating applications [39]. Nowadays, PHAs are mainly applied in packaging, in the form of containers and films [40] but also in the medical field. For example, PHB can be used as repair patches, orthopaedic pins, adhesion barriers, stents, nerve guides, and bone marrow scaffolds, since it is compatible with mammal's blood and tissues [41].

2. PHAs biodegradation in soil

The biodegradation of PHA has been studied in the last 3 decades in several types of soils and under different conditions. According to the definition of the International Standardization Organization (ISO), plastics need to undergo significant changes in their chemical structure by the activity of naturally occurring microorganisms to be considered biodegradable [42]. On the other hand, the European Standardization Committee (CEN) is stricter and considers that a biodegradable plastic needs to be converted into microbial metabolic products. Concerning aerobic biodegradation of plastics in soil, the most used standard testing methods for laboratories are the ASTM D5988-18 [43], ISO 17556 [44], and the French and Italian norms NF U 52-001 [45] and UNI 11462 [46], respectively [47]. However, the NF U52-001 [45] was recently superseded by EN 17033 [48] for "Plastics - Biodegradable mulch films for use in agriculture and horticulture e Requirements and test methods". EN 17033 [48] was created using the methodology of ISO 17556. The ISO 17566 uses a method where the CO₂ production is measured in a system aerated with continuous CO₂-free air [49]. This method is equivalent to the ASTM D5988 for determining the aerobic biodegradation of plastic materials in the soil. In this method, closed flasks (bioreactors) are used and are aerated only periodically. The evolved CO₂ from the sample is trapped in an alkaline solution (BaOH or KOH) and measured by titration [49]. Alternatively, the biochemical oxygen demand can also be implemented [44].

A significant amount of plastic waste can be found on soil. The soil pollution problem, caused in a greater extent by plastic waste, can be somewhat alleviated by the manufacture and use of advanced bio-based plastics that can be biodegradable [47]. The high diversity of microorganisms present in soil increases the possibility of finding microbes capable of degrading biodegradable plastic waste. Because soil may hold microorganisms with this ability, it has been considered an environment with an excellent capacity for degrading PHA [50]. Two different studies estimated that the percentage of bacteria capable of degrading scl-PHA is between 0.8 to 11.0 %, and 2 to 18 % of the total colonies formed from soil plating [51,52]. Table 1 summarizes the laboratory biodegradation tests conducted with PHA polymers. Gómez and Michel [5] tested the biodegradability of several commercially available alternative materials for conventional plastic, according to the ASTM D5988-03. During incubation in soil for 660 days (Table 1), CO₂ production was monitored, and the results showed that the maximum biodegradability was obtained for PHA films (around 70 %), which was not statistically different from that of cellulose paper (control). SEM analysis presented considerable disintegration of PHA-based plastic compared to the other materials tested [5]. Scanning electron microscopy (SEM) is often considered a valuable tool to study colonization and biodegradation of PHA films. For example, it was utilized to evaluate the biodegradability of PHBV by soil microorganisms, since the polymer deterioration generally occurs through

surface erosion, due to microbial activity [53–55]. In natural soils, polymers from the PHA family present higher biodegradation rates (evaluated by weight loss) when compared to other polymers such as poly-DL-lactide and ethyl cellulose [56]. These results may be due to higher biofilm development on PHA plastic films [56].

PHB tested in different laboratories using the ASTM D5988 and some specifications of the ISO 17556 for plastics exhibit a similar degree of biodegradation in both natural and standard soils (Table 1). Other polymers that resist more to biodegradation, such as PBSeT and PBSe, present some differences, being biodegraded slowly in natural soils [49]. In another work, where the ASTM 5988 system was used, PHA biodegradation results were significantly different between 2 laboratories [57]. These authors also discovered that PHA degraded faster at 25 °C than at 37 °C in both laboratories. However, this may be due to higher biomass build-up and consequent carbon retention [57]. Kim et al. [58] demonstrated that the biodegradation of PHB was higher in different soils at 37 °C than at 28 °C, and the worst temperature for biodegradation was the highest tested, 60 °C (Table 1) [58]. PHBV (copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate) degraded at a faster rate at 30°C than at 52°C in soil under aerobic conditions [59]. Mergaert et al. [60] discovered that the biodegradation rate in soils (laboratory testing) of PHB and PHBV (10 mol % HV) was enhanced at higher temperatures (40 °C), and similar results were obtained by other authors [61]. The differences among these studies may be related to different microbial activities in soil, which are strongly influenced by temperature.

Several studies have demonstrated that other conditions and properties of the PHA materials can also affect the biodegradation rate. Specifically, copolymers (polymers derived from more than one species of monomer) are degraded at a faster rate than the homopolymers, although the differences varied widely between soils with several pH values (neutral or acid) and temperatures. Copolymers (e.g., PHBV) usually have a higher degradation rate compared to homopolymers (e.g., PHB) of the PHA family [60]. This higher biodegradation capability is attributed to the surface morphology of copolymers, which combines a low crystallinity and a porous surface, allowing a faster degradation [62].

The biodegradation of PHAs with different chemical compositions was tested in soil for 35 days. These polymers could be ordered as following according to the biodegradation rate (from higher biodegradation rate to lower biodegradation rate): PHB/4HB > PHB3HHx > PHBV > PHB. In this work, biodegradation was higher for all polymers at 28 °C than at 21 °C (Table 1) [31]. PHB films, the most crystalline ones, remained nearly unchanged, suggesting that all regions (crystalline and amorphous) were degraded at similar rates [30]. But for the 3 copolymers, the crystallinity increased, demonstrating that the amorphous regions were degraded at higher rates. Other works, including studies performed in natural environments (where the biodegradation was evaluated by weight loss), present the same pattern, indicating that PHB is more resistant to biodegradation due to its high crystallinity in comparison with the copolymers [50,63,64].

In these works, plastics biodegradation has been evaluated not only by CO₂ production or O₂ consumption but also by weight loss or loss of mechanical properties over time. Although some works use weight loss to assess biodegradation in soil, sometimes it is difficult to adequately clean the samples following soil burial and obtain the exact weight loss [65]. Sometimes it is impossible to use weight loss in later stages of the tests due to a high biodegradation level of the material, hindering a proper weight evaluation [66]. The tested material may be biodegradable in testing conditions at a specific rate, however because the properties in real environments vary extensively (e.g., microbial communities' composition and environmental conditions), the results may be not representative of those environments.

Table 1
Laboratory soil biodegradation tests for PHA polymers.

Type of Plastic	Type of material	Type of environment	Conditions	Test method	Biodegradation (%)	Length of test (days)	Reference
PHB PHB/CAB (50 %/50 %)	Films	Garden soil	Not indicated	Weight loss	64.3 31.5	180	[1]
PHA	Films	Alluvial-type soil	35 % soil moisture	Weight loss	~35	60	[67]
PHA/Rice Husk (60/40 wt %)					> 90		
PHA-g-AA/Rice Husk (60/40 wt %)					84		
PHBV (HV of 12; 43; 47; 52; 64; 72 mol %)	Powder	Soil/compost (90 % / 10 %)	25 °C, 65 % humidity, 80 % soil moisture holding capacity	CO ₂ , ASTM D5988-03	67; 54; 48; 62; 49; 49;	112	[68]
PHA	Films	43 % certified organic topsoil, 43 % no-till farm soil, and 14 % sand	20°C, 60 % moisture	CO ₂ , ASTM D5988-03	74.2	660	[5]
PHBV/WF (50/50 wt %)	Films	Soil sub-tropical	80 % of soil water holding capacity	CO ₂ ASTM D5988-03	36	330	[69]
PHBV/WF (80/20 wt %)					35		
PHBV	Films	Garden soil	23°C, 21 % soil humidity	Weight loss	~20	180	[66]
PHBV /Sep straw					~23		
PHB	Films	Garden soil	Not described	Weight loss	100	180	[32]
PHB + Acrylate					10		
PHBV	Films	Garden Soil	Room temperature	Weight loss	3	30	[70]
					18	30	
PDLLA/PHBV/PEG (30/70/20 wt %)							
PHB	Films	Hardwood, Pinewood, Sandy, Clay, Loamy soil	28°C, pH 3.9; 3.5; 6.5; 7.1; 3.3, 14-22 % water content	Weight loss	77; 74; 88; 78; 93	200	[61]
PHBV (HV of 10 %)					67; 64; 90; 53; 69		
PHB	Films	Garden soil	pH 7.3 ± 0.2	Weight loss	83	77	[71]
PHBV	Films	Organic compound humidified using poultry feces and plant-origin organic materials	pH 6, 40 % maximum humidity, nitrogen of 1 %, minimum organic material of 40 %, C:N maximum of 18:1	Weight loss	9.77 ± 2.77	90	[72]
PHBV/WF (80/20 wt %)					25.55 ± 4.05	60	
PHBV/Sisal fiber (80/20 wt %)					25.02 ± 8.23	60	
PHBV-F0	Particles around 1-2mm	Soil park (2.3 wt % of organic matter, 16.85 wt % of clay, 26.85 wt % of lime, and 56.3 wt % of sand)	28 °C, pH 6.8, 80 % of the soil water retention capacity	CO ₂ , ASTM D5988-96	100	75	[73]
PHBV-SF					100	79	
PHBV-PF					100	87	
PHBV (HV of 3%)					91	123	
PHB-starch (75/25 wt %)	Films	Garden soil	25 ± 2°C, pH 6.8, soil water content 45 %,	Weight loss	50 – 60	14-21	[74]
PHB-starch (60/40 wt %)							
PHB-starch (40/60 wt %)							
PHA (peanut oil)	Films	Commercial soil containing 39 % average organic matter	23°C ± 4°C, pH 6.8, 33 % soil moisture	Weight loss	75	80	[75]

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Table 1 (continued)

Type of Plastic	Type of material	Type of environment	Conditions	Test method	Biodegradation (%)	Length of test (days)	Reference
PHB	Films	Soil of temperate zone of Siberia, with high total exchangeable bases (40.0–45.2 mequiv/100 g), and with nitrate nitrogen N-NO ₃ 6, P ₂ O ₅ 60, and K ₂ O 220 mg/kg soil	28°C, pH 7.1–7.8 and 50 % soil moisture	Weight loss	93	35	[31]
PHBV PHB3HHx PHB/4HB PHB	Powder-Granules	Agro-transformed soil with 280 mg/kg of phosphorus and 250 mg/kg of potassium	25°C; 50 % soil moisture content	Weight loss	100 100 100 32 – 31.6	28 35	[76]
PHB/peat PHB/clay PHB/WF PHB	Films	Forest soil	28°C; 37°C; 60°C	Weight loss	43,6–53.6 36 - 26 33 -23 10.5 ± 1.4; 7.1 ± 0.7; 4.9 ± 0.3	25	[58]
		Sandy soil	28°C; 37°C; 60°C		5.8 ± 0.4; 10.0 ± 1.2; 4.5 ± 0.5		
		Farm soil	28°C; 37°C; 60°C		41.3 ± 3.7; 68.8 ± 4.8; 14.8 ± 1.2		
PHA (MirelTM)	Films	Soil from the experimental field in Spata	30°C, 40 % water content	Biological oxygen demand	26.3	10	[77]
			40°C, 40 % water content		49.5	12	
PHBV (HV of 10 %)	Films	Soil from the Nagoya University Farm and 2 % (w/w) of Farmyard manure	30°C, pH (H ₂ O) = 6.2, 40 % water content total C = 1.2 %, total N= 0.11 %, 40°C, total C = 1.2 %, total N= 0.11 %, pH (H ₂ O) = 6.2, 40 % water content	Weight loss	50	10	[59]
			40°C, total C = 1.2 %, total N= 0.11 %, pH (H ₂ O) = 6.2, 40 % water content		40	17	
PHB	Films	Soil	28°C 37°C 60°C	Weight loss	57.3 86.7 25.9	56	[78]
PHBV (HV of 11 %)	Films	1:1 mixture of black soil and leaf mold for gardening	25 - 30°C	Weight loss	~28	180	[79]
					~48	90	
PHBV/untreated abaca fiber PHBV/Aa-abaca fiber PHBV 6.2 mol % HV content	Films	Garden soil 270.4 g/kg of organic matter, 35.6 % humidity and pH (CaCl ₂) 5.1	23°C	Weight loss	100	30	[53]

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Table 1 (continued)

Type of Plastic	Type of material	Type of environment	Conditions	Test method	Biodegradation (%)	Length of test (days)	Reference
PHB	Films	Clay soil	20°C; 30°C; 40°C	Weight loss	19.7 ± 0.8; 38.7 ± 2.6; 36.5 ± 1.5	200	[80]
		Laterite soil	20°C; 30°C; 40°C		21.7 ± 1.0; 35.7 ± 1.5; 34.0 ± 1.2		
		Saline soil	20°C; 30°C; 40°C		13.9 ± 0.7; 43.5 ± 1.7; 39.0 ± 1.4		
		Sandy soil	20°C; 30°C; 40°C		17.6 ± 1.0; 33.5 ± 1.3; 26.5 ± 1.3		
		Tarine soil	20°C; 30°C; 40°C		16.6 ± 0.8; 23.9 ± 0.9; 20.6 ± 0.9		
PHB/4HB (4HB of 5 mol %) PHB/4HB (4HB of 7 mol %) PHB/4HB (4HB of 10 mol %) PHB/4HB (4HB of 15 mol %) PHB/4HB (4HB of 20 mol %) PHBV (HV of 5 mol %)	Films	Garden soil	Room temperature, 20 % water content	Weight loss	54.38 69.69 79.91 93.39 82.03	60	[81]
PLA/PHBV (70/30 wt %)	Films	Farm soil	Not indicated	Weight loss	15.68	480	[82]
		Infertile soil			1.12		
		Fresh soil from the surface layer of an agricultural field	23–25°C, soil supplemented with 10 ml of 0.1 % (NH ₄) ₂ HPO ₄ solution	CO ₂ , ASTM D5988-12	32	200	[83]
PHBV PHBV (HV of 6.2 %) / PP-co-PE (80/20 w/w) PHBV (HV of 6.2 %) / PP-co-PE/add (80/1 9/1 w/w/w) (with pro-oxidant additive)	Films	Soil rich in humus	60 % of humidity	Weight loss	35 100 100	180	[84]
PHBV	Films	75 g of soil, 10 g of thin expanded perlite, 20 mL of deionized water	28 °C ± 1°C	Weight loss	67 ± 18	34	[85]
PHBV/CNT (99/1 wt %) PHBV/CNT (98/2 wt %)					57 ± 6 40 ± 15		
PHB	Films	Agro-transformed field soil, village Minino, Krasnoyarsk Territory	28°C and soil humidity of 50 %	Weight loss	13	35	[64]
PHBV PHB3HHx PHB/4HB PHB/WF (WF content 0 wt %)	Films	Alluvial-type soil, from farmland topsoil before planting		Weight loss	82 90 97 4	28 28 21 84	[86]

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Table 1 (continued)

Type of Plastic	Type of material	Type of environment	Conditions	Test method	Biodegradation (%)	Length of test (days)	Reference
PHB-g-AA/ WF (WF content 0 wt %)					5		
PHB/WF (WF content 30 wt %)					28		
PHB-g-AA/WF (WF content 30 wt %)			20 % soil moisture		24		
PHB/WF (WF content 50 wt %)					49		
PHB-g-AA/WF (WF content 50 wt %)					42		
PHB	Films	Vegetable experimental field under organic farming (Spain)	25 °C, 50 % of soil water-holding capacity	Weight loss	100	120	[87]
PHB	Films	Commercial soil	30 ± 0.1 °C, under moisture-controlled conditions	Weight loss	~72	30	[88]
PHBV (HV of 24 %)					~40		
PHB/Eastar Bio blend (75/25 %) + 30 % WF	Films	Soil (fertilizer organic compound)	40 % maximum humidity, pH 6, maximum C:N 18:1, N (minimum) 1 %, minimum organic matter	Weight loss	29.32 ± 4.58	90	[89]
PHB/Ecoflex blend (75/25 %) + 30 % WF					13.98 ± 1.83		
PHB					19.17 ± 3.43		
PHB/Eastar Bio blend (75/25 %)					2.83 ± 0.23		
PHB/Ecoflex blend (75/25 %)					1.86 ± 0.22		
PHBV (HV of 4 %)	Films	garden soil	28 ± 2 °C, 15 % soil moisture content	Weight loss	12	60	[90]
			28 ± 2 °C, 20 % soil moisture content		23.6		
			28 ± 2 °C, 25 % soil moisture content		95	45	
			28 ± 2 °C, 30 % soil moisture content		95		
PLA/PHB_b 90D sun	Foils	Soil from agricultural region mixed with perlite in equivalent amounts	20 °C, pH 7.5	CO ₂ ASTM D5988-12 and ISO 17556-2012	55	365	[91]
PLA/PHB_b for original carbon black filled foil					42		
PLA/PHB_w for original transparent foil					57		
PHB/OMWR (100/0 w/w)	Films	Red soil - white soil	pH 8.2 - 8.3	Weight loss	12.0 - 15.0	56	[92]
PHB/OMWR (70/30 w/w)		Red soil - white soil			21.9 - 22.6		
PHB/OMWR (60/40 w/w)		Red soil - white soil			24.6 - 26.2		
PHB	Films	Soil from Kolkata municipal solid-waste landfill (T1; T2; T3; T4; T5)	30 °C, pH 6.17, total phosphorous 0.79 %, total nitrogen 0.18 %, organic carbon 3.42 %	Weight loss	~12; ~19; ~12; ~15; ~10	28	[93]

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Table 1 (continued)

Type of Plastic	Type of material	Type of environment	Conditions	Test method	Biodegradation (%)	Length of test (days)	Reference
PHB	Films	Paleudult soil	pH 6.17	Weight loss	100	180	[94]
PHB3HHx	Films	Soil composted in the farm of Chubu University	34 °C, pH 5.3, relative humidity of 90 %	Weight loss	1.91 - 7.41	28	[95]
PHA	Films	Alluvial-type soil obtained from Taiwan Kaohsiung farmland topsoil	30 - 40 % soil moisture	Weight loss	25	60	[96]
PHA-g-MA					~30		
PHA-g-MA/TPLF (20 % w/w)					~65		
PHA-g-MA/PF (20 % w/w)					~70		
PHA-g-MA/TPLF (40 % w/w)					~85		
PHA-g-MA/PF (40 % w/w)					~90		
PHA	Films	Alluvial soil obtained from farmland topsoil before planting	35 % soil moisture	Weight loss	33	140	[97]
PHA-g-MA					35		
PHA-g-MA/TPF (20 % w/w)					75		
PHA-g-MA/t - TPF (20 % w/w)					66		
PHA-g-MA/TPF (40 % w/w)					88		
PHA-g-MA/t-TPF (40 % w/w)					84		
PHA	Powder	Natural soils, collected from the surface layer of one field and two forests or soil (15 g, plough layer, haplic chernozem; dry weight 88.4 %; soil texture, silty loam, volatile solids 5.55 %; soil organic matter 3.05 %)	25 °C	CO ₂ ISO 17556 (2019)	85.8 - 96.4	150 - 170	[57]
PHB	Films	Artificial soil according to ASTM G 160-03	37 °C	Weight loss	71.1 - 93.0	90-170	[98]
			ASTM G 160-03		17.8 ± 0.64	86	
PHB/PP-g-MA/clay (92 % / 5 % / 3 %)					22.5 ± 0.24		
PHB/PP-g-MA/clay (94.5 % / 2.5 % / 3 %)					25.9 ± 0.67		
PHBV	Films	Red clay latosol soil from a 0 to 15 cm depth profile	28°C, 60 % of the moisture capacity	Weight loss	10	28	[99]

(continued on next page)

Table 1 (continued)

Type of Plastic	Type of material	Type of environment	Conditions	Test method	Biodegradation (%)	Length of test (days)	Reference
PHBV-AgNP PHB	Films	Natural soil from agricultural land of the clay-loam type (clay 29 %, silt 28 %, and sand 43 %)	25°C, C:N = 8, pH 7.9, water holding capacity 80 %, total nitrogen 2 %, organic carbon 0.13 %	CO ₂ , ISO 17556 2019	8	120 360	[49]
		Natural soil mixture from three sources: sandy and forest	25°C, C:N = 8, pH 7.9, water holding capacity 53 %, total nitrogen 7.9 %, organic carbon 0.51 %		~88 -97		
	Standard soil: Prepared according to ISO 17556:2012	25°C, C:N = 10, pH 8.4, water holding capacity 50 %, total nitrogen 3.7 %, organic carbon 0.22 %	~90				
	Natural soil, mixture from three sources: sandy and forest	25°C, C:N = 11, pH 7.4, water holding capacity 80 %, total nitrogen 6.8 %, organic carbon 0.31 %	~86				
Powder	Natural soil: Sandy loam (Sand 62 %, Silt 27 %)	28°C, C:N = 52, pH 7.6, water holding capacity 60 %, total nitrogen 4.2 %, organic carbon 0.03 %		~95	120		
				~98			

Abbreviations: Wood flour (WF), 4-hydroxybutyrate (4HB), 3-hydroxyhexanoate (3HHx), olive pomace stone-rich fraction (SF), olive pomace pulp-rich fraction (PF) olive pomace crude pomace (FO), anhydride-treated (AA-), poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHB3HHx), maleic anhydride-grafted polyhydroxyalkanoate (PHA-g-MA), PHA-g-AA, coupling agent-treated palm fibre (TPLF), palm fibre (PF), tea plant fibre (TPF) treated (crosslinked) tea plant fibre (t - TPF), carbon nanotubes (CNT), cellulose acetate butyrate (CAB), Sep straw (wheat straw), acrylic acid-grafted poly(hydroxyalkanoate) (PHA-g-AA), silver nanoparticles (AgNP), polypropylene-co-polyethylene (PP co-PE), olive mill wastewater (OMW), poly(ethylene glycol) (PEG), poly(D,L-lactide) (PDLLA), PLA/PHB outdoor weathering foil exposed 90 days to sun light (PLA/PHB_b 90D), graphitized polypropylene with maleic anhydride (PP-g-MA), PHB-g-acrylic acid PHB-g-AA), acrylic acid-grafted poly(hydroxyalkanoate) (PHA-g-AA).

3. PHA field tests

The most significant weakness of the existing standards for testing biodegradation of plastics in the soil is their weak reproducibility [47]. Several factors, including the soil type and biodiversity, the conditions such as temperature, water content, or pH, and the measuring method may affect the reproducibility of the results [47]. For example, if the plastic is the only carbon source available for microbial growth, the test may present a higher rate of biodegradation compared to an environment where other carbon sources, and easier to biodegrade, are available. Testing the biodegradation of plastics in real environmental conditions is essential to understand the impact of plastics pollution better.

Changes in soil temperature, humidity, and even microbial composition can lead to different PHA biodegradation rates [50]. In a study conducted in Vietnamese soil, and contradictory to most of the results herein presented, PHB (evaluated by weight loss) degraded better than PHBV [100]. The authors justified these results with the differences in the composition of soil microbial communities and in the diversity of their PHA depolymerases [80]. In this study, the considerable decrease in the molecular weight and the increase in PHA polydispersity indicated that the polymers were cleaved, originating minor fragments with different polymerization degrees.

The shape of the films, soil pH, soil water content, and moisture content of the polymer can also influence the PHA biodegradation rate. PHA films were found to be degraded at a faster rate than polymer pellets due to their higher surface area, which permitted a better microbial attachment and faster biofilms formation [100]. In this study, at weakly acidic pH (e.g., 5.48) the PHA biodegradation rate was higher than the biodegradation rate registered in an-

other soil with a pH value close to neutrality (6.63), which may be linked to differences in the microbial diversity and activity. Soil pH can indeed determine microbial activity, since some species have narrow pH tolerance and cannot survive in acidic environments, and also because pH may influence the availability of soil nutrients (e.g., ammonia and nitrate), which are essential to certain microbial species [101]. However, a weakly acidic soil is a favourable condition for the development of fungi, that were the major PHA degraders in this soil, while bacteria were dominant in a soil with a neutral pH value [100].

Some microorganisms have enzymes with broad substrate specificity and can degrade both scl-PHA and mcl-PHA. However, most PHA-degrading microorganisms produce enzymes specific for a particular type of PHA substrate [102]. Thus, the same polymers in different soils may present different biodegradation rates [103]. The water content affected the PHB biodegradation which seems to be favoured in soils with higher water content [104]. The water absorption capacity of the polymer seems to influence biodegradation. Indeed, PHB3HHx/KF composites (which have higher water absorption capacity than PHB3HHx) presented a higher percentage of biodegradation (determined by weight loss) than PHB3HHx [104].

Another factor affecting the biodegradation rate of PHA films in soils is whether they are buried or not. PHA films on the sediment surface are degraded at a slower rate than those buried in the sediment [62]. The biodegradation rate (evaluated by weight loss) of PHB films placed on the soil surface was 50 % slower than when the PHB was buried [105]. Buried films are surrounded by soil, and thus the exposed surface area for microbial attack is higher, which results in higher biodegradation rates [62]. Moreover, the microbial communities colonizing buried, and non-buried films are different

(e.g., buried films are in contact with both aerobic and anaerobic bacteria), which may also influence the biodegradation rates [62].

Light, especially UV, was found to influence the biodegradation process. With UV pre-treatment, PHB and its films degraded faster than the PHB films untreated [106]. The UV treatment damaged the films creating increased cracks, which accelerated the biodegradation process [106]. The techniques used to produce the films may also influence the biodegradation rate due to changes in their three-dimensional structures and surface area, which can benefit or hamper the microbial colonization of the polymeric films [106]. For example, PHB nanofiber films prepared by an electrospinning technique presented higher weight loss due to the three-dimensional structures and large surface area of nanofibers compared to the PHB produced with the solvent-cast technique [106].

4. PHA blends biodegradation in soil

The cost of bio-based plastics is still higher than that of the plastics produced from petroleum raw materials. The blending of PHAs with other bioplastics or with naturally decomposable materials, such as agricultural waste and natural fibers, is a strategy to reduce production cost or change the properties according to the goals established [32]. This process also changes the biodegradation properties and requires a new assessment of the blends biodegradability. Some blends can be used to change the properties of PHA, but these blends can also change the biodegradation behaviour of PHA, increasing or decreasing the biodegradation rate, depending on the material blended to the PHA [62,65,70,86,104–113]. These different effects can be the result of 1) an overall crystallinity change since crystalline zones are less accessible for the microorganisms, 2) microstructural defects that facilitate the adhesion of bacteria, or even to 3) increased hydrophilicity of the blends that facilitates water adsorption that is fundamental for the microorganism biodegradation [65,73].

The biodegradation behaviours of the PHB/lignin blends (films) were analysed in a soil field study. The results indicated that PHB films disintegrated with 45 % of weight loss within 12 months, however, the PHB/lignin blends had only a weight loss of 12 % when 10 % of lignin was present. These results suggest that the presence of lignin can reduce the PHB biodegradation, probably by hampering the colonization by microorganisms (most likely due to its hydrophobicity), which improved the resistance of the blends to microbial activity [114].

Jeszeova et al. [91] tested PLA/PHB foils with the ASTM D5988 biodegradation test (CO₂ production), and the PLA/PHB white foils showed the best biodegradation (57 %), followed by the PLA/PHB black foils previous exposed to outdoor weathering for 90 days (55 %) and finally by the PLA/PHB black foils (42 %) (Table 1). The microorganisms present in the soil, and potentially involved in the biodegradation of the films, were identified by using culture-dependent methods (i.e., microbial strains were cultivated in three different growth media containing PLA/PHB blend, PHB or PLA) and culture-independent methods (i.e., 16S rRNA gene analysis by denaturing gradient gel electrophoresis (DGGE) and cloning). This strategy allowed the identification and isolation of several PLA/PHB blend degrading microorganisms assigned to several genera, including *Bacillus*, *Streptomyces*, *Rhodococcus*, *Saccharothrix*, *Fusarium*, *Trichoderma*, and *Penicillium*. This approach can be useful to get insights into the biodegradation process because the microorganisms are the main responsible for the biodegradation of the plastics.

The biodegradation of PHBV/wood flour (WF) was more significant in the laboratory (PHA50WF -35 %, PHA20WF - 36 %) using ASTM D5988 than in the field study. Chan et al. [67] explained this result due to the higher moisture content of the soil laboratory test, which has been shown to accelerate the biodegrada-

tion of both wood and PHA. But contradictory results demonstrating higher biodegradation of PHB in garden soil than in laboratory settings (using the same soil) have also been reported [71].

As previously indicated, the temperature can significantly influence the biodegradation process and PLA/PHA mulches degraded more extensively in the soil during the summer than in winter-time because the warmer temperatures promoted microbial activities [115].

In conclusion: the rate of biodegradation in soil is influenced by several factors, including the properties of the PHA material such as crystallinity, surface area, type of PHA, composition, and shape, the environmental factors such as temperature, moisture level, pH, and nutrient supply, the microbial communities and the activity and specificity of microbial depolymerases. These factors interact, creating different soil environments and different biodegradation potentials from place to place, from season to season.

5. Microorganisms involved in PHA biodegradation

Microorganisms are the main responsible for the biodegradation of PHA-based plastics in several ecosystems. Biodegradation depends on the existence of PHA degrading enzymes (PHA depolymerases) produced by microorganisms that can hydrolyse water-insoluble PHA into water-soluble forms, so it can be used by these microorganisms [116]. In soil, differences in the rate of PHA biodegradation can be due to several factors, including the composition of the microbial communities and to type and specificity of the depolymerases that they produce [31].

Almost 600 PHA depolymerases with different substrate specificities have been identified in various microorganisms [117]. Among intracellular and extracellular depolymerases and through the analysis of their sequences, they were classified into 8 superfamilies and in 38 homologous families [117]. Several bacteria capable of PHA biodegradation are assigned to genera: *Stenotrophomonas*, *Alcaligenes*, *Comamonas*, *Rhodococcus*, *Rhodocyclus*, *Variovorax*, *Acinetobacter*, *Pseudomonas*, *Bacillus*, *Xanthomonas*, *Syntrophomonas*, *Ilyobacter*, and *Ralstonia* [36,50,118].

In several studies, fungi were able to biodegrade PHA polymers and were the dominant microorganisms colonizing the surface of the polymer [57,119]. Fungi have higher biodegradation capability compared to bacteria because their PHA-depolymerases have higher mobility [120]. Several groups of fungi, including ascomycetes, basidiomycetes, deuteromycetes (e.g., *Penicillium simplicissimum*) zygomycetes, and micromycetes (e.g., *Penicillium*, *Paezilomyces*, *Acremonium*, *Verticillium*, and *Zygosporium*), among others, have been identified as capable of degrading PHA polymers [50,121].

A rough surface allows better adhesion of the microorganisms and water, which accelerates the biodegradation of the polymers. A smooth surface decreases adhesion, which delays the biodegradation process [114]. The lag phase preceding the biodegradation of PHA films in soil may take days, weeks, or even months, depending on the composition and shape of the PHA and on the environmental conditions. This lag period is the time needed for microbial adhesion to the material's surface and for expression and release of extracellular depolymerases [31]. Usually, a higher degree of crystallinity decreases microbial degradation, while the amorphous regions are easily degraded [122]. In analogous ecosystems, in different regions, the biodegradability of PHBV is related to the numbers of PHBV degraders and is dependent on the growth conditions for PHBV degraders [83,123,124]. Soil microcosms with higher functional diversity present better PHA biodegradation capacity than soil microcosms with lower functional diversity [94,103].

It has been verified that the percentage of true PHA-degrading microorganisms is relatively low in the microbial community colonizing the surface of the polymer [50,64]. Other types of microor-

ganisms (commensal organisms) may develop on the surface by growing on intermediate compounds of PHA biodegradation, such as oligomers, monomers, acetoacetate, and other compounds produced by the true PHA degraders [31]. The isolation of the PHA degraders from the non-PHA degraders can be accomplished by collecting the microbial biofilms developed on the surface of the polymers, followed by cultivation on typical microbiological media containing PHA as the unique carbon source. This strategy is useful and widely used and is designated by the “clear zone technique”. Clear zones around the colonies of microorganisms with PHA-depolymerase activity will develop and indicate PHA biodegradation [31,102,125]. The microorganisms forming these colonies can be further cultivated and identified.

Interestingly, some microorganisms can degrade several types of PHA in soil, but others can only degrade a specific PHA type. Volova et al. [31] use the clear zone technique together with molecular-genetic methods (rRNA gene sequence) and found that PHB was degraded by bacteria of the genera *Mitsuaria*, *Chitinophaga*, and *Acidovorax*, but they were not detected on the surface of the copolymers PHB/4HB, PHB3HHx, and PHBV. *Roseateles depolymerans*, *Streptomyces gardneri*, and *Cupriavidus sp.* were specific degraders of PHB/4HB, *Roseomonas massiliae*, and *Delftia acidovorans* degraded PHBV, and *Pseudoxanthomonas sp.*, *Pseudomonas fluorescens*, *Ensifer adhaerens*, and *Bacillus pumilus* degraded specifically PHB3HHx [31]. *Streptomyces* were capable of degrading all PHA polymers [31]. Some microorganisms can produce several types of depolymerases, and thus have a broader range of PHA biodegradation potential, while others only produce one kind of depolymerase capable of PHA biodegradation [102].

6. Conclusion

Several factors can influence the PHAs biodegradation in soil, including environmental conditions, the properties of the materials, and the presence of PHA-degrading microorganisms, which are the key factor to achieve biodegradation. The blend of PHAs with other types of materials makes these polymers more competitive and allows their application in several areas. However, testing and validating the biodegradation of those new blends is necessary to determine if they meet the environmental requirements. In order to understand the biodegradation process in soil, it is essential to conduct laboratory and field tests in different conditions and control or evaluate all the factors that can influence the process. It is also fundamental to identify the microorganisms and the enzymes in those environments since they have different specificities and biodegradation capabilities.

Declaration of Competing Interest

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

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