

# Polyhydroxyalkanoate (PHA) Bio-polyesters – Circular Materials for Sustainable Development and Growth



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doi: <https://doi.org/10.15255/CABEQ.2022.2124>

Review

Received: July 18, 2022

Accepted: December 16, 2022

Achieving circularity in materials requires fundamental changes in the polymers we use today and the way they are produced. Functional polymeric materials from renewable feedstocks that do not conflict with food and animal feed, and their renewal through biodegradation under diverse environmental conditions as the desired end-of-life option indeed constitute a paradigm shift for today's plastics industry. Considering the ever-increasing environmental problems associated with the disposal or incineration of fossil plastics, the increasing microplastic formation, food contamination, and rising atmospheric CO<sub>2</sub> concentrations, have made it clear that the time is ripe for alternative, innovative, and sustainable polymers with plastic-like properties. In this nexus, the present review shines new light on the benefits of biobased and, at the same time, biodegradable microbial polyhydroxyalkanoate (PHA) biopolyesters. Special emphasis is dedicated to carbon recyclability through biodegradability and compostability of these fascinating natural materials, which are slowly but surely being commercialized as replacement for fossil plastics that are produced and disposed of in multi-million-ton scale annually, resulting in a growing environmental threat. This review highlights that end-of-life options of PHA are analogous or even superior to another well-known polymer from nature, cellulose, while PHA offer the additional attributes of plastics in use with tailor-made properties. Finally, the review demonstrates how PHA biopolyesters can contribute to reaching many of the heavily discussed and desired UN Sustainable Development Goals.

## Keywords:

biopolymers, biodegradable polymers, circular bioeconomy, circular materials, microbial biopolyesters, microplastics, polyhydroxyalkanoate, UN Sustainable Development goals

## Introduction

Almost all full carbon backbone (“carbon–carbon backbone”) plastics of fossil origin are highly resistant to bio-mediated degradation. The current practice of plastic production and disposal is generating enduring impairment to the marine and terrestrial environment<sup>1</sup>. Estimates of global emissions of plastic waste to rivers, lakes, and the ocean range from 7 to 9 million metric tons per year, with a similar amount emitted as carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) into the atmosphere as of 2016. These quantities are forecasted to double by 2025. Noteworthy, almost 80 % of all conventional plastic ever made is still present on our planet, amounting to roughly 6.3 Gt as of 2016<sup>2</sup>. Pollution by plastic

waste accumulating in the environment is a “hardly reversible” development, as natural mineralization processes for such materials are extremely slow. While reports on biological degradation of the polyester poly (ethene terephthalate) (PET) exist<sup>3,4</sup>, such processes are far from industrial realization, and do not apply to highly durable polyolefins like poly (ethene) (PE), poly (propene) (PP), poly (vinyl chloride) (PVC), or poly (styrene) (PS); these polymers fragment under diverse environmental conditions (solar irradiation, abrasion, etc.) to nano- and microparticles, but do not undergo biodegradation<sup>5</sup>. Despite having brought numerous benefits to society, fossil plastics have a dark side: “Plastics are Forever”<sup>6</sup>!

In addition, engineered remediation solutions (chemical and mechanical recycling), though needed, are still embryonic, inadequate or insufficient to handle the steadily growing volumes of plastic

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waste<sup>7</sup>. Hence, negative consequences of plastic pollution are almost irreversible. Potential impacts from hardly reversible fossil plastic contamination encompass, *inter alia*, changes to the global carbon cycle, deterioration of soil quality, disturbed aquatic ecosystems, and negative consequences to human health. These impacts coincide with threats to biodiversity, which ultimately is connected to severe societal impacts<sup>8,9</sup>.

On the raw material side, we currently witness unstable geopolitics, resulting in disrupted global supply chains causing increasing supply impairment of fossil resources required for production of established fossil plastics.

The combination of negative environmental impact of fossil plastics and the disruption to their primary feedstocks provides society the ideal opportunity for step change to move towards alternatives<sup>10</sup>. These alternatives utilize local, renewable, and waste carbon sources to produce materials of value: Materials that at their end of life are recycled (the material and the carbon) without causing the societal and environmental impacts of fossil plastics. The two principles – use of renewable/waste/local carbon feedstocks to produce materials of value and the ability to recycle them (material as well as carbon) – without disrupting nature need to be at the core of any step change model<sup>11</sup>.

This review, for the first time, combines the key features of polyhydroxyalkanoate (PHA) biopolyesters, namely, their bio-based origin, their biosynthesis, and their appealing end-of-life options in a holistic way, putting special emphasis on circularity aspects of these materials. In addition, it shines new light on the potential of PHA to solve the current microplastic predicament, and attempts to remove widespread contradictions and incorrect assumptions on the topical subject of “bioplastic”. As a unique feature, the article comprehensively compares circularity of PHA with the prototype natural circular material, namely cellulose, thus demonstrating that PHA is indeed embedded into nature’s closed cycle from every criterion imaginable. The authors further endeavor to demonstrate that switching from fossil plastics to PHA biopolyesters can contribute to achieving the UN Sustainable Development Goals (SDGs) as long as PHA is produced from renewable carbon sources as it is prevalent in nature.

### **Fossil plastics accumulation and persistence – implications for the future**

Global fossil plastics production is estimated at 400 million tons of virgin plastic per year and is expected to reach 1 Gt per year in 2050<sup>2</sup>. Typically, most of this fossil plastic is disposed as macroplastic

waste in landfills and in the environment, which, through erosion, abrasion, and other weather-related acts would turn into micro- and nanoplastic particles<sup>12</sup>. Such nano- and microplastic particles are also generated during the use of the fossil plastics by abrasion during diverse industrial processes, from vehicle tires, and even from shoe soles. Two types of plastic particles were thought to be created; they are defined as “macroplastics” for particles of a size of more than 5 mm, “microplastics” or plastic particles of less than 5000  $\mu\text{m}$  (5 mm) in size. More recently, it was discovered that a third category, defined as “nanoplastics” are also formed, having particles as small as 1–100 nm<sup>13</sup>. Nanoplastic and microplastic particles have the appropriate size to enter the food chain starting with planktons which ingest them. Plankton serves to nourish higher animals like fish, and the nanoplastic and microplastic particles finally end up on our table<sup>14,15</sup>. According to a study by Wilcox *et al.*, plastic particles are present in the intestines of about 90 % of investigated seabirds from 186 different species<sup>16</sup>. It is important to note that not all micro- and nanoplastic particles taken up via nutrition and respiration leave our bodies: A recent study estimates that until the age of 70 we accumulate about 50,000 plastic particles 1–10  $\mu\text{m}$  in size, corresponding to about 40 ng, in our tissue<sup>17</sup>.

Mechanical plastic recycling, a process extolled by plastics manufactures, especially in the field of beverage bottling, is an excellent source of microplastic formation: A study conducted by Schymanski *et al.* demonstrated that mineral water sold in plastic bottles contains microplastic particles consisting of PET, the same material as the bottle itself; the more recycling cycles the material undergoes the higher the quantity of microplastic particles found in the beverage packaged in the plastic bottle<sup>18</sup>. This was illustrated by Cox *et al.*, who calculated that the recommended daily intake of 1.5 – 2 L water, if bottled in plastic, causes the consumption of 90,000 microplastic particles per year per person<sup>19</sup>. In addition to mineral water, beer connoisseurs should also be warned by a study by Liebezeit and Liebezeit, where authors described that all investigated samples of German beer display microplastic pollution; between 16 and 254 particles per liter on average that include fibers, granules, and other fragments, were found per liter of beer<sup>20</sup>.

Surprisingly, microplastic particles are found in many other food products, where we would not expect their occurrence *a priori*. A study by Peixoto *et al.* reported the detection of microplastic particles of different types and geometry in samples of table salt, both of marine (which is rather obvious considering the discussed marine plastic pollution) as well as of alpine origin, which was rather unexpected<sup>21</sup>.

Detailed studies demonstrated microplastic fallout in most remote areas in the Pyrenean mountains, far away from any civilization or industrial activity, which in turn proves the ease by which such lightweight particles are transported not only by fresh and marine water<sup>22</sup>, but also by wind and clouds<sup>23</sup>. More recently, a study reported the detection of microplastics in the polar regions (both Arctic and Antarctic)<sup>24</sup>, which shows that microplastics are already omnipresent in the ecosphere and the biosphere.

Only in the last few years, we have started to understand the effects caused by microplastic particles in animals and humans. The intestinal toxicity of microplastic fibers of different geometry and length is already well described for the zebrafish *Danio rerio*, a model organism to study toxicity of microplastics<sup>25</sup>. For this species, it was recently shown that exposure to microplastic prevents sufficient nutrients uptake<sup>26</sup>. In 2019, it was shown that plastic particles are found ubiquitously in the human digestive tract<sup>27</sup>. Now, in 2022, we even have proof that microplastic circulates in our bloodstream (1.6 µg of microplastic particles  $\geq 700$  nm, mainly PE, PET, and PS, was found per mL of blood taken from test persons), and evidence that this *in vivo* presence of microplastic causes devastating effects like inflammation and cancer<sup>28</sup>.

Besides the negative effects of microplastic formation due to plastics use, recycling of recalcitrant plastics is only a partial remedy to get rid of plastic waste: Recycling decreases the quality of the plastics, and the spent plastic after one or two recycling steps turns into real waste. Hence, mechanical recycling of plastics serves to only postpone the waste problem but does not constitute a remedy. Chemical recycling has been promoted as a solution to this issue, whereby the monomers or even the carbon could be reclaimed to upgrade into polymers and plastics for reuse. These processes have been known since the early days of polymer synthesis and production. While considerable research and scale-up efforts are underway here, most are still in their infancy. Moreover, chemical recycling is severely challenged by the mixed plastics waste streams that are available today, making the job of separating various monomers difficult and uneconomical<sup>1,2,7</sup>. In addition, chemical recycling involves energy-intensive processes, and while one can use renewable energy, this, in itself is emerging, and we are far from an ideal renewable energy use state.

To get rid of spent plastic waste completely, it needs to undergo thermochemical conversion into energy (incineration), which in turn increases atmospheric CO<sub>2</sub> concentrations, thus fueling global warming. This global warming has a momentum of its own: The warmer the oceans get, the less CO<sub>2</sub>

they can fix in a dissolved form. Importantly, the oceans serve as a CO<sub>2</sub> sink for roughly  $4 \cdot 10^{13}$  tons of carbon, about 60 times the carbon amount of the pre-industrial atmosphere! Oceans fix about 25–30 % of the CO<sub>2</sub> generated by anthropogenic activity, summing up to an excess of about 1,300 Gt CO<sub>2</sub> per year. Thus, they are less effective in counteracting the increase in atmospheric CO<sub>2</sub> level, which ultimately leads to a vicious spiral, in which the rise in ocean temperature and CO<sub>2</sub> emissions from the oceans fuel each other<sup>29</sup>. Despite the lower solubility of CO<sub>2</sub> in oceans at higher temperature, the entire quantity of CO<sub>2</sub> dissolved there has elevated due to the higher overall amounts of CO<sub>2</sub>, which are exchanged between the atmosphere and the oceans; this, in turn, leads to an acidification of the oceans by a pH-value drop of about 0.1 since the onset of industrialization, a rate that is 10 times faster than during the last 300 million years. The impact of this ongoing acidification on the ecosystem and its socioeconomic consequences cannot be even roughly estimated at the moment<sup>30,31</sup>.

Hence, the only rational response to all the global threat posed by heavily aggravating and poorly reversible plastic pollution is to rapidly reduce plastic emissions through reduction in consumption of virgin plastic materials, replace them where necessary by using biobased and biodegradable alternatives, along with internationally coordinated strategies for waste management<sup>8</sup>.

### Alternatives and their benefits: so-called “bioplastics”

Estimates published by European Bioplastics for 2021 report a global “bioplastics” production of about 2.42 million tons per year, which is by far less than 1 % of the entire global plastic production<sup>32</sup>.

It is important to note that this quantity also includes (partly) biobased, but not biodegradable/compostable materials like biobased PE (“bio-PE”), a material originating from fermentatively generated ethanol; “bio-PE” does not biodegrade in the environment, and is produced from food carbon sources such as cane sugar. The global production of “bio-PE” already amounts to about 230 kt per year, and its consumption is expected to more than double by 2026. Another example of “bioplastics” is “bio-PET”, a material used by famous beverage producers to manufacture “plant bottles” or “green bottles” despite only 30 % of the carbon present in them being of biological origin (ethylene glycol stemming from ethanol via oxirane), while terephthalic acid, the aromatic co-monomer in “bio-PET”, is produced from fossil resources. “Plant bottles” or “green bottles” made of “bio-PET” are advertised to be readily recyclable, while this process leads to in-



creased recalcitrant PET-microplastic formation, as observed for its fossil version. Currently, about 35.8 % of all materials termed “bioplastics” account for such biobased, but not biodegradable materials like “bio-PE” and “bio-PET”. This clearly demonstrates that using the term “bioplastics” in their context is nothing more than greenwashing, misleading the consumer, and continuing along the same environmentally destructive path. For the sake of differentiation, the authors of present review would like to term those “bioplastics” that are identical molecules to fossil plastics but use partly or entirely renewable carbon sources, but are neither biodegradable nor compostable as “Type II Bioplastics”

About 64.2 % of today’s “bioplastics” account for materials like PHA, poly (lactic acid) (PLA), poly (butylene adipate terephthalate) (PBAT), poly (butylene succinate) (PBS), poly ( $\epsilon$ -caprolactone) (PCL), thermoplastic starch (TPS), polysaccharides, and various cellulose-based materials and their blends. This group of “bioplastics” have their origins and attributes so diverse that it likely prompted the use of the word “bioplastics”. The authors would like to define these materials as “Type I Bioplastics”. PBAT, PLA, and TPS are the current market leaders, amounting to 19.2 %, 18.9 %, and 16 %, respectively, of the entire “bioplastic” market in 2021. For microbial PHA biopolyesters, a current share of less than 2 % of the bioplastic market is reported, accounting for a global production for 2021 of not more than an estimated 40,000 to 50,000 tons<sup>32</sup>. Among the second group of “bioplastics” some are of entirely biological origin – PHA, PLA, TPS, polysaccharides, and cellulotics. PBAT is industrially compostable, but does not degrade during anaerobic digestion. It undergoes biodegradation in soil, albeit at rather low rates. In addition, PBAT is not marine biodegradable, and its required raw materials (terephthalic acid, adipic acid, 1,4-butane diol) are currently produced from fossil raw materials. The aliphatic copolyester PBS is biodegradable in both fresh and sea water, while its raw materials are only partly accessible from biological processes: succinic acid is produced by anaerobic fermentation using biological carbon, while 1,4-butandiol is a fossil-carbon product. PCL is a fossil carbon-based product although it is biodegradable and compostable. Cellulotics, TPS, and polysaccharides are biological in origin, and although they are all chemically modified, they biodegrade in nature. However, this group has limited commercial use due to their property profiles; nonetheless, they are commercially produced. Lactic acid, the monomer for producing PLA, is of biological origin, although the subsequent polymerization of lactic acid into PLA is a chemical synthesis process<sup>33</sup>.

The diversity of products, product origin, and end-of-life profiles of this second group of “bioplastics” is perhaps the reason for the term “bioplastics”, an offshoot of the term “biopolymer”. “Biopolymer” is defined as a polymer that is biosynthesized from biological carbon and is biodegradable in the environment including in the marine environment. The term “bioplastics” cannot claim to have the same meaning, since it encompasses products with such diversity. Therefore, it is dangerous and misleading, especially to the layperson and to the consumer to use a term like “bioplastics”, as it gives the impression that materials termed as such are biosynthesized and biodegradable when they are not. Some are of biological origin – PHA, PLA, TPS, polysaccharides, and cellulotics. PBAT is produced from fossil origin monomers, PBS has one monomer that is biobased, although its comonomer is also being produced from biological carbon in limited quantities. Therefore, the so-called “bioplastics” like PBAT, PBS, or PCL are not circular materials.

However, the situation is expected to change fundamentally very soon. European Bioplastics expects the entire “bioplastic” production to more than triple in volume between 2020 (about 2.1 million tons) and 2026 (about 7.6 million tons). The “bioplastics” trend is here to stay and it will even increase! PBAT is anticipated to take 30 % of the volume produced until 2026, second being PBS (16 %), and significantly overtaking PLA (10.4 %). PHA, a truly biological and circular material, most likely will rank among the top 5 “bioplastics” in 2026, with an estimated market share of 6.4 % of all “bioplastics” (approximately 0.5 million tons), a tenfold increase in just five years! Importantly, for 2026, it is also estimated that PHA will be produced at higher quantities than the recalcitrant biobased polyolefin “bio-PE”<sup>32</sup>.

### **Eliminating microplastics: Follow nature’s cycle of life – biodegradation**

Industrially and commercially relevant biopolymers to replace established thermoplastics and elastomers are of growing interest for the scientific community as well as the Industry<sup>34–36</sup>. For the obvious reason, their production and entire life cycle are embedded into nature’s closed material cycles. Being based on renewable resources, which ultimately stem from the photosynthetic fixation of CO<sub>2</sub> by green plants, cyanobacteria, or algae, their production requires no depletion of fossil resources fixed in Earth’s interior. In the case of microbial PHA biopolyesters, the most essential and diverse class of biopolymers with plastic-like properties, conversion of these renewable resources (carbohydrates, lipids, alcohols, CO<sub>2</sub>, or CH<sub>4</sub>) occurs *in vivo*

– inside the microbial cells as intracellular products of the cell's secondary metabolism; hence, such cells serve as “cellular bioplastic factories”<sup>37,38</sup>. From microbial biomass, these intracellular products are isolated using multiple available strategies, refined, and processed into vendible materials using the same techniques and machineries as are currently used for the processing of fossil plastics<sup>39</sup>. The PHA biopolymers, when spent, can undergo end-of-life scenarios fundamentally different from the fate of spent fossil plastics: Under aerobic conditions, they are mineralized by the enzymatic toolbox of diverse organisms (bacteria, fungi) towards CO<sub>2</sub> and water as the sole products of their oxidative breakdown and biomass that is being used as organic fertilizer. CO<sub>2</sub> and water, in turn, are the starting materials for regeneration of the raw materials needed for PHA production (carbohydrates, lipids) by phototrophic organisms, closing the carbon cycle, thus balancing carbon! In case of anaerobic treatment of spent PHA, as it is the case in biogas plants, CH<sub>4</sub> is also generated. This renewable CH<sub>4</sub>, sometimes also called renewable natural gas (RNG), in turn, not only serves as potential green energy carrier, but is accepted by a number of microbes (predominantly type II methanotrophs) as substrate for biomass growth and PHA accumulation, again closing the carbon cycle<sup>38</sup> (see Fig. 1). This is in contrast with linear fossil plastics, which, after use, remain on Earth as macro-, micro-, and nanoplastic waste, or are thermally converted to surplus CO<sub>2</sub>.

The circularity of PHA biopolyesters is already being commercially exploited in many uses, including for replacing intentionally added fossil microplastics in cosmetics to offer UV protection (sun screen), and in skin peeling and scrubbing<sup>40</sup>. This light scattering effect of such products emulates living cells harboring PHA granules: here, microbes are protected by the PHA inclusion bodies against excessive UV exposure, known to cause fatal cell damage by formation of reactive oxidative species (ROS)<sup>41</sup>. When intentionally added, PHA microparticles in shower gels and in cosmetic peeling and scrubbing agents are released into the environment, such as in sewage and wastewater treatment plants, lakes, rivers and in the marine environment, they undergo biodegradation, unlike fossil microparticles which remain recalcitrant and persistent microplastics causing excessive microplastic loads in sewage sludge generated in wastewater treatment plants. Wastewater treatment plants filter up to 99 % of the microplastic from domestic wastewater, but with PHA microparticles this would not be needed. Sewage sludge is an excellent organic fertilizer<sup>42</sup>; however, currently they contain significant microplastics, which end up in agricultural fields when used as fertilizer. Replacing fossil microparticles with

PHA microparticles would allow the PHA microparticles in sewage sludge to readily biodegrade in fields by the microflora present there. The presence of PHA microparticles has an added benefit, especially in sewage and wastewater treatment plants. PHA, while undergoing biodegradation, converts the high nitrogen content present in sewage sludge into nitrogen gas, a process also called “denitrification”, thus aiding in improved sewage treatment<sup>43</sup>.

Products containing PHA microparticles as an additive are already on the market, e.g., “Naturetics™” products by Nafigate Cooperation, which were launched in Czech Republic in 2021, and are based on the Hydal technology to produce PHA from waste cooking oil<sup>44</sup>. Considering current and expected legislative regulations, such as the amendment to the REACH Regulation on Intentionally added Microplastics, The European Green Deal or the new Circular Economy Action Plan by the European Commission, which aim at drastically reducing microplastic release into the environment<sup>45</sup>, it is expected that biodegradable PHA granules would soon replace primary fossil microplastics in many more technological applications.

Other “Type I Bioplastics” such as PLA, PBS, and PBAT, form bioplastics that are recalcitrant and persistent; however, their lifetime as microplastics is generally accepted to be shorter than the non-biodegradable fossil plastics. Large pieces of items produced from these “bioplastics” would disintegrate into smaller particles forming microplastics and microparticles from these “bioplastics” (“biomicroplastics”) and would persist in nature. Their persistence as “biomicroplastics” is being studied, and the academic and the industrial communities need to overcome present rudimentary and incomplete data situation on the formation rate of microplastics, their degradation rate, sorption behavior, and eventual detrimental effects. This will remain a central field of research in the near future, especially by the manufacturers of these materials, primarily due to the aforementioned European legislation<sup>46</sup>. Present knowledge in this field, although undeveloped and mostly publicized by the manufacturers, indicates that microplastics created by materials such as PLA, PBS, PBAT would biodegrade sooner than fossil plastics and their microplastics.

### Definition and examples of biodegradable materials from nature

Prior to continuing the discussion on “biodegradable materials”, it is important to start with its definition and some related points. *Per definitionem*, “biodegradable materials” are a class of material, which can be degraded by the action of living systems like bacteria, fungi, animals, etc., or by isolated enzymes stemming from these organisms. Hy-

hydrolytic enzymes, be they in living cells or in isolated form (free or immobilized enzymes) are key for biodegradation of biopolymers, and biodegradable materials are the only ones that are defined as biopolymers. Important examples for such hydrolytic enzymes are hydrolases, recognizable by a four-digit enzyme commission (E.C.) number beginning with 3. Key representatives for hydrolases running biodegradation of polymers are presented and discussed in Table 1.

In the biodegradation of PHA, esterases play a key role. Among esterases breaking down PHA (“PHA depolymerases”), we need to differentiate between intracellular and extracellular PHA depolymerases. Intracellular depolymerases (i-PhaZ, E.C. 3.1.1.7x), on the one hand, serve the organisms accumulating PHA as storage material to re-utilize the stored PHA as a carbon and energy source during periods of starvation. On the other hand, extracellular PHA depolymerases (e-PhaZ) are those enzymes excreted by microflora (both prokaryotes and eukaryotes) to convert PHA that is released by PHA-accumulating microbes after cell lysis and spent PHA items into accessible substrates (Fig. 1). Hence, e-PhaZ are the enzymes accomplishing biodegradation of PHA materials in diverse environments! Typically, such e-PhaZ are less specific than i-PhaZ, and are able to hydrolyze the ester bonds in different polymers, such as in PLA<sup>54</sup>. *Nota bene*: There are no enzymes like intracellular PLA depolymerases; PLA is not produced as intracellular product by natural organism, hence, there was no need for Nature to develop such enzymes. This also

makes it clear why PLA is not a “biopolyester” *sensu stricto*: Only the monomer, lactic acid, is produced biologically, while its polymerization to PLA occurs chemically in the presence of expensive and often toxic catalysts. In addition, highly crystalline PLA shows slow biodegradation, which is catalyzed by unspecific esterases<sup>55</sup>. Indeed, PLA should really be regarded as xenobiotic rather than a biopolymer; it simply does not exist as a natural material. This contrasts with PHA, which is a biopolymer and a biopolyester, whose entire life cycle is biological: based on renewable raw materials, intracellular biosynthesis of both the monomers (hydroxy acids) and the polymers (PHA), and is biodegradable and compostable. Moreover, PHA is known as biocompatible, which means that it exerts no harmful effects on the environment and living organisms, while PLA, e.g., when applied *in vivo* as implant material, is reported to cause inflammatory reactions by the generation of highly acidic degradation products (lactic acid)<sup>56</sup>. In addition to polyester depolymerases, lipases (E.C. 3.1.1.x) are also an important class of esterases, pivotal for digestion of fats and oils. Different from other esterases, lipases are focused on lipophilic substrates like triacylglycerides<sup>57</sup>. Among the class of esterases, there are also enzymes able to hydrolyze PET, such as PETase (E.C. 3.1.1.101) isolated in 2016 from *Ideonella sakaiensis*, a bacterium that has adapted over the past half century to thrive on PET as the sole carbon source. However, large-scale application of such enzymes for PET biodegradation requires additional development due to their very low catalytic activity<sup>58</sup>.

Table 1 – Hydrolases responsible for biodegradation of diverse polymers

Glycosidases (polysaccharases; E.C. 3.2.x.x)	<p>Glycosidases are cofactor-independent enzymes, which catalyze the hydrolytic conversion of polysaccharides to mono-, di-, and oligomeric sugars.</p> <p>Important examples from nature are cellulases (E.C. 3.2.1.4, E.C. 3.2.1.21, and E.C. 3.2.1.91), which hydrolyze the <math>\beta</math>-1,4-glycosidic bonds in cellulose, the most frequently occurring polymer on Earth, which is essential as cell wall material of plants.</p> <p>Amylases, especially E.C. 3.2.1.1 (<math>\alpha</math>-amylase) and E.C. 3.2.1.2 (<math>\beta</math>-amylase), are well known as enzymes that hydrolyze starch<sup>47</sup>; we become aware of the result of the action of amylases when chewing old bakery products: They will become sweet in our mouth due to sugar formation!</p> <p>As another hydrolase, chitinase (E.C. 3.2.1.14) breaks down the glycosidic bonds in chitin, an important component of the cell walls of fungi and exoskeletal elements of many animals like mollusks or arthropods<sup>48</sup>. Chitin, the second most abundant natural polysaccharide, in turn is also an important raw material to manufacture biodegradable biopolymer films, and, together with its deacetylation product chitosan, is used to size and strengthen paper<sup>49</sup>.</p> <p>Finally, pectinase (E.C. 3.2.1.15) should be mentioned as the enzyme hydrolyzing the 1,4-<math>\alpha</math>-D-galactosiduronic bonds in pectin, an important structural polymer in the non-woody parts of plants; its hydrolysis by pectinase makes fruits to become mushy during ripening. Technologically, pectin is widely used, mainly in the food sector as a gelling and thickening agent, particularly in jams and jellies, and as dietary fiber<sup>50</sup>.</p>
Proteases (polyamidases; E.C. 3.4.x.x)	<p>This group of hydrolases (“proteolytic enzymes”) hydrolyses peptide bonds, such as those linking amino acids in proteins. In the contexts of “bioplastics”, they hydrolyze polymeric films made of gelatine<sup>51</sup> or processed whey retentate proteins<sup>52</sup>.</p>
Esterases (E.C. 3.2.x.x)	<p>Esterases are an essential group of hydrolases when it comes to biodegradation of biopolyesters like PHA or poly (lactic acid) (PLA); these enzymes catalyze the conversion of biopolyesters into hydroxycarboxylic acids<sup>53</sup>.</p>



The degradation products generated by the biocatalytic action of listed hydrolases in turn undergo conversion by diverse organisms using well-known catabolic pathways, such as the glycolysis pathway (Emden-Meyerhoff-Parnas pathway, fructose-1,6-bisphosphate pathway), or the 2-keto-3-desoxy-6-phosphogluconate (KDPG) pathway (Ender-Doudoroff pathway) to generate pyruvate, which finally undergoes decarboxylation to yield acetyl-CoA. Acetyl-CoA, in turn, is the central compound in the metabolism of organisms. PHA hydrolysis produces oligomers of hydroxyalkanoates, which are further depolymerized by oligomer hydrolases generating hydroxyalkanoic acid monomers. These are then converted to acetyl-CoA by the microorganisms<sup>59</sup>.

In aerobic processes, acetyl-CoA undergoes conversion in the tricarboxylic acid cycle, where it is oxidized to CO<sub>2</sub>, and reduction equivalents and energy are generated, beside electrons (bound to coenzymes), which react with molecular oxygen as the terminal electron acceptor in the respiratory chain. Biodegradation through this metabolic sequence is exactly what happens in PHA-producing microbes during cultivation in a nutritionally balanced medium. The degradation products of PHA biopolymers (sugars, amino acids, or hydroxy acids in the case of spent PHA as substrate) are converted by the cells as substrate for biomass and energy formation<sup>60</sup>.

The situation fundamentally changes when such PHA-producing organisms are exerted to environmental stress conditions, such as a deprivation of nitrogen or phosphate source, or low oxygen tension. Under such conditions, the tricarboxylic acid is blocked for acetyl-CoA, but PHA-accumulating organisms have an exit strategy to convert this intermediate compound: By the anabolic PHA enzyme toolbox, acetyl-CoA undergoes a condensation reaction to acetoacetyl-CoA (catalyzed by 3-ketothiolase, previously known as  $\beta$ -ketothiolase; E.C. 2.3.1.9)<sup>61</sup>, which is reduced to 3-(*R*)-hydroxybutyryl-CoA (catalyzed by the NADH-preferring acetoacetyl-CoA reductase; E.C. 1.1.1.36)<sup>62</sup>, serving as substrate for the enzyme PHA synthase (E.C. 2.3.1.304), which catalyzes the formation of the PHA homopolymer poly(3-(*R*)-hydroxybutyrate) (P(3HB))<sup>63</sup>. The metabolic rationale behind this PHA biosynthesis is the fact that redox equivalents (NAD<sup>+</sup>) need to be regenerated (hence, NADH needs to be oxidized) even under conditions of the TCC being inactive. NAD<sup>+</sup> regeneration in turn is needed to warrant further oxidative substrate breakdown, which is dependent on availability of redox equivalents in oxidized form (NAD<sup>+</sup>). The “pseudo-fermentation” reaction from acetoacetyl-CoA to 3-(*R*)-hydroxybutyryl-CoA, which converts NADH, provides a tool for NAD<sup>+</sup> regeneration<sup>59</sup>. Now, PHA

is accumulated as intracellular inclusion bodies (“PHA granules”, also called “carbonosomes”)<sup>64</sup>, which act as carbon- and electron sinks, serving the cells as storage for energy and carbon, and as protectant against various stressors (UV-exposure, hypo- and hypertonic stress, desiccation, heat, oxidation, heavy metal exposure, etc.), in addition to other metabolic and biological roles<sup>65</sup>. These “biopolymeric organelles” consist of a hydrophobic PHA core, surrounded by a hydrophilic layer made of enzymes involved in generation (PHA synthases), degradation (PHA depolymerases) and intracellular organization (phasins) of PHA<sup>64</sup>.

Intracellularly, storage PHA is degraded (“mobilized”) by previously discussed i-PhaZ depolymerases under conditions favoring intracellular PHA degradation (low energy charge of cells, typically under conditions of exogenous carbon limitation); hence, microbial cells harboring PHA have a survival advantage under conditions of starvation compared to PHA-negative cells. When present as extracellular PHA, such as in form of a spent PHA bioplastic item, e-PhaZ depolymerases degrade the material to accessible carbon sources (hydroxy acids), also for PHA accumulating strains, and the cycle of PHA biosynthesis and breakdown starts *de novo*<sup>54</sup>.

Fig. 1 provides a visualization of the previously discussed circularity of PHA in nature.

### **Biodegradation in the modern world – compost to recover carbon present in PHA biopolyester – biogas and organic fertilizer**

“Biodegradable” is frequently connected to environmentally friendly products, capable of decomposing back into those natural elements from which they were once produced. Therefore, by reasonably modifying and controlling (bio)degradation, biodegradable materials can play a key role in reducing ecological pollution<sup>66,67</sup>. Sustainable production of materials, like PHA that has such end-of-life options, easily comply with current policies and actions set by the European circular economy strategy and the 17 United Nation’s Sustainable Development Goals (SDGs), which are elucidated later in this discussion.

However, it is important to emphasize that the property “biodegradability” is not identical to “compostability”, although these two expressions are frequently used interchangeably. Both properties are defined in norms that form the basis for a commercialized polymeric material to reach established certifications. In Europe, such certification processes are regulated at the national level in cooperation with the European Bioplastics Industry Association. As a prime example, the European norm EN 13432, the standard norm to assess a “bioplastic” on a ho-

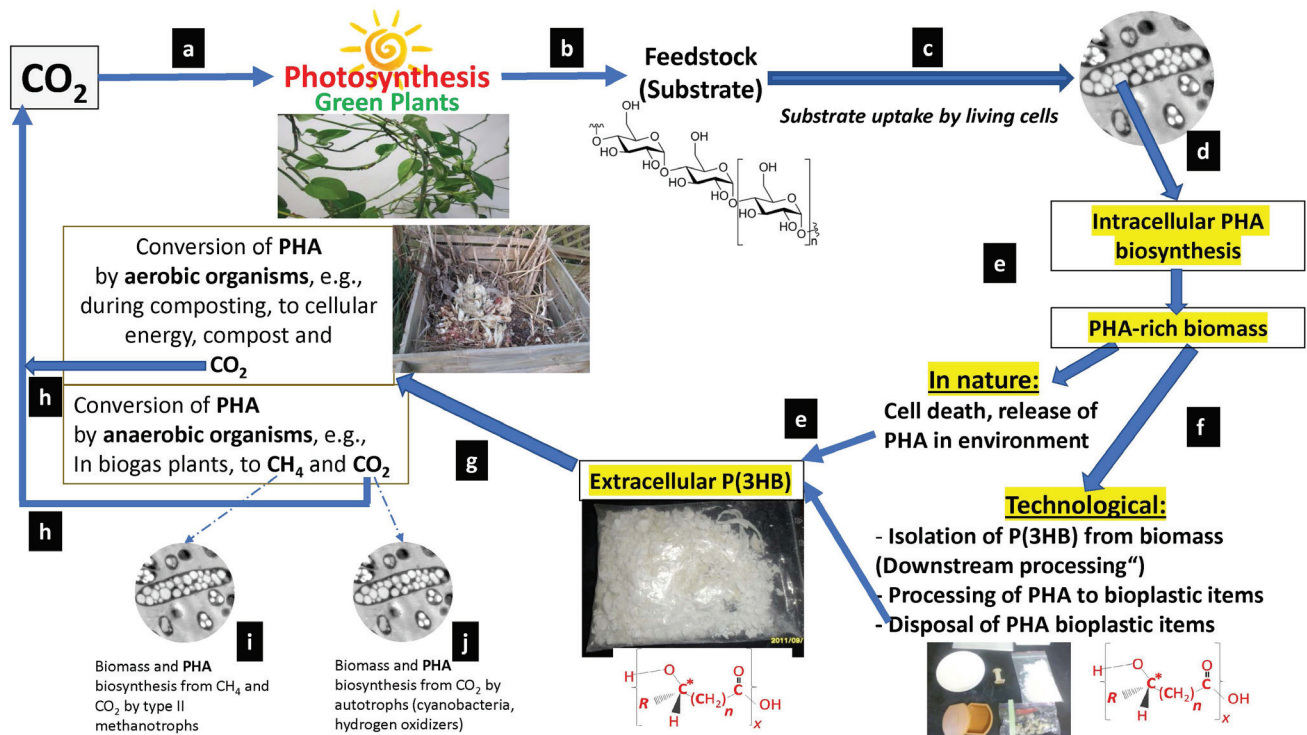


Fig. 1 – Circularity of PHA biopolyesters. (a) Photosynthetic fixation of CO<sub>2</sub> by green plants; (b) Formation of carbonaceous feedstocks (e.g., carbohydrates) by green plants based on photosynthetic fixation of CO<sub>2</sub>; (c) Feedstock (substrates) are metabolized by PHA-producing microbes; (d) Intracellular accumulation of PHA granules by microbes based on consumed substrates; (e) Release of PHA granules into the environment after cell death; (f) Technological downstream processing for recovery of PHA from cell biomass, followed by processing of recovered PHA to vendible bioplastic items, and disposal of PHA-based items after use; (g) End-of-life fate of disposed PHA-based items by aerobic biodegradation via composting (generates CO<sub>2</sub>) or anaerobic fermentation to CO<sub>2</sub> plus CH<sub>4</sub> in biogas plants; (h) CO<sub>2</sub> generated during these processes is again photosynthetically fixed by green plants: The cycle is closed! (i) Some type II methanotrophic microbes can directly metabolize CH<sub>4</sub> stemming from anaerobic PHA digestion to PHA; (j) Several autotrophic microbes like various cyanobacteria directly convert CO<sub>2</sub> stemming from PHA digestion to PHA.

lastic basis, addresses both biodegradability and compostability of packaging materials made of polymers. According to norm EN 13432, a “biodegradable” material has 90 % of its carbon metabolized under standardized conditions of humidity, temperature, and pH-value within 180 days. A “compostable” material, in turn, disintegrates within 180 days of composting into leftovers passing through a sieve of 2 mm pore size by at least 90 %. At that point, it should be stressed that all commercialized PHA biopolyesters meet the requirements for both “biodegradability” and “compostability” according to norm EN 13432<sup>67</sup>.

Composting, also known as “rotting”, describes a process of the natural nutrient cycle where organic materials (often containing high-molecular biopolymers) are aerobically disintegrated by heterotrophic soil organisms (bacteria, archaea, protozoa, fungi, etc.). In addition to CO<sub>2</sub>, water-soluble minerals are also released from composting of organic materials, such as nitrates, ammonium salts, phosphates, potassium, and magnesium compounds, which act as precious biological fertilizers. Moreover, finished compost can be used in multiple other ways, such as for mulch, amending soil, and

compost tea. Some of the intermediate products generated during this degradation process are converted into humus, which, in the scientific field of pedology, refers to all of the finely decomposed organic matter present in a soil sample<sup>67</sup>.

When discussing “compostability”, one needs to distinguish between “industrial composting” and “home composting”. In this context, norm EN 13432 refers explicitly and exclusively to the composting process in industrial composting facilities (“industrial” or “technical composting”). Here, conversion of biogenic materials to compost occurs by the controlled, aerobic, exothermic (with a thermophilic phase >55 °C) biological degradation and conversion. Such industrially compostable materials can undergo separate organic waste collection; subsequently, they can be converted in industrial composting facilities, or, alternatively in anaerobic digestion plants into the green energy carrier biogas and organic fertilizer or humus or compost. However, many materials disposed of in industrial composters and anaerobic digestors are not “home compostable”, hence, they should not be disposed in the private compost heap in the garden, where the process is not controlled, and is determined by the giv-



en environmental and weather conditions. Such “home composting” can be understood as upgrading household waste to produce compost at home. This process can be practiced at home with various environmental advantages, such as increasing soil fertility, reducing landfills, minimizing CH<sub>4</sub> contribution to the environment, and limiting food waste. Incidentally, PHA biopolymers are also home compostable, and as such, home composting of spent PHA items like packaging materials also reduces the amount of packaging in domestic waste<sup>67</sup>.

### Standards for the measurement of biodegradability and compostability of materials

Until 2020, no EU-wide standard for “home composting” was available, until the norm prEN 17427 (“*Packaging – Requirements and test scheme for carrier bags suitable for treatment in well managed household composting plants*”) was established. It refers to the end-of-life fate of plastic bags. Prior to the establishment of the EU-wide norm, several standards and corresponding certificates regulated home compostability of bioplastics on national levels, mainly based on EN 13432. For example, a home compostability certification scheme is offered by the certifier Vinçotte. Based on Vinçotte’s certification protocol, TÜV Austria offers the label “Ok compost HOME” for home compostable packaging, in addition to the label “OK biodegradable”; hence, two different certifications make a bioplastic material both “biodegradable” and “home compostable”. It is important to note that TÜV Austria’s “Ok compost HOME” certification must not be considered as a specific standard; it rather constitutes a list of all the technical demands made to a packaging material required to obtain this certification; it constitutes the basis of subsequent “home compostability” standards established in other countries. As an example, the Australian standard AS 5810 (“*Biodegradable plastics – Biodegradable plastics that are suitable for home composting*”) from 2010 is also based on TÜV Austria’s “Ok compost HOME”, while DIN CERTCO, in turn, provides a “home compostability” certification based on AS 5810. Italy has the standard UNI 11183:2006, which is another national standard for “*composting at ambient temperature*”, In 2015, the French Standard “*NF T 51-800 Plastics – Specifications for plastics suitable for home composting*” was introduced, which is also part of the DIN CERTCO certification scheme<sup>67</sup>.

Hence, several standards exist to inform whether a material is biodegradable and/or compostable under different conditions (aerobic/anaerobic, industrial/home, etc.). Those international standards prescribe the test schemes that need to be applied in

order to evaluate and determine the compostability and biodegradability of PHA, cellulose- or starch-based plastic-like materials. In general, those standards comprise the requirements to test parameters regarding the characterization of the material (e.g., chemical composition like the assessment of heavy metal levels), its disintegration ability, its aerobic biodegradation into CO<sub>2</sub>, biomass and water within a defined period (typically six months), anaerobic digestion for CH<sub>4</sub> and CO<sub>2</sub> formation, and ecotoxicity tests. Bioplastics certified according to EN 13432 can be recognized by conformity marks such as the “Seedling”, “OK compost”, or “DIN geprüft”. Standards and specifications have been developed by several authorities, such as the European Committee for Standardization (EN), the American Society for Testing and Material (ASTM), the International Organization for Standardization (ISO), or the British Standard Institution (BSI)<sup>67</sup>.

In this context, biodegradability and compostability of PHA biopolyesters have been scrutinized under diverse environments and test conditions, i.e., soil, water, marine, as well as industrial and home composting. PHA-producing companies have tested their PHA products according to corresponding standards by certain certification organizations in order to verify the claims of biodegradability and compostability of each product, and to obtain the respective logos and labels.<sup>36</sup>

### Comparing biodegradability and compostability of PHA and cellulose

Cellulose makes up nearly 50 % of the biomass synthesized on Earth by photosynthetic CO<sub>2</sub> fixation. Wood fibers and cotton are the most common cellulose sources. It makes up for about 90 % of cotton fibers and about 45 % of typical wood fibers<sup>68</sup>. Most cellulolytic, hence, cellulose-biodegrading, microbes belong to bacteria and fungi; in addition, some slime molds and anaerobic protozoa also have been reported to decompose cellulose. In cellulosic waste, such cellulolytic organisms often establish synergism with non-cellulolytic species; interaction between such different organisms finally results in complete degradation of cellulose, releasing CO<sub>2</sub> and water under aerobic conditions, and CO<sub>2</sub>, CH<sub>4</sub> and water under anaerobic conditions<sup>69</sup>. Therefore, it is important to compare the biodegradability and compostability of PHA with cellulose.

Composting of cellulosic leftovers is considered an effective strategy to manage such waste streams, in order to make them harmless and to tap their carbon content. Microbes capable of cellulose degradation have a well-equipped toolbox of different synergistically functioning enzymes of different specificities. Cellulases (E.C. 3.2.1.4 and E.C. 3.2.91) hydrolyze the β-1,4-glycosidic bonds of cel-

lulose. Endoglucanases (endo-1,4- $\beta$ -glucanases, EGs, E.C. 3.2.1.4) catalyze endohydrolysis of bonds, predominantly in amorphous regions of cellulose, and form new terminal ends, where cellobiohydrolases (exo-1,4- $\beta$ -glucanases, CBHs; E.C. 3.2.91) take action, which hydrolyze only from the non-reducing chain ends. Both types of enzymes can act on amorphous cellulose, while crystalline cellulose is efficiently degraded only by CBH enzymes. For both types of enzymes, cellobiose molecules, hence, 1,4- $\beta$ -glycosidically linked glucose dimers, are released. Cellobiose, in turn, is hydrolyzed by  $\beta$ -glucosidases (E.C. 3.2.1.21) into two glucose monomers. Such products of cellulose breakdown act as carbon and energy sources for both cellulolytic and non-cellulolytic organisms in environments where cellulose degradation occurs<sup>70</sup>. This generation of sugars from cellulose catabolism can be considered the basis of microbial interactions taking place in such environments<sup>71</sup>. For correct functioning, EG enzymes, CBH enzymes, and  $\beta$ -glycosidases need to be in a stable form in the exocellular environment; often, these enzymes form a ternary complex with cellulose. Best-studied cellulase systems are those of the mesophilic fungi *Trichoderma reesei* and *Phanerochaete chrysosporium* (reviewed by<sup>72</sup>), while species from the genera *Cellulomonas*, *Pseudomonas*, and *Streptomyces* are the best-studied aerobic cellulolytic bacteria<sup>73</sup>.

Noteworthy, around 5–10 % of natural cellulose is degraded under anaerobic conditions. The enzymatic system of anaerobic cellulose degraders evidently differs from the system described for aerobic fungi and bacteria. *Clostridium thermocellum*, an obligate anaerobic Gram-positive, spore-forming bacterium constitutes the best-characterized species among these organisms<sup>72</sup>.

For anaerobic cellulose breakdown, involved enzymes are organized into large functional entities, so-called “cellulosomes”, which are attached to the cell surface. This organization in “cellulosome” orients enzymes, especially their biocatalytic regions, in a way promoting maximum synergism among catalytic units. Due to the attachment of “cellulosomes” at the interface between the cell and the substrate (cellulose), which is water-insoluble, their hydrolysis products (such as cellobiose) enter the interior of bacteria via extended proteinaceous fibers, which exist between the cell and cellulose. It is noteworthy that these microbes need elevated temperatures for growth and cellulose biodegradation, which explains why the anaerobic cellulolytic processes play a minor role in natural cellulose biodegradation.

Several cellulolytic anaerobic organisms have been isolated from typical anaerobic habitats, such as compost, sewage sludge, soil, sediments, and an-

aerobic digestors. Other well-described anaerobic cellulolytic microbes encompass rumen bacteria, fungi, and protozoa<sup>72</sup>.

Park *et al.* investigated biodegradability of diverse cellulose products (linen, cotton, rayon, and cellulose acetate) by different methods, such as activated sewage sludge test (according to ASTM D 5209-92; CO<sub>2</sub> evolution measured during 24 days), soil burial test (according to AATCC Soil Burial Method 30-1993; 28 days in natural soil), and enzymatic (cellulase) hydrolysis. Appearance of samples was studied microscopically with the progress of degradation time. In addition, the changes of internal structure were analyzed from X-ray diffraction patterns. It turned out that the rate of biodegradability in activated sewage sludge-, soil burial-, and enzyme hydrolysis tests was highest in rayon, which was followed by cotton and cellulose acetate, while linen showed varying behavior: It showed best biodegradability in the soil burial test, while it exhibited lower biodegradability than rayon and cotton in other tests. In sewage sludge tests, about 27 % of rayon was degraded after 24 days, which was more than for cotton (about 21 %), linen (18 %), and cellulose acetate (10 %). Remarkably, cotton, despite its high hydrophilicity, showed lower biodegradability than rayon because of linen's higher crystallinity. From the correlation analysis between the properties of cellulose fibers and the biodegradability, moisture regains, and biodegradability were represented to be most closely correlated<sup>74</sup>.

To conclude, it can be stated that, although mechanistically diverse and catalyzed by different enzymatic machineries, biodegradation of cellulose and PHA, respectively, results in the same final products, and occurs within reasonable periods in both cases. Fig. 2 compares the degradation pathways for PHA and cellulose, both under anaerobic and aerobic conditions.

### PHA's place in the world of biodegradability and compostability

PHA, according to the discussed norms and certificates, comply with the norms for biodegradability in soil, fresh and sea water, industrial compostability, home compostability, and anaerobic digestion. This is confirmed by the strict certification processes to which many commercially available types of PHA were subjected<sup>38</sup>. However, not all types of PHA are prone to biodegradation to the same extent; there are several factors impacting biodegradability of PHA, such as:

– Environmental conditions (temperature, humidity, pH-value, and availability of diverse macro- and micro-nutrients, *inter alia* oxygen, for the degrading microflora)<sup>75–80</sup>,

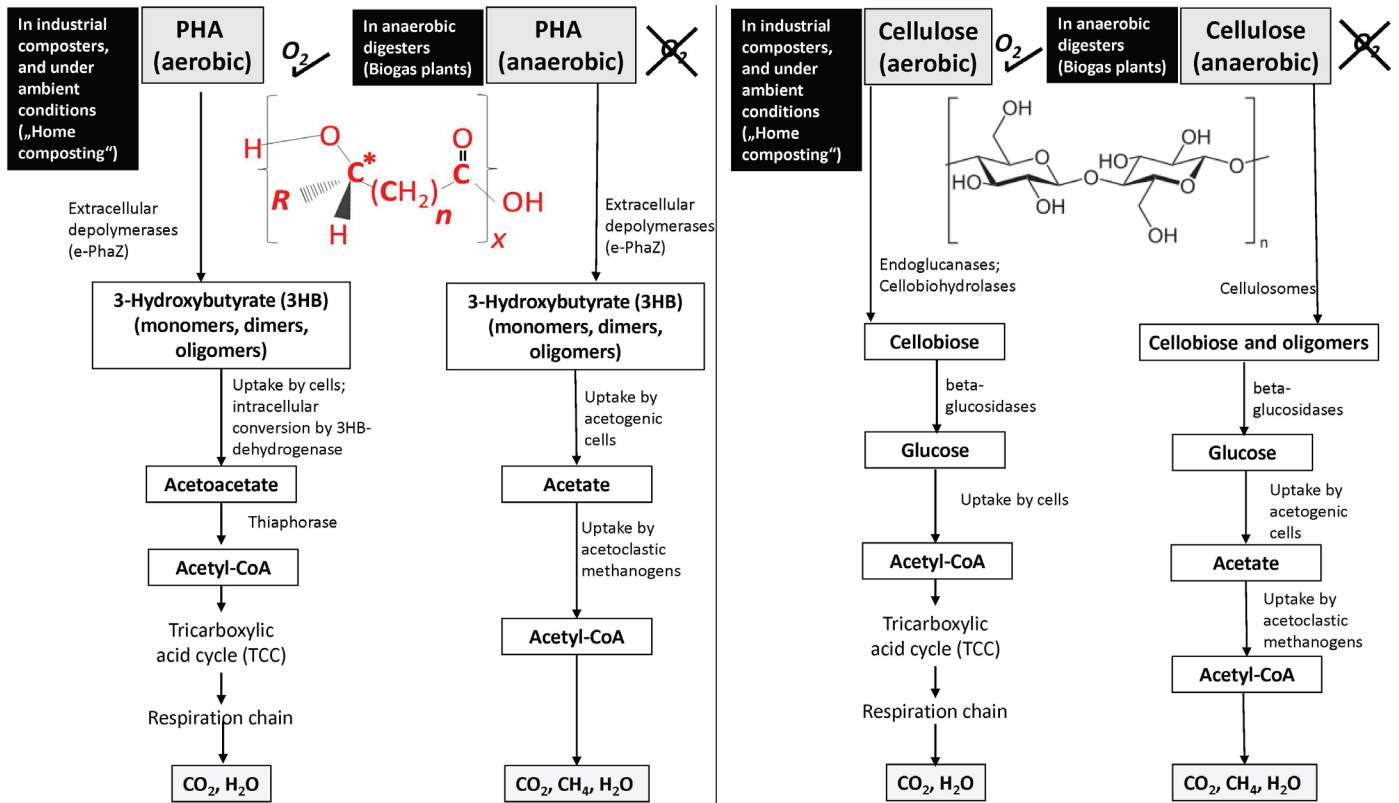


Fig. 2 – Comparison of biodegradation of PHA and cellulose under aerobic (industrial and home composting) and anaerobic (biogas plant) conditions

– The composition of the biopolyester on the monomeric level (PHA homopolyesters with highly structured crystal lattice like P(3HB) typically degrade slower than copolyesters)<sup>76,81–85</sup>,

– PHA's degree of crystallinity ( $X_c$ ; the higher the crystallinity, the slower the degradation rate; P(3HB) homopolyester, a rather crystalline material, reveals slower biodegradation than other members of the PHA biopolyester family with more amorphous regions)<sup>81–86</sup>,

– Molar mass (PHA polymers with lower molar mass typically are more prone towards biodegradation than those with higher molar mass)<sup>87</sup>,

– Stereo-regularity of the PHA biopolyester<sup>88</sup>,

– PHA surface area<sup>89</sup>.

Even the highly crystalline P(3HB) homopolyester is biodegradable and compostable. According to the German company Biomer, a globally leading P(3HB) producer, P(3HB) is “fully biodegradable” and compostable<sup>89</sup>. In terms of biodegradability, industrial P(3HB) homopolyester from Imperial Chemical Industries (ICI), UK, even outperformed Novamont's TPS-based composite material Mater-Bi®, and SK Chemical's (Republic of Korea) chemosynthetic biodegradable polymer SkyGreen®, a material consisting of succinic acid, adipic acid, butanediol, and ethane-1,2-diol, most of these compounds being of fossil origin. At different tempera-

tures (28, 37, and 60 °C), biodegradability of these materials was studied by burying thin sheets made of these materials in forest soil, sandy soil, activated sludge soil, and in farm soil. P(3HB) showed almost 100 % degradation (average mass loss of five parallel samples: 98.9 %) in activated sludge soil at 37 °C after only 25 days, while degradation in farm soil (68.8 % mass loss after 25 days), sandy soil (10 % mass loss), and forest soil (7 % mass loss) was considerably slower, again showing the high impact of the environment and microflora on PHA biodegradability. Importantly, no complete degradation was achieved for TPS (72.1 % degradation in activated sludge after 25 days at 60 °C as highest degradability), and SkyGreen® TPS (max. 69.1 % degradation in activated sludge after 25 days at 60 °C) at any condition<sup>75</sup>!

Choi *et al.* demonstrated the high impact of the degree of crystallinity and composition on biodegradation of extracellular PHA, namely, the bacterial copolyester poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P(3HB-co-3HV)) with different 3HV fractions; the renewable resources glucose (main carbon source) and the 3HV-precursor levulinic acid were used as feedstocks. The higher the 3HV fractions in P(3HB-co-3HV) copolyesters, the lower the biopolyesters' crystallinity, which in turn resulted in faster extracellular degradation in tests with fungal e-PhaZ enzyme solution. Importantly,



when carrying out these biodegradability tests without enzyme addition, the biopolyester samples were not degraded at all, which demonstrated the need for the appropriate microflora with the right enzymatic machinery for PHA degradation<sup>81</sup>. This matches findings by Kumagai *et al.*, who also demonstrated that P(3HB) homopolyester degradability is highly dependent on the degree of crystallinity. Incubation studies with extracellular bacterial depolymerase revealed faster degradation for PHA samples of lower crystallinity; importantly, during degradation, molar masses remained mostly stable, indicating that the attack by depolymerases first hit those P(3HB) chains in a polymer sample present in an amorphous state on the surface of the polymer specimen. Only after that, P(3HB) chains in the crystalline regions were depolymerized<sup>86</sup>. Hence, PHA depolymerization occurs in two main steps: first, adsorption of the PHA depolymerase's binding domain onto the PHA surface takes place, while in the second step, hydrolysis of biopolyester chains by the catalytic domain of the enzyme occurs. Polymer chain scission is initiated by endo-scissions, which occur randomly throughout the polyester chain, followed by exo-scissions from the ends of the polyester chain<sup>91</sup>.

In 1989, Kunioka *et al.* noticed that P(3HB-co-4HB) film samples with 0 to 37 mol-% 4HB displayed faster biodegradation in soil and activated sludge at lower crystallinity, caused by higher shares of 4HB units<sup>82</sup>. Later, P(3HB-co-4HB) films (0, 6, 10, 28, 85, and 94 % 4HB) were subjected towards degradation by extracellular PHA depolymerase at 37 °C and pH-value 7.5. Also here, the rate of biodegradation strongly increased with increasing 4HB fraction, which correlated well with decreasing crystallinity. However, samples with very high 4HB content (85 % and 94 %) degraded considerably slower than P(3HB) due to the hard susceptibility of the depolymerase enzyme to the 4HB moieties<sup>83</sup>. Only very recently, Vodicka *et al.* demonstrated that P(3HB-co-4HB) copolyesters with different 4HB fractions, obtained from cultivation of the thermophilic bacterium *Aneurinibacillus* sp. H1, had degraded faster in simulated body fluids (artificial gastric juice, simulated colonic fluid) than P(3HB) homopolyester. Importantly, degradation mechanisms differed between studied biopolyesters. While incubated in artificial gastric juice, samples were disintegrated due to fast hydrolysis caused by the low pH-value; here, degradation was caused by abiotic factors. Incubation in simulated colonic fluid, in contrast, resulted in biodegradation by enzymatic hydrolysis. These outcomes are pivotal for selection of PHA of defined composition for special *in vivo* applications<sup>76</sup>.

In 1992, Luzier showed that standardized injection-molten specimens of ICI's BIOPOL® P(3HB-co-3HV) products were completely degraded in seawater after 350 weeks; in anaerobic sewage, only 6 weeks were needed for complete degradation of such P(3HB-co-3HV) specimens. Complete degradation of these materials in estuarine sediments, aerobic sewage, and soil occurred within periods between 6 and 350 weeks. Again, without the presence of microflora, such as in humid or dry air, no degradation was observed, which is analogous to aforementioned findings for P(3HB) homopolyester. PHA biodegradation needs the appropriate surrounding!<sup>77</sup> In 2004, Rosa *et al.* studied biodegradation of P(3HB) ( $X_c$ : 72 %), and P(3HB-co-3HV) ( $X_c$ : 50 % crystallinity), obtained from the Brazilian company PHB Industrial Brasil SA (PHB/ISA), during about 10 months by mass loss of PHA samples. In soil composting medium at 46 °C and at 24 °C, as well as in a soil simulator containing *inter alia* manure, both types of PHA showed similar biodegradation rates<sup>78</sup>. In marine environment, Doi *et al.* studied degradability of P(3HB), P(3HB-co-3HV), and P(3HB-co-4HB) films during one year. Also here, it was shown that all polymers were degraded *via* surface erosion. Moreover, the temperature of seawater was more important for the rate of surface erosion than PHA's composition<sup>79</sup>. In this context, GreenBio's P(3HB-co-4HB) grade "Sogreen-00X" is "completely degraded into CO<sub>2</sub> and water at the environment of soil, rivers, sewage, and marine water in 3–6 months" according to manufacturer information<sup>80</sup>.

A detailed comparison of the biodegradability of thin solvent-casted films of P(3HB), P(3HB-co-3HV), P(3HB-co-20 %-3HV), P(3HB-co-40 %-3HV), and P(3HB-co-4HB), all of them manufactured by Ningbo TianAn Biomaterials Co. Ltd., PR China, was accomplished according to ISO 14855-1 under controlled composting conditions at 58 °C. This test method is based on the measurement of evolved CO<sub>2</sub> during biodegradation, with cellulose acting as biodegradable reference material. Already after five days, all tested PHA samples and the cellulose standard started to decompose. The order of biodegradability was P(3HB-co-4HB)  $\approx$  P(3HB-co-40 %-3HV) > P(3HB-co-20 %-3HV) > P(3HB-co-3 %-3HV) > P(3HB), which correlated well with the lower degree of crystallinity of copolyesters with higher comonomer fraction. After 110 days of testing, the final degrees of biodegradation amounted to 90.5 %, 89.3 %, 80.2 %, 90.3 %, and 79.7 %, and 83.1 % for P(3HB-co-40 %-3HV), P(3HB-co-20 %-3HV), P(3HB-co-3 %-3HV), P(3HB-co-4HB), P(3HB), and cellulose. In the broad context of PHA biodegradability and compostability, this is an important outcome; it shows that biodegradability of

most tested PHA copolyesters even outperformed cellulose, a well-known biological, biodegradable material, and biodegradation of the more crystalline P(3HB-*co*-3 % -3HV) and P(3HB) was almost as complete as for cellulose under the same conditions<sup>84</sup>.

Moreover, PHBH<sup>®</sup>, a poly (3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (P(3HB-*co*-3HHx)) copolyester, developed and commercialized by the company Kaneka, is “certified to biodegrade in seawater” and received the label “OK Biodegradable MARINE”, “OK Compost Industrial”, “OK Compost Home”, “OK Biodegradable Soil”, and “OK Biobased” by TÜV Austria, and the “Compostable” certification from the Biodegradable products Institute of the U.S. Composting Council<sup>92</sup>. According to information provided on Kaneka’s internet page, “PHBH<sup>®</sup> was faster degraded under marine conditions than PCL, PBSA (note: poly (butylene succinate-*co*-butylene adipate)), PBAT, PBS, and PLA (not degraded in seawater at all after 28 days)”. About 23 % of the material was completely vanished in marine environment after only 28 days. Importantly, PHBH<sup>®</sup> shows even better aerobic biodegradability in compost than cellulose, a well-known biodegradable material, according to ISO 14855 tests, and almost identical anaerobic biodegradation in aqueous phase according to ISO 14853 than cellulose<sup>92</sup>. Similar properties are reported Solon<sup>®</sup> P(3HB-*co*-3HHx), manufactured by the U.S.-based company RWDC Industries from waste cooking oil. This product is certified for “OK biodegradable SOIL”, “OK biodegradable MARINE”, “OK biodegradable WATER”, “OK compost HOME”, “OK compost”, and “OK biobased”<sup>93</sup>. The same goes for the P(3HB-*co*-3HHx) biopolyesters made by Danimer Scientific, and commercialized under the trade mark Nodax<sup>™</sup>. They are certified with “OK biobased”, “OK biodegradable SOIL”, marine biodegradable according to ASTM D6691, “OK compost HOME”, “OK compost”, and U.S. Food and Drug Administration (FDA) approved for food contact<sup>94</sup>. Wang *et al.* directly compared biodegradability of P(3HB) homopolyester and P(3HB-*co*-12 % -3HHx) copolyester. While 40 % of the copolyester was degraded within only 18 days, no more than 20 % of the higher crystalline homopolyester degraded in parallel setups under the same conditions<sup>85</sup>.

Since 2008, the P(3HB-*co*-3HV) product ENMAT Y1000P, produced by TianAn Biologic Materials, PR China, is certified as “compostable” by the US-Biodegradable Products Institute (BPI); moreover, it is listed as Food Contact Material (“FCM”) substance No. 744 in Table 1 of Annex I of the Plastics Regulation of the EU, and, since 2008, it complies with EU REACH (Registration, Evaluation,

Authorisation and Restriction of Chemicals). In 2022, TianAn ENMAT biopolyesters received “OK compost HOME” and “OK biodegradable MARINE” certification according to TÜV Austria<sup>95</sup>. Shenzhen Ecomann’s Ambio<sup>®</sup> P(3HB-*co*-4HB) products are TÜV Austria-certified for “industrial and home composting”, and also FDA-approved<sup>96</sup>. In addition, P(3HB-*co*-3HV)’s excellent biocompatibility was only recently verified by Mohandas *et al.*, who assessed attachment behavior, viability, and proliferation of fibroblast cells on microbial P(3HB-*co*-3HV) films; high compatibility of cells with P(3HB-*co*-3HV) films was shown, in addition to excellent blood compatibility as revealed by hemolysis-, *in vitro* platelet adhesion-, and coagulation assays<sup>97</sup>.

The same biodegradability was ascertained for Danimer Scientific’s Nodax<sup>®</sup> P(3HB-*co*-3HHx), which is certificated, besides its “biobased” nature (ASTM D6866) regarding its anaerobic and aerobic degradability in soil (TÜV Austria, ASTM D5988), freshwater (TÜV Austria, ASTM D5271, EN 29408), and marine water (TÜV Austria, ASTM D6691), and its suitability for industrial (TÜV Austria, ASTM D6400, EN 13432), and home composting (TÜV Austria, ASTM D6400, EN 13432)<sup>98</sup>.

In 2014, Meredian Inc. (joint later Danimer Scientific) received a Food and Substance Contact Notification approval from FDA, certifying that their P(3HB-*co*-3HHx) biopolyesters are “safe to use for food contact” and can be classified as “non-hazardous waste” after disposal<sup>99</sup>.

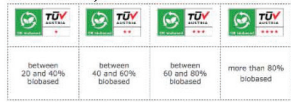





In contrast to microbial PHA, PLA biodegrades in industrial composting facilities at elevated temperature, while it does not biodegrade under home-composting conditions. This high recalcitrance of PLA was demonstrated a long time ago<sup>100</sup>, and was substantiated more recently by direct comparison of PLA and other biopolymers like Kaneka’s copolyester PHBH<sup>®</sup>; in contrast to PHBH<sup>®</sup>, PLA was not degraded at all under these ISO 14853 (anaerobic biodegradation in aqueous phase) and ISO 14855 (aerobic biodegradation in compost) test conditions during a 28-days test span<sup>101</sup>. However, in the public perception, PLA is probably still the prototype “bioplastic”. PHA biopolyesters should take this role at earliest convenience!<sup>102</sup>

Table 2 provides an overview of described norms and standards applicable for PHA biopolyesters.

### Reaching UN’s Sustainable Development Goals (SDG) through the use of PHA

The 17 UN Sustainable Development Goals (SDGs) encompass a universal approach towards reaching a concerted global sustainable develop-

Table 2 – Definitions, standards, norms and certifications applicable for PHA biopolyesters

Criterion	Definition	Standard/Norm	Label
“Biobased”	Defines the biobased carbon content in a product; 1 star: between 20 and 40 %; 2 stars: between 40 and 60 %; 3 stars: between 60 and 80 %; 4 stars: more than 80 %.  “OK biobased Certification Scheme TS OK20“: based on radiocarbon method.	EN 16640, ASTM D6866, CEN/TS 1613	“OK Biobased” (TÜV Austria)  
“Biodegradable”	A “biodegradable” material has 90 % of its carbon metabolized under standardized conditions of humidity, temperature, and pH-value within 180 days.	EN 13432	“OK Biodegradable” (TÜV Austria)
Biodegradable in water	Degradation in aqueous medium is determined by measuring the amount of CO <sub>2</sub> produced during exposure.	DIN EN ISO 14853 ASTM D5271 EN 29408	“OK Biodegradable WATER”  
Biodegradable in sea water	Marine degradation is determined by measuring the amount of CO <sub>2</sub> produced during exposure.	ASTM D6691 (“Aerobic Biodegradation of Plastic Materials in Marine Environment”)	“OK Biodegradable MARINE” (TÜV Austria)  
Biodegradable in soil	Aerobic biodegradability of a plastic material in the environment determines the extent to which and the time in which plastic materials are mineralized by soil microorganisms.	ASTM D5988 (“Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in Soil”)  AATCC Soil Burial Method 30-1993	“OK Biodegradable Soil” (TÜV Austria)  
“Compostable”	A “compostable” material disintegrates within 180 days of composting into leftovers passing through a sieve of 2 mm pore size by at least 90 %.	EN 13432 ASTM D6400 ISO 14855	“OK Compost” (TÜV Austria)
“Industrial compostable”	Conversion of an “industrial compostable” biogenic material to compost occurs by the controlled, aerobic, exothermic (with a thermophilic phase > 55 °C) biological degradation and conversion.	EN 13432 ASTM D6400	“OK Compost Industrial” (TÜV Austria)  
“Home compostable”	Upgrading of “home compostable” household waste to produce compost at home.	prEN 17427 (“Packaging – Requirements and test scheme for carrier bags suitable for treatment in well managed household composting plants”)  AS 5810 (Australia) (“Biodegradable plastics – Biodegradable plastics that are suitable for home composting”) (basis for DIN CERTCO, Germany)  UNI 11183:2006 (Italy)  NF T 51-800 (France) (“Plastics – Specifications for plastics suitable for home composting”)  ASTM D6400	“Ok Compost HOME” (TÜV Austria)  



ment agenda. They constitute the core of “The 2030 Agenda for Sustainable Development”, adopted in 2015 by all UN member nations<sup>103</sup>. In essence, these 17 goals were dedicated to help spur economic and social progress, prevent war and starvation, while reducing and eliminating the environmental threats posed by climate change and pollution. Historically, the 2030 Agenda has its roots in the Earth Summit in Rio de Janeiro, Brazil, where in 1992, more than 178 countries agreed to “Agenda 21”, an all-inclusive strategy for sustainable development to improve human lives and protect the environment<sup>104</sup>. Principle 3 of the Rio Declaration postulates: “*The right to development must be fulfilled so as to equitably meet developmental and environmental needs of present and future generations.*” This principle can be regarded a catchy definition of the heavily used terminus “Sustainability”, and “Industrial Biotechnology” through its production of PHA is one example of this principle. The production and use of PHA aims to: reduce and eventually eliminate fossil resource exploitation at low cost for short-term economic benefit, reduce and eliminate negative environmental legacy (global warming, polluted environments, microplastic formation, etc.) to subsequent generations, and switching to truly circular, carbon-neutral material and processes. “Take from nature only what gets returned to nature by means of nature!” is another saying that fits here. Principle 4 of the Rio Declaration states that “*In order to achieve sustainable development, environmental protection shall constitute an integral part of the development process and cannot be considered in isolation from it.*”<sup>104</sup> Here again, PHA’s renewable and favorable end-of-life attributes fit with the stated principle.

Of the 17 UN Sustainable Development Goals, at least eight have a direct link to industrial-scale PHA production:

**Goal 1: “No Poverty”:** “Industrial Biotechnology”, such as PHA production, can offer generation of differently qualified employment options, distributed all over the process chain from the raw material to the marketable product. Most importantly, these jobs can be generated in underprivileged global regions, and locally, where material streams, agro-industrial and other processes currently treated as waste, can be upgraded to raw materials for “Industrial Biotechnology”, such as for PHA biopolyester production.

**Goal 2: “Zero Hunger”:** Production of bioproducts from edible resources like purified sugars is more or less optimized for many different products such as PHA; however, PHA production on an industrial scale can resort to raw materials not competing with food resources. Using such “second

generation feedstocks” for manufacturing bioplastic would not deplete materials of relevance for human nutrition. Thus, food reserves can be protected instead of being, e.g., burned into biofuels, as seen in 2005–2007, when a dramatic biofuel boom resulted in shortage of affordable cereals, especially corn. In this context, we should remember, e.g., the 2006 “Tortilla riots” in Mexico caused by a 25 % increase in food prices<sup>105</sup>; as a consequence, bioethanol production from the first-generation feedstock, corn, in Mexico is now prohibited by law<sup>106</sup>.

**Goal 7: “Affordable and Clean Energy”:** Spent PHA can be incinerated to generate thermal energy in a carbon-neutral process, or can be digested in biogas plants, generating the renewable energy carrier CH<sub>4</sub>.

**Goal 9: “Industry, Innovation, and Infrastructure”:** Biobased industry indispensably resorts to fundamental changes in the way products are manufactured, starting from raw materials, equipment (bioreactors to be operated in discontinuous and continuous mode, adapted downstream processing facilities, etc.), new ideas how the wealth of natural products can be tapped to transform them to desired marketable end products (e.g., lignin-first approaches for holistic utilization of lignocellulose waste<sup>107</sup>), and provision of secure raw material supply chains; the latter involves the establishment of decentralized production facilities. In a best-case scenario, PHA production facilities should be integrated into existing production lines for other products, where current industrial waste streams accrue at large quantities, and can be directly used in-house for PHA production processes. Such concepts, also called “Biorefinery”, already exist in many parts of the world and are becoming increasingly popular to exploit renewable feedstocks as a whole. For PHA, (semi)industrial examples already exist, as shown for the PHA production process at the company PHA/ISA in Brazil, where PHA production is integrated in the existing infrastructure for sugar and ethanol production, and runs economically based on in-house available raw materials (molasses from sugar production as main carbon source), energy sources (combustion of bagasse from sugar cane residues), solvents for downstream processing (PHA extraction from biomass by fusel alcohols, a by-product of ethanol distillery), and bagasse fibers to be used as filler materials to generate low-price – high-performance biocomposites with PHA. This way, the entire sugar cane plant is converted within a biorefinery concept<sup>108</sup>.

**Goal 12: “Responsible Production and Consumption”:** The first part (“responsible production”) covers already discussed aspects like mindful choices of raw materials (2<sup>nd</sup> instead of 1<sup>st</sup> genera-

tion feedstocks) and energy sources for PHA production, while the second part (“responsible consumption”) addresses the responsibility of the end consumer, hence, the client buying products in supermarkets. Here, it should come to mindful choices when purchasing polymeric articles: Is there willingness of customers to pay some “green bonus” for renewable and circular products, which cannot be produced that inexpensively, initially, as from established fossil carbon sources? Are consumers practicing “home composting” of packaging materials for appropriate disposal of spent home compostable packaging materials? Are (fossil) microplastics in cosmetic products replaced by biodegradable microparticles, e.g., made of PHA? Utilizing biopolymers like PHA as packaging materials aligns with Goal Number 12 to ensure sustainable consumption and production patterns<sup>109</sup>.

**Goal 13: “Climate Action”:** Rising greenhouse gas emissions call for the switch to carbon-neutral production processes in order to ultimately meet the 1.5 °C scenario of global warming in 2050; in this context, PHA production, if appropriately designed as a whole, is considered a carbon-neutral process, thus contributing to reaching this goal. PHA production does not result in formation of additional greenhouse gases; therefore, its production does not contribute to global warming and climate crises, if

renewable energy can be used in their production. An important point to note here: Every ton of fossil plastic replaced by PHA saves about 2–3 tons of CO<sub>2</sub> emissions!<sup>110</sup>

**Goal 14: “Life below Water”:** Fossil plastics waste severely threatens all forms of marine life and in turn humanity’s well-being. This goes for macroplastic waste, which is steadily consumed by marine mammals like dolphins or highly endangered whales, destroying their digestion system or preventing the digestion of convertible organic food, and sea birds, which also confuse smaller plastic particles with food. Moreover, microplastic particles are consumed by zooplankton and other marine invertebrates, which confuse it with phytoplankton, the origin of the food chain. This zooplankton, in turn, is consumed by higher organisms like fish, birds, mammals, and that way climbs up the trophic chain, finally reaching our dinner plates and the human digestion system. Shells were shown to filter microplastic particles; consuming these plastic-enriched mollusks also enriches our menu with microplastic particles<sup>111</sup>. Moreover, seaweeds are compromised in their photosynthetic activity when covered by layers made of microplastic particles<sup>112</sup>. PHA produced in running industrial processes, in contrast, is marine biodegradable within not more than 180 days according to valid norms and certifica-

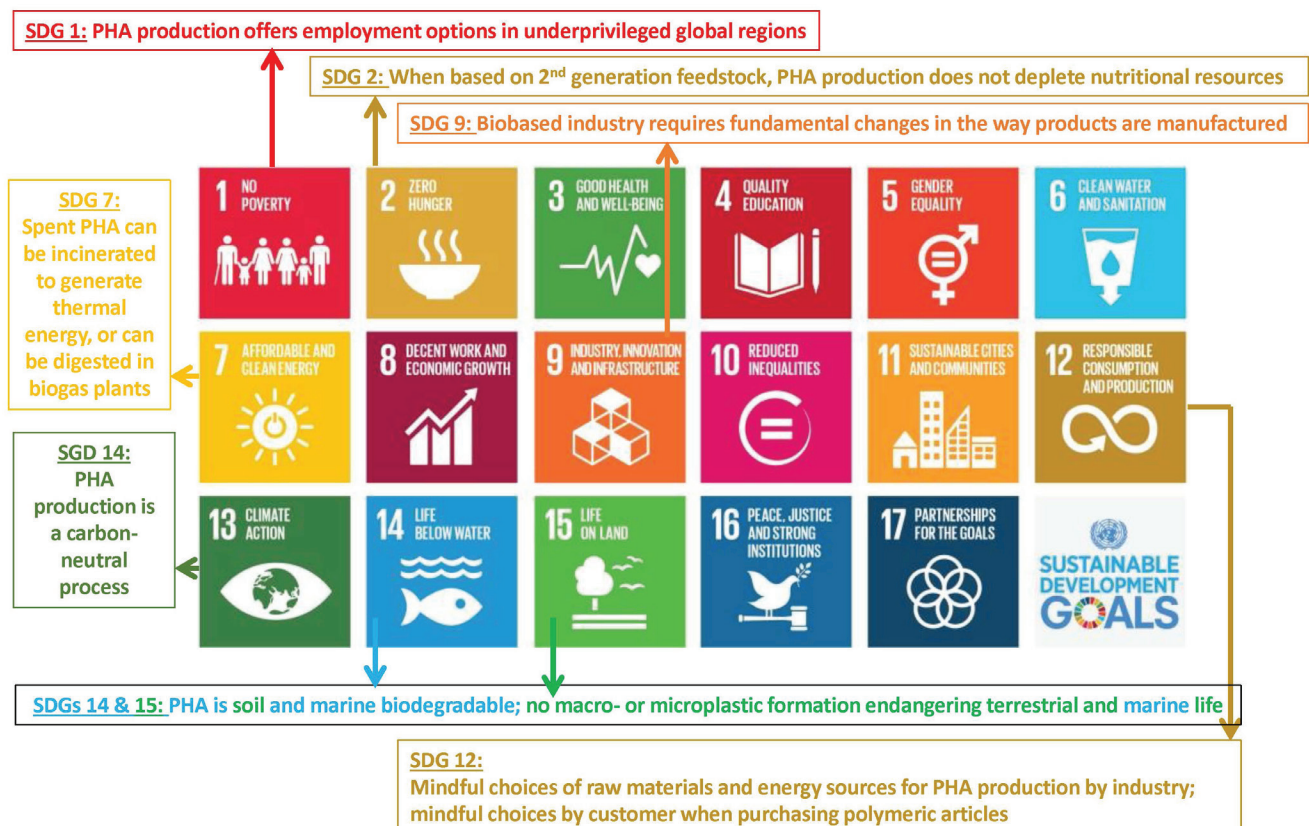


Fig. 3 – The 17 UN Goals for Sustainable Development and their relation to PHA biopolyesters

tions (e.g., those that authorize the granting of the “OK biodegradable MARINE” label), as reported by leading companies in this sector, such as Danimer Scientific, BluePha, Bio-On, or Nafigate (*vide supra*). PHA therefore does not contribute to endangering marine life forms.

**Goal 15: “Life on Land”:** PHA biopolyesters are biodegradable in soil and compostable; they leave no toxic remnants in terrestrial environments, agriculture, or silviculture, and form no recalcitrant macro- and microplastic particles remaining in forests, meadows, fields, mountains, etc., where terrestrial life forms could come in contact with them.

Fig. 3 presents the 17 UN SDGs, and highlights those goals to which industrial PHA production and use can contribute

## Conclusions and outlook

PHA biopolymers, as a class of materials, are poised to play a leading role in our use of sustainable plastic-like materials in the future. Their functional attributes as a material for packaging, fibers and fabrics, and for durable long-term use has already been demonstrated. Spent PHA biopolyesters fulfill our desire to have all end-of-life options available – recyclability and biodegradability in all environments, which allows them to be industrially and home composted. PHA’s use of renewable carbon as a starting raw material makes them carbon-neutral, establishing their credential as truly circular and functional.

PHA biopolymers are also the most versatile class of polymer materials available to humanity. To date, more than 150 different monomers have been discovered<sup>113</sup>, making infinite numbers and types of PHA biopolymers possible, with functional attributes mimicking the top 7 best-selling fossil plastics in the world. Over 28 different current and potential manufacturers and start-ups are engaged in the development and commercialization of PHA biopolymers worldwide<sup>114</sup>, a testament to their potential for proliferation and use, and to the potential for renewed economic development and growth that are sustainable and circular. PHA, like no other single material, can stand out in terms of being able to fulfill our desire to combine circularity and sustainability with continued economic development, growth, and collective prosperity.

Despite the promise of PHA, significant challenges still exist. The proliferation of a new product requires their availability in substantial quantities to spur innovation in downstream application development, and the availability of new materials in large quantities is firmly tied to reasonable material and production costs. In turn, reasonable material cost is tied to investments in the development of production technologies for PHA, and the availability of

large volumes of renewable carbon raw materials that do not compete with food and animal feed. Advances in synthetic biology and process engineering have allowed PHA production to evolve; however, they are still significantly more expensive than the fossil plastics they would eventually replace. Only through investments in the science and innovation of PHA production and their use can we achieve the breakthroughs we so desire and need to bring to the masses a material that fits neatly into nature’s “Circle of Life”.

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