

# Polyhydroxyalkanoates – Linking Properties, Applications, and End-of-life Options



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<https://doi.org/10.15255/CABEQ.2020.1819>

Review

Received: May 6, 2020

Accepted: October 5, 2020

When it comes to “bioplastics”, we currently notice an immense complexity of this topic, and, most of all, a plethora of contradictory legislations, which confuses or even misleads insufficiently informed consumers. The present article therefore showcases microbial polyhydroxyalkanoate (PHA) biopolyesters as the prime class of “bioplastics” *sensu stricto*. In particular, biodegradability of PHA as its central benefit in elevating the current plastic waste scenario is elaborated on the biochemical basis: this covers aspects of the enzymatic machinery involved both in intra- and extracellular PHA degradation, and environmental factors impacting biodegradability. Importantly, PHA degradability is contextualized with potential fields of application of these materials. It is further shown how the particularities of PHA in terms of feedstocks, mode of synthesis, degradability, and compostability differ from other polymeric materials sold as “bioplastics”, highlighting the unique selling points of PHA as “green” plastic products in the circular economy. Moreover, current standards, norms, and certificates applicable to PHA are presented as basis for a straight-forward, scientifically grounded classification of “bioplastics”.

## Keywords:

biodegradability, biopolymers, certifications, composting, depolymerases, polyhydroxyalkanoates

## Introduction: The plastics pollution – origin and effects

Being useful, practical and inexpensive, polymeric materials or plastics are indeed ubiquitous in our daily lives. During the decades between 1940 and now, synthetic plastics that are not biodegradable in nature and of petrochemical origin have dominated growth. However, their uncritical disposal and accumulation in the ecosphere have resulted in an environmental catastrophe. In this context, Geyer *et al.* published a seminal paper in 2017, outlining the use and fate of all plastics. They concluded that up until 2015,  $8.3 \cdot 10^9$  metric tons of plastics were produced globally, with only 9 % of it being recycled, 12 % incinerated, but 79 % (corresponding to  $6.3 \cdot 10^9$  metric tons) discarded in landfills or simply dumped in the environment. Furthermore, they estimated that by 2050,  $12 \cdot 10^9$  metric tons of plastic waste will be accumulated in land-

fills and the environment – double the amount that existed in 2015.<sup>1</sup> In 2016, the Ellen MacArthur Foundation published a report on plastics production with input from numerous industry experts and organizations. Authors came up with the conclusion that, in 2015, almost 95 million tons of packaging plastics were consumed, constituting the primary source of plastics pollution. They also estimated that plastics production uses 6 % of the total fossil crude oil extracted, and contribute 1 % of the total carbon emission when assuming its post-use incineration as common fate of all plastic. Without major mitigation efforts, by 2050, plastics would consume 20 % of the world fossil feedstock production and represent 15 % of the carbon emissions.<sup>2</sup>

At some point in Earth’s history, massive amounts of carbon-containing plant and animal biomass were buried, which then, under pressure, turned into what we describe today as fossil fuels, such as coal, petroleum, and natural gas. After the discovery of these fossil fuels, which was accompanied by the onset of the industrial revolution, mankind started to release the carbon that was seques-

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tered over a period of hundreds of millions of years. This has caused the increase in greenhouse gases (GHG), particularly  $\text{CO}_2$ ,  $\text{CH}_4$  and  $\text{NO}_x$ , contributing to the climate change we are experiencing today. Plastics, a natural offshoot of petroleum and natural gas, have played their part, although their contribution to global warming has been relatively small compared to GHG emissions due to energy generation, transportation, or even agriculture. However, plastics have had another more serious and deleterious effect on the planet, including its oceans and life therein, through the accumulation of these fossil-based materials that do not degrade into  $\text{CO}_2$ ,  $\text{CH}_4$  or water anytime soon. Solutions to plastics accumulation or pollution is a necessary and urgent issue that needs large-scale innovative solutions. While numerous methods have been developed over decades and are being employed today, such as collection and then recycling or incineration, as Geyer *et al.* notes, these methods do not address the entire breadth of the problem of plastics pollution described above. While these methods and processes can be broadened to cover additional plastics waste streams, they will not cover numerous sources of plastics pollution given their ubiquitous nature.<sup>1</sup>

Recycling is necessary and needs to be improved, but it also comes at substantial costs, including huge energy consumption and in turn more GHG emissions, and it would not address the entire plastics waste crisis, including microplastics, generated from disintegrating larger plastic articles, and from fibers and fabrics, paints and coatings, adhesives, shoes, and car tires.<sup>1</sup> As a consequence of the plastics pollution, 127 countries have banned single-use or other plastics.<sup>3–6</sup> However, banning plastics use or relying solely on recycling and incineration are insufficient strategies to eliminate the negative environmental impacts of plastic waste and GHG emissions, now or in the future. The scale of the problem requires additional innovative solutions, especially such solutions resorting to biodegradable materials.

### Biodegradation of polymers

Biodegradability can be considered an end-of-life option for consumer plastics produced for single-use. Known natural polymeric materials typically biodegrade in the environment under various conditions; they turn into  $\text{CO}_2$ , water, and biomass in the presence of  $\text{O}_2$ , and into  $\text{CO}_2$ ,  $\text{CH}_4$ , water and biomass during anaerobic digestion. Importantly, the process of biodegradation of natural materials is circular: photochemotrophs like plants use  $\text{CO}_2$ , water, and nutrients to grow, and, at the end of life, they biodegrade into the very products they consumed to grow.

In the meanwhile, mankind has transformed biodegradation into an industrial process by increasing the temperature and/or by adding specific microorganisms; we call it “industrial composting”. In industrial composting facilities, compost temperatures exceed  $50\text{ }^\circ\text{C}$  due to the metabolic heat generated by the microbes thriving in it. This high temperature is also a legal requirement because it ensures sufficient pasteurization of the final product (compost). The same process of biodegrading natural materials can be carried out at lower temperatures; this process is known as “home composting”. Here, temperatures as high as in industrial composters are typically not reached or only in the core, because the compost is agitated/turned less often, and has a surface-to-volume ratio beneficial for cooling by the ambient. Also, due to a lower aeration, microbial growth is less intensive and less heat is generated. The timeline is also important in biodegradation and/or composting performance, because higher temperatures in industrial composting facilities speed up biodegradation, while the process of home composting takes longer; however, in both processes, biodegradation occurs.

Circularity of natural materials has been at play since the origin of life, and the Earth has kept a balance with respect to GHG. Therefore, mimicking nature is the best solution when it comes to materials production and plastics pollution prevention, in order to ensure early in advance that accumulation of these materials, which have a devastating effect on life on Earth, cannot occur beforehand. This includes producing materials using renewable sources, such as  $\text{CO}_2$  and/or  $\text{CH}_4$  and water, and, at the end of life span, returning the material to nature, i.e., allowing nature to convert these materials again into  $\text{CO}_2$ ,  $\text{CH}_4$ , water, and biomass. There are numerous materials that can and are being made using renewable  $\text{CO}_2$  or  $\text{CH}_4$  as the carbon source; however, there are only few materials that act like plastics and also turn into  $\text{CO}_2$ ,  $\text{CH}_4$ , water, and biomass. Polyhydroxyalkanoates (PHA), a family of naturally occurring microbial polyesters, are one such class of materials, which also have many of the beneficial properties of the top seven best-selling types of petro-plastics (PET, HDPE, PVC, LDPE, PP, PS, PA), thereby making them the most suitable material to replace many different plastics used today, especially but not exclusively those used in single use applications and packaging that are difficult to collect or recycle.<sup>7–9</sup> The wide range of chemical, physical, and mechanical properties of PHA is due to the fact that these biopolymers can differ very much based on their monomer composition and on molecular weight and molecular weight distribution. Different types of PHA may also show differences in biodegradability (*vide infra*). Some

properties, e.g., chemical functionalization by inserting monomers with specific function groups in growing PHA chain during *status nascendi*, are only obtained by providing chemically related precursors in the production medium. However, these substances are often not biobased (e.g., halogenated precursors, monomers of polythioesters [PTEs; *vide infra*]); in some cases, this makes the resulting PHA harder to degrade, or even non-degradable.<sup>10</sup>

### General aspects, properties, and end-of-life options of polyhydroxyalkanoates (PHA)

PHA are a naturally occurring family of polymers, which are synthesized by about 40 % of the world's microorganisms. Their chemical structure resembles a linear polyoxoester.<sup>10</sup> Microorganisms produce PHA when they experience certain nutritional deficiencies. The type and structure of the PHA they produce depend on their raw material source.<sup>9,11</sup> By 1995, 95 different types of PHA polymers and their corresponding unique building blocks (hydroxyalkanoic acids) were discovered,<sup>12</sup> and to date about 150 different types of PHA polymers and their corresponding building blocks have been described.<sup>13</sup> Microorganisms biosynthesize PHA when they experience deficiencies in certain nutrients, which they associate to be a precursor to an upcoming substrate/food shortage. When these microbes are exposed to such shortage, they then consume the accumulated PHA as an energy and carbon source to survive during starvation. More recently, additional unexpected functions of PHA have been discovered, all of them showing that PHA biosynthesis is a result of microbes' "SOS-response" to stress provoked by, e.g., osmotic imbalance, heavy metal contamination, or UV-irradiation.<sup>14,15</sup> When recovered from microbial biomass and appropriately processed, PHA have the properties of established plastics. Here, it should be emphasized that the PHA recovery process still is a challenging task considering the entire ecological footprint of these biopolyesters; as recently reviewed, there are numerous R&D efforts ongoing to optimize PHA recovery in terms of reducing solvent, chemicals, and energy input, avoiding traditional halogenated PHA-extraction solvents, and reaching higher recovery yields in shorter time and optimized product quality.<sup>16</sup>

Depending on the type of building block or monomer, different types of PHA mimic different plastics, ranging from thermoplastics to elastomers. To date, only around 15 different building blocks and their corresponding PHA polymers have been thoroughly studied, and these PHA polymers demonstrate the beneficial properties of the top seven best-selling petro-plastics (*vide supra*). A lot of work has gone into research of their properties and

applicability in various industrial and consumer applications where plastics are used. These include films, fibers, thermoformed and molded parts for use in packaging, food service, agriculture, medical devices, electronics, leisure industry, fabrics, paints and coatings, adhesives, etc.<sup>7,8</sup>

Mechanical properties such as elasticity modulus, tensile strain and tensile strength of PHA like poly(3-hydroxybutyrate) (PHB) and its composite materials are in a similar range like measured for bones; thus, these biomaterials hold promise for application as implant materials, e.g., for femoral fractures.<sup>17</sup> In comparison to surgically used polymers like PLA, poly(glycolate) (PGA), or poly(lactid-*co*-glycolid) (PLGA), implants based on PHA have the added advantage of not reducing the local pH-value during *in vivo* degradation; this lacking of acidogenesis makes PHA well accepted by cells and the immune system. As drawbacks, the low *in vivo* degradation rate of PHA-based implants and the high crystallinity, especially of PHB, complicate the enzymatic degradation of the implants, as shown before by the remarkable recalcitrance of tiny bar-shaped PHB-based femoral implants against *in vivo* degradation in living rats.<sup>17</sup> In this context, modern implantation surgery often faces the problem of biomaterial-associated microbial infections, which requires the improvement of implant surfaces to prevent bacterial adhesion as the start of biofilm formation. To overcome this issue, a recent study developed drug delivery systems consisting of antibiotic-embedding PHB and PHBHV for coating titanium implants. A simple multi-layer dip-coating technique was used for optimal coating of the implants. Drug delivery, antibacterial effect, toxicity, and cell adhesion were studied for individual coated implants. Both antibiotic-loaded PHA coatings resulted in protection against microbial adhesion, PHBHV coatings displayed a better drug release profile by faster degradation compared to coatings with the homopolymer PHB. When coatings with different antibiotic concentration per layer were used, a better controlled and more homogeneous release was noticed. Because the PHA-coatings degrade with time under physiological conditions, these new drug delivery systems performed in an outstanding and expedient fashion, not only by preventing the initial bacterial adhesion, but also by inhibiting the subsequent bacterial reproduction and biofilm formation, which serves for a prolonged drug release.<sup>18,19</sup> Because of the high versatility of their mechanical properties, combined with excellent biocompatibility and *in vivo* degradability, PHA biopolyesters are among the most auspicious biomaterials for tissue engineering, and are being used to replace and heal different types of hard or soft tissue; PHA-based tissue engineering is described

for restoring cartilage, skin, cardiovascular tissue, bone marrow, and nerve conduits (recent reviews by<sup>20–26</sup>).

PHA being produced naturally are *biobased* (building blocks originating from renewable resources, such as sugars, organic acids, alcohols, lipids, CO<sub>2</sub>, or CH<sub>4</sub>) and, at the same time, *biosynthesized* (building blocks converted to polymers by the enzymatic machinery of living organisms). Since they are naturally consumed by microorganisms, PHA are *biodegradable* and *compostable* as well as *biocompatible* and *bioresorbable*, hence, they exert no negative effect on the biosphere surrounding them (e.g., on living organisms, cell lines, living tissue, ecosystems). In fact, they act as energy/nutrition providers to microorganisms and, in that sense, have a positive effect on the environment. Other natural polymers, which can be used to manufacture materials with plastic-like properties include starch (also known as thermoplastic starch – TPS), proteins (gelatin, etc.), chitin, or cellulose. These materials are also renewable, biodegradable, and biocompatible. These polymers belong to the group termed biopolymers *sensu stricto*, and the PHA family belongs to this group of materials.<sup>27</sup>

As mentioned previously, aerobic (oxidative) degradation of PHA by microbes like bacteria or fungi generates CO<sub>2</sub> and water, while anaerobic PHA consumption by living organisms, e.g., in biogas plants, results in generation of CH<sub>4</sub> in addition to water and CO<sub>2</sub>.<sup>27</sup> While biodegradability of PHA *per se* has been long established, decisive factors influencing biodegradability of PHA, such as shape and thickness of polymer specimens, crystallinity, composition on the level of monomers, environmental factors (humidity, pH-value, temperature, UV-radiation), and surrounding microflora, though comprehensively studied and reviewed, require further elucidation.<sup>28</sup> For example, most studies have suggested that crystallinity can negatively impact the rate of biodegradation of PHA,<sup>29</sup> while others have suggested that crystallinity has no impact on biodegradation.<sup>30</sup> In addition, there are reports stating that certain, yet scarcely studied, types of PHA, such as poly(3-hydroxyoctanoate) (PHO) were not biodegradable at all.<sup>31</sup> While more studies are needed to further refine biodegradation timelines for various types of PHA, the fundamental fact that those types of PHA produced to date at reasonable quantities are biodegradable has already been established.<sup>32</sup> This variability in biodegradation of PHA follows all other natural materials including starch, cellulose, proteins, or chitin.

In addition to their biodegradability, PHA are manufactured from renewable resources, hence, they do not deplete limited fossil reserves. This fact, coupled with their biodegradability as discussed

earlier, make PHA truly circular; therefore, these biomaterials are an integral part of the closed carbon cycle of our planet; this means that their biodegradation does not further increase the atmospheric concentration of CO<sub>2</sub> in analogy to other natural materials that we know and understand well, such as carbohydrates (e.g., starch), alcohols (e.g., glycerol), fatty acids, or lipids, which are typically utilized for production of PHA today. These “renewables” in turn are products of the natural metabolism of plants and microorganisms; their carbon content was not entrapped in our planet’s interior for millions of years. This is yet another fundamental difference from conventional plastics that are produced from fossil fuels like petroleum or natural gas. At end of life, PHA undergoes biodegradation – aerobic or anaerobic – depending on the disposal method generating biomass, water, CO<sub>2</sub> and CH<sub>4</sub>, all of which are fixed by plants and phototrophic microbes, or by methanotrophs, respectively, thus continuing along the natural carbon cycle. This circularity of PHA is the essential and critical difference between plastics from petroleum whose degradation by incineration deprives the world of its natural carbon cycle, thus releasing sequestered carbon that was fixed millions of years ago by suddenly releasing them into the atmosphere.<sup>27</sup>

The fundamental differences between fossil-based conventional plastics and renewable and biodegradable PHA, as outlined previously, make the fate of fossil plastics linear, while PHA are circular and excellently match the topical vision of “The Circular Economy” espoused by numerous communities and organizations worldwide, especially when considering the option of recycling PHA after use, which might be the *conditio sine qua non* to avoid the limitation of PHA to low value applications. However, PHA offer much more than just their renewability and biodegradability as important attributes in their quest to replace fossil plastics. They can also be collected after use just like fossil fuel-based plastics, and upon a substantial stream of PHAs available, they could be recycled just like fossil-based plastics. Mechanical recycling of PHA is predominantly accomplished via extrusion and injection molding (demonstrated by Zaverl *et al.* for the copolyester poly(3-hydroxybutyrate-co-3-hydroxyvalerate) PHBHV), which can undergo five subsequent recycling cycles,<sup>33</sup> or PHBHV/PLA blends, which were recycled six times by Zembouai *et al.* without significant loss in quality.<sup>34</sup> In addition, combination of extrusion and compression molding was demonstrated for PHB homopolymer, which, however, displayed significant quality loss already after two recycling cycles, as shown by Rivas *et al.*<sup>35</sup> We should consider that mechanical recycling of PHA-based materials is

still in its infancy, and various studies will be needed to assess the parameters determining recyclability of these materials, such as composition on the monomeric level, molecular mass, etc. As recently reviewed by Vu *et al.*, there are still considerable gaps of knowledge about the relationships between PHA composition on the monomer level, and accessibility of differently composed PHA items to recycling strategies under investigation.<sup>36</sup> Understanding this relationship, however, is crucial since it will decide if separation of the bioplastic materials already at the source is needed or not. As an alternative to mechanical recycling, chemical upgrading of spent PHA is feasible via pyrolysis, which can generate, dependent on the PHA composition, chemically relevant compounds like crotonic acid and oligomers of 3HB when starting from PHB, 2-pentenoic acid when using PHBV,<sup>37</sup> or 2-decenoic acid when pyrolyzing the *mcl*-PHA poly(3-hydroxydecanoate).<sup>38</sup> Another intriguing recycling strategy is based on microwave-assisted conversion with green solvents like alkaline methanol; as shown by Yang *et al.*, PHB homopolymer can be converted this way to 3HB, 3-methoxybutyrate, and crotonic acid.<sup>39</sup> When recycling of PHA is not feasible, which is the case with over 80 % of collected fossil-based plastic waste today, PHA can be incinerated with other fossil plastics to generate energy or left in industrial composters to generate biomass for agricultural use, something that fossil plastics cannot claim as an attribute. In addition, PHA-based products, when left accidentally in the environment (which, of course should be avoided!), will biodegrade into CO<sub>2</sub>, CH<sub>4</sub> and water just like other natural polymeric materials, which is an attribute that would go a long way in fulfilling society's desire of getting back to the natural cycle of circularity and renewability. Moreover, spent PHA can also undergo hydrolysis to generate optically pure building blocks, which in turn can be used as chiral synthons for synthesis of marketable compounds, such as pharmaceuticals, synthons for organic synthesis, or fragrances.<sup>40</sup>

### Mechanisms of PHA degradation

In accepting PHA as biodegradable, it is important to understand the biodegradation mechanisms at play in all details. Biodegradation of polymers in nature is driven by a specific group of (mainly hydrolytic) enzymes called depolymerases. These enzymes break down the complex molecules into constituent natural building blocks (monomers), which the microbes then consume to generate energy. Numerous microorganisms possess the ability to secrete such enzymes. Some microorganisms secrete them extracellularly, while others pos-

sess intracellular enzymes for polymer degradation.<sup>40</sup>

In this context, the group of PHA depolymerases (PhaZs), encoded by *phaZ* genes, biodegrade PHA polyoxoesters. There are two types of PHA depolymerases, those that are nonspecific and are excreted extracellularly (e-PhaZs), and intracellular depolymerases (i-PhaZs). The former are of lower specificity and degrade PHA into microbially convertible substrates, namely, small oligomers and monomeric repeat units. PHA is typically degraded *ex vivo* by microbial depolymerases produced by bacteria, streptomycetes, and fungi, and by other hydrolytic effects during the span of up to one year.<sup>17,21</sup> Moreover, e-PhaZs are typically of low molecular mass and have their pH-optimum in the alkaline range.<sup>40</sup> Biodegradability of PHA has been studied over the last 40 years and has been compared to numerous (semi)synthetic polymers. A variety of review papers, some of which are referenced here, have been published, clearly establishing PHA biodegradability in soil, fresh water and marine environments.<sup>41,42</sup> In the context of biodegradability of PHB, the best studied PHA, even under non-biocatalytic conditions in pure water or phosphate buffer saline at 37 °C, a progressive decrease in molar mass was described; after 650 days of immersion, molar mass had reduced by almost 50 %.<sup>43</sup>

Biodegradability of PHA depends on various factors, such as the composition of the biopolyesters on the monomeric building block level (PHA homopolyesters like PHB typically degrade slower than copolyesters), the stereo-regularity, crystallinity (higher degradability at lower crystallinity), molar mass (biopolymers of lower molar mass are typically biodegraded faster than their counterparts of higher molar mass), and environmental conditions (temperature, pH-value, humidity, and availability of nutrients for the microorganisms carrying out the degradation).<sup>28,32</sup> Moreover, one has to differentiate between depolymerases degrading short-chain-length PHA (*scl*-PHA; building blocks with three to five carbon atoms), and those enzymes degrading medium-chain-length PHA (*mcl*-PHA; building blocks with six to 14 carbon atoms). While many *scl*-PHA depolymerases (EC 3.1.1.75) have already been isolated and characterized, there is only a limited number of *mcl*-PHA depolymerases (EC 3.1.1.76) described in detail. Indeed, *mcl*-PHA-degrading microorganisms have been scarcely found in the environment, including predominantly some Gram-negative *Pseudomonas* sp. and thermophiles, and several Gram-positive *Streptomyces* sp., as recently comprehensively reviewed by Urbanek *et al.*<sup>43</sup> In the context of *mcl*-PHA, one should add that these materials feature intriguing material properties resembling those of elastomers and sticky res-

ins, such as low crystallinity and glass transition, and melting temperature. Such *mcl*-PHAs are not yet produced on a larger industrial scale, but are expected to enter the market as smart materials, e.g., thermosensitive adhesives and glues, “bio-latexes”, or carrier materials for bioactive compounds.<sup>44</sup> From *mcl*-PHA harboring unsaturated groups, rubber-like materials displaying constant product properties, but still expedient biodegradability, can be produced by post-synthetic modification, i.e., via cross-linking.<sup>45</sup>

Intracellularly, PHA can be depolymerized by the catalytic action of *i*-PhaZs produced by the host strain; cells use the carbon and energy content bound in their PHA reserves by mobilizing it in periods where no convertible exogenous carbonaceous nutrients are available. This *in vivo* PHA degradation starts by the reaction catalyzed by *i*-PhaZs (EC 3.1.1.7x), which generates monomeric (*R*)-3-hydroxyalkanoates and their oligomers; (*R*)-3-hydroxybutyrate dehydrogenase (EC 1.1.1.30), an oxidoreductase, reversibly oxidizes them to acetoacetate, which is then converted to acetoacetyl-CoA by the catalysis of the transferase acetoacetyl-CoA synthetase (EC 2.3.1.194). Finally, acetoacetyl-CoA is hydrolyzed to the central metabolic compound acetyl-CoA by the reversible enzyme 3-ketothiolase (acetyl-CoA–acetyltransferase, formerly known as  $\beta$ -ketothiolase; EC 2.3.1.9).<sup>46,47</sup>

In addition to *i*-PhaZs, which keep the intracellular cycle of PHA biosynthesis and degradation running, also extracellular PHA depolymerases (*e*-PhaZs) are reported. These enzymes are required for PHA biodegradation by other organisms; they catalyze biodegradation and composting of items consisting of PHA.<sup>48</sup> In a nutshell, *i*-PhaZs display higher substrate specificity, while their extracellular counterparts are rather unspecific hydrolytic enzymes (e.g., esterases), which also occur in eukaryotic organisms. Notably, *i*-PhaZs do not hydrolyze isolated, extracellular PHA, while extracellular depolymerases, when expressed *in vivo*, are not able to hydrolyze intracellular granules, since there exist considerable differences in the physical structures of intact, native intracellular granules and denatured extracellular PHA, especially regarding the different crystallinities.<sup>49</sup> In addition, depolymerization of intracellular PHA takes place to a certain extent simultaneously to PHA biosynthesis, even under conditions which are beneficial for PHA formation (excess carbon source in parallel to nutrient deprivation). This results in a steady biosynthesis and degradation of PHA in living cells, hence, a permanent modification and re-organization of the polyester chains, the frequently cited “cyclic nature of the PHA metabolism”, takes place.<sup>50</sup> Yet, under conditions favoring PHA biosynthesis, intracellular PHA depolymerases have considerably lower activ-

ity than measured at the same time for PHA synthases. If *i*-PhaZs are not active at all, which happens, e.g., in the case of recombinant *Escherichia coli* containing PHA synthesis genes (*phaCAB*), but no PHA depolymerases-encoding genes (*phaZ*), ultra-high molar mass PHA can be produced by the cells.<sup>51</sup> This effect was also demonstrated when inactivating (knocking out) PHA depolymerase in the *Azotobacter vinelandii* genome, which resulted in generation of PHA of highly uniform molar mass.<sup>52</sup>

Choi *et al.* demonstrated the high impact of PHA crystallinity on its extracellular degradability. These authors produced the *scl*-PHA copolyester poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBHV) with different 3HV fractions by cultivation of the strain *Alcaligenes* sp. MT-16 on glucose plus the 3-hydroxyvalerate (3HV) precursor levulinic acid (4-oxopentanoic acid). It was shown that increasing 3HV fractions in PHBHV samples drastically decreased the polyesters' crystallinity, which resulted in faster extracellular degradation when incubating the polyesters in solutions of *e*-PhaZ isolated from the fungus *Emericellopsis minima* W2. This demonstrated the impact of crystallinity, which in turn is dependent on PHA's monomeric composition, on PHA degradability. Enzyme-free degradation experiments carried out for 20 weeks with the same types of PHA in alkaline medium showed no degradation at all.<sup>53</sup>

In addition, it was shown that all PHA depolymerases discovered so far, both *i*- and *e*-PhaZs, are specifically hydrolyzing the oxoester bonds of PHA, while thioester bonds in polythioesters (PTEs), a group of sulfur-containing PHA analogues, are not cleaved by these enzymes.<sup>54</sup> PTE copolyesters of 3-hydroxyalkanoates and 3-mercaptoalkanoates, such as poly(3HB-*co*-3MP) or poly(3HB-*co*-3MB), are biosynthesized when supplying PHA producers like *C. necator* precursor substrates accessible only chemically, such as 3-mercaptobutyrate (3MB) or 3-mercaptovalerate (3MV), in addition to gluconate as main carbon source.<sup>55</sup> These copolyesters were shown to be degraded by only a limited number of bacteria like *Schlegellella thermodepolymerans*,<sup>56</sup> while for poly(3-mercaptopropionate) (P3MP) and other PTE homopolyesters, which are accessible by cultivating recombinant *E. coli* on appropriate precursor substrates, no degradation was observed at all, even when exposing the polyester to soil, compost, or activated sludge for half a year.<sup>57</sup>

### Combining PHA degradability with applications

Among the most prominent fields of application, PHA-based biodegradable packaging materials come in first. This is of high significance, especially in the field of food packaging, where it is often desired to have compostable, transparent packaging

with high barrier for O<sub>2</sub>, CO<sub>2</sub>, and moisture.<sup>58</sup> Considering PHA's complete pro-benign nature, expedient compostability and plastic-like processability, it is indeed reasonable to pack perishable food in PHA materials; after unpacking, the PHA packaging material, which is contaminated with food remains, can be easily disposed of as organic waste. This drastically reduces plastic waste. In addition, when degrading spent PHA packaging in biogas plants, the generated CH<sub>4</sub> can act not only as a carbon-neutral energy carrier, but even as feedstock for biosynthesis of new PHA by using methanotrophic PHA production strains.<sup>59–61</sup> Particularly PHA's excellent resistance to oxygen permeability attracts huge interest in using these materials for development of packaging materials preventing oxidative spoilage of wrapped goods.<sup>62</sup> In direct comparison to the long-established petrochemical packaging plastic high density poly(ethylene) (HDPE), it was demonstrated that quality preservation of food packed in PHA-based packaging materials is at least as good.<sup>63</sup> Already in 1996, the PHA copolyester PHBHV, which can be processed to plastic films, trays and containers for food packaging, was EU-approved for food contact application.<sup>64</sup> Also, other consumer applications for PHA exist, where biodegradability could be a relevant feature, such as diapers, various hygiene articles, drinking straws, cups, etc.<sup>10</sup>

The biodegradability of PHA under diverse environmental conditions makes this biopolymer family expedient candidates for drug carriers.<sup>65,66</sup> As described above, extracellular enzymes like PHA depolymerases and other biocatalysts, which are not that specific, are excreted by various microbes, and degrade PHA into small oligomers and monomers, which are microbially convertible substrates. The drug-retarding properties of PHA-based delivery systems can be controlled primarily by PHA's composition on the monomeric level and its molar mass. Additionally, PHA has demonstrated a substantial impact on the bioavailability of bioactive compounds, enhanced drug encapsulation, and reduced toxicity in comparison to other biodegradable polymers, as recently reviewed.<sup>20</sup> As another example for use of PHA in drug delivery, Rhodamine-B-loaded nanoparticles of randomly distributed poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) copolyesters of a mean size of about 150 nm were prepared by Wu *et al.* These nanoparticles were coated with sub-cytotoxic concentrations of poly(ethylene imine) to assist attachment to and uptake by different cell types. Cell response to this nanoparticle system was studied *in vitro* and *ex vivo*. It was shown that the nanoparticles were transported along endolysosomal cell compartments, the endoplasmic reticulum and the Golgi complex, without negatively affecting cell morphology or respiration.<sup>67</sup>

### Standards assessing PHA's degradability, compostability and biocompatibility

Most consumers are not aware of the differences in the types of plastics available on the market, and rightfully it's no trivial task to distinguish different plastic products. The expression "bioplastic", "green plastic", "biopolymer" and other similar words may sound sustainable and are effective in advertising and labelling a product due to the association of "bio" with plastics or polymers. However, the truth surrounding renewability, compostability or biodegradability, and in turn, sustainability is far from reality. Not all materials termed "bioplastics" or "biopolymers" are environmentally beneficial! Table 1 provides an overview on concepts for distinguishing between different types of "bioplastics".

Fig. 1 illustrates the categorization of PHA biopolyesters, among other heavily used plastic-like polymers, based on the categories "biobased", "biodegradable/compostable", and "biosynthesized".

In this context, logos and labels linked to a harmonized certification system are to an increasing extent applied to unambiguously inform the consumer whether a commercialized "bioplastic" is bio-based and/or compostable, and, even more importantly, how the consumer should dispose of it after its use ("(home) composting it or not?").<sup>74</sup> Indeed, several standards exist to define whether a material is biodegradable and/or compostable under different conditions (aerobic/anaerobic, industrial/home, etc.). Those international standards (most important examples: EN 13432:2000, ISO 17088:2012, ASTM D6400-12; details *vide infra*) prescribe the test schemes that need to be applied in order to evaluate and determine the compostability and biodegradability of "bioplastics" such as PHA or 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.<sup>75</sup> Bioplastics certified according to EN 13432 can be recognized by conformity marks, such as "Seedling", "OK compost", or "DIN geprüft".

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

Table 1 – Definitions applicable to “bioplastics”

<b>Biobased</b>	<p>Material is fully or at least partly derived from renewable biomass (e.g., plants, CO<sub>2</sub>, CH<sub>4</sub>). Bio-based carbon content is the decisive variable describing the amount of bio-based carbon (in relation to fossil-based carbon) present in a material or product. This biobased carbon content can routinely be determined via the <sup>14</sup>C method (according to <i>ASTM D6866: Standard test methods for determining the biobased content of solid, liquid, and gaseous samples using radiocarbon analysis</i>).<sup>68</sup></p> <p>Applies for: PHA, PLA, starch, cellulose, proteins, chitin, “bio-PE”.</p> <p><i>Nota bene:</i> Partly, this group of plastics also encompasses the PlantBottle™ commercialized by The Coca Cola® company, which consists of so-called “bio-based” PET; however, this material has a bio-based carbon content stemming from renewable resources (the ethylene part chemically produced starting from ethanol via oxirane and glycol) of only 23 % based on the carbon footprint!</p>
<b>Biodegradation</b>	<p>Chemical process during which microorganisms or parts thereof (enzymes) available in the environment convert materials into natural substances such as water, CO<sub>2</sub>, and compost (no artificial catalytic additives needed to accelerate degradation). This process depends on the surrounding environmental conditions (e.g., location, pH-value, humidity, or temperature), on the chemical properties of the material itself and on the material shape.</p> <p>Applies for PHA, starch, cellulose, proteins, chitin, PLA (requires higher than ambient temperatures!), PBS, PBAT, PBSe, or PBSA.</p>
<b>Biodegradable plastic</b>	<p>Bio-based or fossil-based plastics that meet standards for biodegradability and compostability. If a material or product is labeled as “biodegradable”, customers should get further information about the timeframe, the degree of biodegradation, and the required environmental conditions. Moreover, a timeframe for biodegradation must be set in order to make claims quantifiable and comparable. This is regulated in the applicable standards (<i>vide infra</i>).</p> <p>Applies for PHA, PLA, PBAT, starch, cellulose, or proteins.</p>
<b>Compostable plastic</b>	<p>Bioplastic that has proven its compostability according to international standards (see text) and can be treated in industrial (!) composting plants (does not imply home compostability). Importantly, thickness of specimens may have a significant role in compostability. Generally, plastics are compostable by successfully meeting the harmonized European standards (ISO 17088, EN 13432 / 14995 or ASTM 6400 or 6868), by having a relevant certification, and an according label (seedling label via Vinçotte or DIN CERTCO, OK compost label via Vinçotte, or TÜV in Austria).</p> <p>Applies for PHA, PLA, PBAT, starch, cellulose, proteins.</p>
<b>Degradable or oxodegradable plastic</b>	<p>Plastics to which (typically catalytically active) additives have been added to improve the degradability; importantly, such materials do not meet biodegradability and compostability standards. E.g., “Oxobiodegradable plastics” used for PE-based plastic bags do not fulfil the requirements of EN 13432 on industrial compostability, and are therefore not allowed to carry the seedling label.</p> <p>Applies to many petrochemistry-derived plastics, e.g., PE or PP, containing respective additives.</p>
<b>Bio-based, nonbio-degradable polymers</b>	<p>Polymers such as bio-based polyamides (PA) like “renewable nylon”,<sup>69</sup> polyesters like PTT or PBT, polyurethanes (PU) and polyepoxides used in technical applications like textile fibers (seat covers, carpets, threads) or automotive applications (foams for seating, casings, cables, hoses), etc. Their operating life lasts several years or even decades and, therefore, biodegradability is not desired.</p> <p>Other examples: BRASKEM’s bio-based poly(ethylene) (“bio-PE”), which resorts to chemical conversion of ethanol to ethylene. Here, polymerization of ethylene to PE occurs chemically via coordination polymerization or radical polymerization. The only “green” step in this process chain is the fermentative conversion of the renewable 1<sup>st</sup>-generation feedstock sucrose to ethanol by yeasts. Such “bio-PE” is currently strongly emerging regarding its market volume, which is expected to amount to estimated 300.000 t per year by 2022.<sup>70</sup> In 2018, even the company LEGO™ switched to “bio-PE” to manufacture their globally famous toy bricks; however, “bio-PE” is not biodegradable, and its production exploits food resources.</p> <p><i>Nota bene:</i> For the “bio-based” PET PlantBottle™ commercialized by The Coca Cola® company, copolymerization of terephthalic acid and glycol to PET occurs chemically, and the product is highly recalcitrant towards biodegradation. The benefit of expedient recyclability, as claimed by the manufacturers, is again partly compensated by the increased microplastic formation in “Re-PET” beverage bottles.<sup>71</sup></p>
<b>Bio-based, biodegradable plastics</b>	<p>Include starch blends made of thermo-plastically modified starch and other biodegradable polymers as well as polyesters such as PLA or PHA. Unlike cellulose, materials such as regenerate-cellulose or cellulose-acetate have been available on an industrial scale only for the past few years and are primarily used for short-lived products.</p> <p><i>Nota bene:</i> Polymerization of lactic acid to the cyclic lactide dimer and the subsequent ring opening polymerization (ROP) as the most widely applied method to generate poly(lactic acid) (PLA), the material still considered as the typical “bioplastic” by the public, requires metal catalysts like tin octoate, hence, PLA is not <i>biosynthesized</i>. Here, one has also to consider high recalcitrance of highly crystalline PLA towards biodegradation, and restrictions regarding its <i>in vivo</i> biocompatibility due to local acidification of tissue during degradation by generated lactic acid. Moreover, PLA is industrially compostable. For a comprehensive review, see reference.<sup>72</sup></p>
<b>Fossil-based, biodegradable plastics</b>	<p>Other plastics are neither biobased nor biosynthesized, but still biodegradable/compostable; however, they have a petrochemical origin. Prime examples are poly(ε-caprolactone) (PCL) used for many biomedical applications, or the random copolyester poly(butylene adipate terephthalate) (PBAT), which is used for materials commercialized by, e.g., the company BASF SE under the trade name Ecoflex®. This material is used for wrapping food, degradable plastic bags, water resistant coatings, or agricultural purposes.<sup>73</sup> These materials enter the natural cycle of carbon after being biodegraded, hence, they do not need to be incinerated or landfilled, but their production still exploits fossil resources (cyclohexanone generated by chemical conversion of cyclohexane originating from petrochemistry in the case of PCL, or adipic acid, 1,4-butandiol, and terephthalic acid in the case of PBAT, respectively).</p>



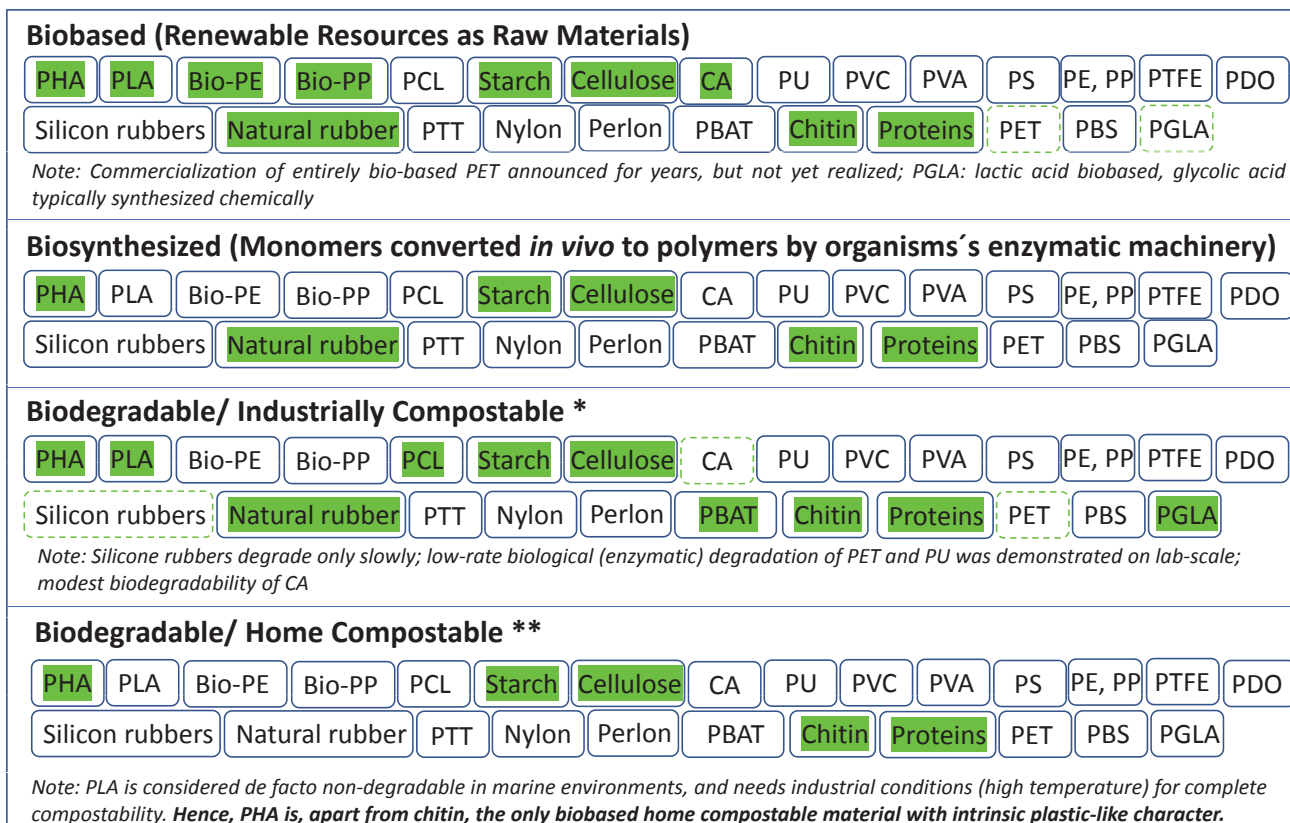


Fig. 1 – Classification of diverse polymers. Polymers marked in green meet the criteria “biobased”, “biosynthesized”, or “biodegradable/compostable”. PHA: Polyhydroxyalkanoates; PLA: poly(lactic acid); Bio-PE: bio-based poly(ethylene); Bio-PP: bio-based poly(propylene); PCL: poly( $\epsilon$ -caprolactone); PU: poly(urethane); PVC: poly(vinyl chloride); PVA: poly(vinyl alcohol); PS: poly(styrene); CA: cellulose acetate; PTFE: poly(tetrafluoroethylene) (Teflon®); PDO: poly(dioxanone); PTT: poly(trimethylene terephthalate); PBAT: poly(butylene adipate terephthalate) (Ecoflex®); PET: poly(ethylene terephthalate); PBS: poly(butylene succinate); PGLA: poly(glycolic-co-lactic acid). *Nota bene*: PHA is the only group marked in green in all categories which displays plastic-like properties without the need for special processing techniques and/or additives.

\* Industrially compostable: “Industrially compostable packaging” refers to the ability of packaging to biodegrade and decompose only in a commercial composting facility. Industrial composting facilities treat the packaging at high temperatures (above 55 °C, much higher than can be achieved in home composting) to accelerate degradation of the material. In accordance to the norm EN 13432.

\*\* Home compostable: Packaging labeled as “home compostable” means that the customer can simply place the packaging in the home compost bin. No EU-wide norm available yet!

compostability of each product, and to obtain the respective logos and labels.<sup>75–78</sup> 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), the British Standard Institution (BSI), etc. Table 2 shows the certifications and labels obtained for selected currently commercialized PHA resins.

In the context of integration of PHA into nature's closed carbon cycle, it should be reminded that “biodegradability” is not equal to “compostability”. Both characteristics are defined via norms and are strictly determined by certificates. Here, the norm EN 13432, which provides a holistic characterization of a “bioplastic”, addresses biodegradation and composting of polymeric packaging materials; it claims that a material is “biodegradable”, if

90 % of its carbon is metabolized within 180 days. In contrast, the same norm postulates that a material is “compostable” when leftovers in a sieve of 2 mm pore size after 180 days of composting do not exceed 10 % of the original material.<sup>10</sup> In the case of PHA, both characteristics are applicable. For Europe, these certifications are regulated at the national level in cooperation with the European Bioplastics industry association.

Importantly, EN 134323 refers specifically to *compostability in industrial composting facilities*. The standard, however, does not claim *home compostability*. Industrially compostable materials are suitable for separate organic waste collection with subsequent treatment in anaerobic digestion (biogas) plants or industrial composting facilities, but not suitable for throwing them on the compost heap in the garden.<sup>79</sup>

It has to be emphasized that no EU-wide standard for “home composting” existed at all until 2020, when the norm prEN 17427 was introduced, which, however, is applicable only for plastic bags (“Packaging – Requirements and test scheme for carrier bags suitable for treatment in well managed household composting plants”).<sup>80</sup> However, there were several national standards for home compostability of bioplastics and corresponding certification schemes, which are mainly based on EN 13432. E.g., the certifier Vinçotte offers such a home compostability certification scheme. Based on Vinçotte, TÜV Austria offers the label “OK biodegradable” specifically also for home compostable packaging (“Ok compost HOME”). Importantly, TÜV Austria’s “Ok compost HOME” certification program does not explicitly refer to a specific standard, but lists all the technical requirements that a product needs to meet in order to obtain this certification; hence, it should not be considered being based on one standard, but constituting the basis of several standards introduced in other countries. In this context, DIN CERTCO offers a certification for home compostability according to the Australian standard AS 5810 (“Biodegradable plastics – Biodegradable plastics that are suitable for home composting”; year 2010; also based on TÜV Austria), while Italy has a national standard for composting at ambient temperature, UNI 11183:2006. In 2015, the French Standard “NF T 51-800 Plastics – Specifications for plastics suitable for home composting” was presented (year 2015), which is also covered in the DIN CERTCO scheme.<sup>81</sup>

#### Details on key standards for “Bioplastics”

Due to its fundamental importance for all national standards regarding “bioplastics”, details of EN 13432 as the standard norm to characterize PHA as a real “bioplastic” are summarized as follows:

a) Chemical analysis: presentation of all ingredients and review of limit values for heavy metals.

b) Biodegradability in aqueous media: 90 % of the organic material must be converted to CO<sub>2</sub> within a period of 6 months.

c) Compostability: After 12 weeks of composting, no more than 10 % residues based on the original mass may remain in a 2 mm sieve. (Practical examination of the technical compostability: There must be no negative effects on the composting process.)

*Nota bene:* This standard refers specifically to compostability in industrial composting facilities. The standard, however, does not cover home compostability! Those materials are suitable only for the separate organic waste collection with subsequent treatment in biogas plants or industrial composting facilities.

d) Ecotoxicity: Investigation of the effects of compost on plants (growth and ecotoxicity).

#### Details of EN 14995

This frequently used European Standard specifies requirements and methods for determining the compostability or anaerobic treatability of plastic materials with the following four properties: 1) biodegradability; 2) disintegration during biological treatment; 3) impact on the biological treatment process; 4) impact on the quality of the compost produced. *Nota bene:* For PHA used as packaging materials, EN 13432 applies.

Details of ISO 17088: this norm was prepared by the Technical Committee ISO/TC 61, Plastics, Subcommittee SC 5, Physical-chemical properties. This specification is intended to establish the requirements for the labelling of plastic products and materials, including packaging made from plastics, as “compostable” or “compostable in municipal and industrial composting facilities” or “biodegradable during composting” (for the purposes of this International Standard, these three expressions are considered to be equivalent). The labelling will, in addition, have to conform to all international, regional, national or local regulations (e.g., European Directive 94/62/EC). All these criteria apply for PHA as well as for other biomaterials like cellulose, starch, etc.

#### Other certificates applicable for PHA, cellulose, or starch (selection)

##### *Biobased:*

– ASTM D6866: Standard test methods for determining the biobased content of solid, liquid, and gaseous samples using radiocarbon analysis.























– Since 2013, the Japan Bioplastics Association (JBPA) offers the “BiomassPla” seal certification system as an identification system for plastics and other products of renewable origin (“Biomass-based plastic ratio requirement minimum 25 wt% of products measured by C14 measurement based on ASTM D6866-05”) (see Table 2).<sup>84</sup>

– The “OK Biobased” label, offered by TÜV Austria and created by Vinçotte, uses a star system to quantify the biobased carbon content of a certified product; one star on the label refers to 20–40 %, two stars indicate 40–60 %, three stars 60–80 %, and four stars more than 80 % biobased carbon.<sup>85</sup>

##### *Biocompatibility:*

– According to the standardized ISO 10993 norm, a material is “biocompatible” if it exerts no negative effect on the biosphere surrounding them (e.g., living organisms, cell lines, tissue, or ecosystems).

Table 2 – Certifications and labels for commercialized PHA Nodax™ PHA (Danimer Scientific),<sup>77</sup> KANEKA BIODEGRADABLE POLYMER PHBH™ (Kaneka Belgium NM),<sup>82</sup> Solon™ PHA (RWDC Industries),<sup>76</sup> and Mirel™ (Telles).<sup>78</sup> Table adapted from<sup>83</sup>.

Product:	Nodax™ PHA P(3HB-co-3HHx) produced from canola oil	KANEKA BIODEGRADABLE POLYMER PHBH™ (AONILEX®) P(3HB-co-3HHx) produced from plant oils by microbial fermentation	Solon™ PHA “Solon is a PHA polymer” (no more info provided by the company)	Mirel™ “Bioplastics by Telles” PHA copolyesters (“Mirel resins are biodegradable plastics made from sugar”; raw material: corn). P3HB and copolyesters were produced
<b>Biodegradability</b>				
Anaerobic conditions	ASTM D5511	YES (manufacturer information) <sup>82</sup>	YES (manufacturer information). “Solon will biodegrade in any natural environment.” <sup>76</sup>	ASTM D5511 ASTM D7081
In soil	ASTM D5988 	YES (manufacturer information) <sup>82</sup>		ASTM D5988-96 
In freshwater	ASTM D5271 EN 29408 	Information not provided by manufacturer		ASTM D5271 ISO 14851 (injection molded and extruded sheets) 
In marine environment (sea water)	ASTM D6691	Vinçotte certificates X151A, X131A, X331N; compliant with ASTM 7081		YES (manufacturer information) <sup>78</sup>
<b>Compostability</b>				
Industrial composting	ASTM D6400 EN 13432:2000 	ASTM D6400 and 6868 (certificates by BPI), EN 13432:2000; Vinçotte certificates X151A, X131A, X331N. Certified as “compostable” also in Japan by JBPA   		EN 13432 ASTM D6400 
Home composting	EN 13432:2000 (Note: under lower temperature conditions) 	EN 13432:2000; Vinçotte certificates X151A, X131A, X331N (Note: under lower temperature conditions) 		Injection molding grades P1003 and P1004 
<b>Biobased (Renewables)</b>				
	ASTM D6866 	ASTM D6866; Vinçotte certificates X151A, X131A, X331N. Certified as “biobased” also in Japan by JBPA  		ASTM D6866 
<b>Approval for food contact</b>				
	Approved by FDA and EFSA. <sup>84–86</sup>	Information not provided by manufacturer	Information not provided by manufacturer	Approved by FDA and EFSA (“F”-series of Mirel™ PHA: “Food contact”). <sup>84–86</sup>

*Aerobic biodegradability under controlled industrial composting conditions:*

– ASTM D5338: Standard test method for determining aerobic biodegradation of plastic materials under controlled composting conditions, including thermophilic temperatures

– ASTM D6400: Standard Specification for Compostable Plastics (industrial composting of PHA and other materials)

– ISO 20200: Plastics – Determination of the degree of disintegration of plastic materials under simulated composting conditions in a laboratory-scale test

– ISO 14855-1: Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions – Method by analysis of evolved carbon dioxide – *Part 1: General method*

– ISO 14855-2: Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions – Method by analysis of evolved carbon dioxide – *Part 2: Gravimetric measurement of carbon dioxide evolved in a laboratory-scale test*

– ISO 16929: Plastics – Determination of the degree of disintegration of plastic materials under defined composting conditions in a pilot-scale test

*Aerobic biodegradation in marine and other aquatic environments:*

– ASTM D6691: Standard test method for determining aerobic biodegradation of plastic materials in the marine environment by a *defined microbial consortium* or natural sea water inoculum

– EN 29408: Determination of the complete aerobic biodegradability of organic substances in an aqueous medium by determining the oxygen demand in a closed respirometer (ISO 9408: 1991)

*Aerobic biodegradation in soil:*

– ASTM D5511: Standard test method for determining anaerobic biodegradation of plastic materials under high-solids anaerobic-digestion conditions

– ASTM D5988: Standard test method for determining aerobic biodegradation of plastic materials in soil

*Aerobic biodegradation in waste water treatment systems:*

– ASTM D5271: Standard test method for determining the aerobic biodegradation of plastic materials in an activated-sludge-wastewater-treatment system (*Note: withdrawn in 2011!*)

*FDA and EFSA Approval*

In the context of using PHA for food packaging, etc., PHA have been certified by the U.S. Food

and Drug Administration (FDA) for use that comes into contact with food.<sup>86</sup> The frequently used expression “FDA approval” means that specific PHA forms can be used in food packaging, caps, utensils, tubs, trays, and hot cup lids, as well as houseware, cosmetic, and medical products. This also means that these PHA grades can be used to store frozen food, and can be used in microwaves and boiling water up to 212 °F.<sup>86</sup> According to European regulations, materials and articles must be inert, in order to prevent the transport of their constituents into foods at levels that endanger human health, and also to avoid altering of food physicochemical composition.<sup>87,88</sup> As mentioned previously, PHBHV copolyesters already achieved EU-wide EFSA food contact application approval in 1996.<sup>64</sup>

In the past, Mirel™ PHA F1005 and F1006 (“F”: for food contact) were the two food-contact injection molding grades commercialized by Telles (joint venture between Metabolix and Archer Daniels Midland), which obtained FDA approval in 2010 (“Mirel F1005 is FDA cleared for use in non-alcoholic food contact applications, from frozen food storage and microwave reheating to boiling water up to 212°F®). In 2014, PHA copolyesters with up to 25 % 3-hydroxyvalerate (3HV), 3-hydroxyhexanoate (3HHx), 3-hydroxyoctanoate (3HO), and/or 3-hydroxydecanoate (3HD) were also approved for use in the manufacture of food contact materials, except for use in contact with infant formula and human milk.<sup>68</sup>

In the context of biocompatibility, Tepha, Inc., USA, sells a range of PHA-based medical monofilament sutures, monofilament and composite meshes, and surgical films.<sup>89</sup> Among them TephaFLEX® sutures made of the highly flexible homopolyester poly(4-hydroxybutyrate) (P4HB), which is absorbed *in vivo* substantially faster than P3HB, were already approved by FDA for *in vivo* use.<sup>90</sup>

## Conclusions

Plastics pollution ranks right up there with climate change, since it affects the entire globe and all living beings, but none more so than our food chain, which is negatively impacted due to (micro)plastics being consumed by marine organisms thus endangering humanity’s wellbeing. Reducing or eliminating plastics pollution requires numerous novel approaches, such as innovations in recycling, including chemical recycling, but none ranks higher than introducing and using natural materials that are just as convenient as plastics and yet completely harmless to the environment at end of life due to their biodegradability.

PHA fall in this class of materials like cellulose, proteins and starch. PHA are circular due to their biodegradability characteristics, and due to the use of renewable sources to manufacture them. PHA have been thoroughly studied for biodegradability and have already demonstrated their expedient commercial potential. Today, several manufacturers are already producing these polymers and their use is growing rapidly.

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