Polyhydroxyalkanoates (PHA): Microbial Synthesis of Natural Polyesters

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Abstract Among materials emulating fossil plastics in functionality and processability, polyhydroxyalkanoates (PHA) stand out as the sole group that is completely integrated into nature's closed loop material cycle. Being biobased, biosynthesized, biodegradable, home and industrial compostable, and biocompatible, PHA biopolymers outperform competing polymeric materials labelled with "bio" attributes claiming sustainability. PHA biopolymers exhibit versatile material characteristics mimicking fossil plastics and are the most auspicious candidates to replace established fossil plastics, resins, and fibers.

Roughly 40% of all prokaryotic strains accumulate PHA biopolymers, and more than 150 different hydroxyalkanoate (HA) monomers that make up PHA biopolymers have been described, making PHA the most versatile family of biopolymers known to humankind. Commercially relevant PHA homo- and heteropolyesters include 3-hydroxybutyrate, 3-hydroxyvalerate, 3-hydroxyhexanoate, and 4-hydroxybutyrate monomers. However, numerous other PHA heteropolyesters having higher number of carbon atoms in the individual building blocks have been studied and found to have reasonably relevant functionalities. Furthermore, PHA biopolymers can also be used in numerous non-plastic applications such as being the source of optically pure chemicals or biologically active substances. Therefore, we currently stand only at the beginning of PHA discovery and industrialization.

While reducing plastic pollution, greenhouse gas emissions and climate change are the current drivers for intensified exploration and commercialization of PHA biopolymers, they have a far greater role to play than just being exploited due to their renewable nature and intrinsic biodegradability which have also been reviewed here.

As consumers, brand owners, converters, waste managers, and policy makers conceive and acknowledge the beneficial attributes of PHA biopolymers in this current wave of commercialization, the next wave consisting of PHA biopolymers for durable applications would irreversibly reduce fossil plastics use helping us to make a quantum leap in reducing plastic pollution, greenhouse gas emissions, and climate change-related to fossil plastics use.

1 Introduction

By affecting our environment and the quality of our life, pollution from plastics has become an urgent issue of our times, ranking right behind Climate Change. Innovation in recycling—mechanical or chemical—continues to make headway; however, recycling rates have remained at around only 9% of all plastics discarded for over a decade, thus clearly showing that current policies and pure market economics are insufficient in increasing recycling rates. It is obvious that mitigating plastic pollution would require a paradigm shift; simply redesigning our use of plastics to reduce consumption or dramatically improving recovery and recycling is insufficient. It will also require significantly expanding waste plastics collection and processing. One solution is using materials that mimic plastics in processing, applicability, and functionality but, at the same time, having environmentally friendly end-of-life options. Therefore, materials are needed that can not only be recycled and reused; they must also have the inherent ability to biodegrade in our environment and not harm our ecosystem if they leak into soil or the aquatic environment (Koller 2019a).

One such material that is ubiquitous in our lives is cellulose, a biopolymer, which is extracted from wood and cotton and is used as viscose, rayon, paper, and other important articles. While the processes to arrive at such utilitarian materials are complex and sometimes involve toxic chemicals, humankind has learned to control them well. Cellulose leaking into the environment biodegrades readily. It also composts, can be recycled, and is renewable. Exactly the same way, polyhydroxyalkanoates (PHA), a versatile class of natural and microbially produced biopolymers (Rehm 2010), act like cellulose in the environment; they biodegrade, they compost, they can be recycled, they are biosynthesized, and they are renewable. However, in contrast to cellulose and other well-known biopolymers, they also have plastic-like functions and processing features, many of which we have learned to appreciate and rely on. Moreover, if erroneously entering thermic recycling systems (incineration), PHA do not harm the environment, in contrast to, e.g., poly(vinyl chloride) (PVC) (Koller and Mukherjee 2020).

2 PHA: General Aspects

2.1 Early Discovery of PHA

PHA's discovery and commercialization has a history similar to many common substances we derive today from fossil carbon, although the protocols for their biosynthesis via fermentation were developed already a century ago. For example, the famous *Clostridia*-based Weizmann process for production of acetone, isopropanol, and 1-butanol starting from renewable feedstocks was used on large scale already during World War I. The discovery of PHA even dates back as far as

1888, when the renowned Dutch microbiologist Martinus Willem Beijerinck observed light-refractive inclusions inside microorganisms that differed from endospores under a light microscope. However, unable to imagine what waves these inclusion bodies will make in the scientific community many decades later, he did not further examine them. Only decades later, Beijerinck's experiments were repeated, and the inclusions were identified as PHA (reviewed by Chowdhury 1963). This was almost 40 years after Beijerinck's observations, when in 1923 Maurice Lemoigne from the Institute Pasteur, the most frequently cited pioneer of PHA, described the excretion of 3-hydroxybutyrate (3HB) by resting *Bacillus* "M" cells under anaerobic conditions (Lemoigne 1923). In 1925, Lemoigne performed quantitative studies of this product and theorized 3HB to be a degradation product of an intracellular polymer (Lemoigne 1925). Indeed, his assumption was correct, and in 1927, he succeeded in isolating solid poly(3-hydroxybutyrate) (P3HB) by chloroform extraction and elucidated its empirical chemical formula $(C_4H_6O_2)_n$ (Lemoigne 1927).

Nevertheless, PHA, despite their beneficial properties, went off the radar of science due to the rapid development of fossil plastics. Interest in PHA started rising again in the 1950s with the elucidation of their predominant biological role as energy and carbon storage in cells of *Bacillus megaterium* and *Bacillus cereus*, their intracellular circularity and their thermoplastic-like properties (Macrae and Wilkinson 1958). An original citation from the abstract of their article postulated for the first time: "The evidence that poly-β-hydroxybutyrate is a reserve carbon and energy source is discussed." In this key study, the authors claimed for the first time the fundamentals of PHA biosynthesis and intracellular degradation:

1. "It (PHA; note by authors) should best be biosynthesized in an environment containing excess external carbon and energy source."
2. "The supposed carbon and/or energy source (PHA; note by authors) should be capable of being broken down in the absence of an external carbon and energy source."
3. "The products of breakdown of the storage compound (PHA; note by authors) is capable of being used as a source of carbon and/or energy to prevent cell autolysis and death."

The recognition that PHA biopolyesters are "materials with plastics-like properties from living organisms" was again almost dismissed as being of merely academic relevance until the work of Prof. Gerhart Braunegg in the 1970s considered one of the pioneers of the modern PHA efforts (and the doctoral advisor of one of the authors of this review) and many other innovative researchers at that time. Prof. Braunegg carried on his pioneering work despite being told as a young scientist at a scientific conference that "plastics are produced from petroleum, not by bacteria" (personal communication G. Braunegg). However, his conviction and labor were rewarded when Imperial Chemical Industries (ICI), UK, in 1989 introduced the first commercial PHA biopolymer and Unilever started making bottles from it to fill in shampoo. Industrial economics, however, won the battle at that time. Given the low price of fossil fuels and plastics thereof in comparison to the costs necessary for

producing PHA biopolymers, ICI stopped their PHA production. It was not until the 1990s when the level of plastic pollution and the beneficial properties of PHA could no longer be ignored, thus triggering companies like PHB Industrial S.A., Monsanto, Metabolix, Proctor and Gamble, or Kaneka to start industrial PHA biopolymer research and production.

The first PHA discovered was the polymer of the chiral repeat unit (monomer) (*R*)-3-hydroxybutyrate (3HB). This poly(3-hydroxybutyrate) (abbreviated in the literature as PHB or P(3HB)) is the homopolyoxoester of the monomer 3HB. P (3HB) is a natural biopolymer just like nucleic acids, proteins, cellulose, starch, or chitin. The 3HB monomer also occurs in the human metabolism as one of the ketone bodies; also, its di- and trimers are rapidly metabolized in vivo and can therefore be used as a carbon and energy source for severely injured persons suffering from hyperglycemia and could replace glucose infusions especially in patients suffering from diabetes (Tasaki et al. 1999; Chen and Wu 2005). In animals and humans, 3HB is not only an intermediate metabolite but also plays an important regulatory role by influencing gene expression, lipid metabolism, neuronal functions, and the overall metabolic rate as recently reviewed by Mierziak et al. (2021). Oligomeric P(3HB) has been found also in many organisms (Seebach et al. 1994) where they can form complexes with polyphosphate and act as a calcium transport channel in membranes (Reusch et al. 1995; Seebach et al. 1996).

2.2 PHA Are Biosynthesized

2.2.1 "Biopolymer" versus "Bioplastic"

We used the term "biopolymer" above which is defined as any polymeric substance that is formed inside a living organism, hence, "biosynthesized". Such biosynthesized materials are typically also biodegradable. Polymers that are both biobased and can biodegrade are defined by Merriam-Webster as a "bioplastic," thereby implying that biopolymers and bioplastics are one and the same. Webster also defines plastics as materials of chemoorganic synthetic origin that can be formed into different shapes by application of heat. Therefore, in the narrowest sense, the word "bioplastics" is wrongly defined, since plastics are synthetically produced, and they do not biodegrade in nature. The expression "bioplastics" was first used in 1989; however, since then that definition has degenerated into including all materials that are either biodegradable or renewable or both. The Swiss Academy of Technical Sciences elaborated a factsheet on bioplastics illustrating the problematic terms used in industry (online resource 1 n.d.). The authors of present chapter, therefore, have restricted themselves to the terms "biopolymers" (not "bioplastics") and "plastics," respectively, to differentiate between naturally synthesized and biodegradable polymers and synthetic fossil polymers that do not biodegrade.

For illustration, materials like polylactic acid (PLA) and poly(butylene succinate) (PBS), and sometimes poly(ε-caprolactone) (PCL) and poly(butylene adipate terephthalate) (PBAT) are also described as "bioplastics," while none of these are either

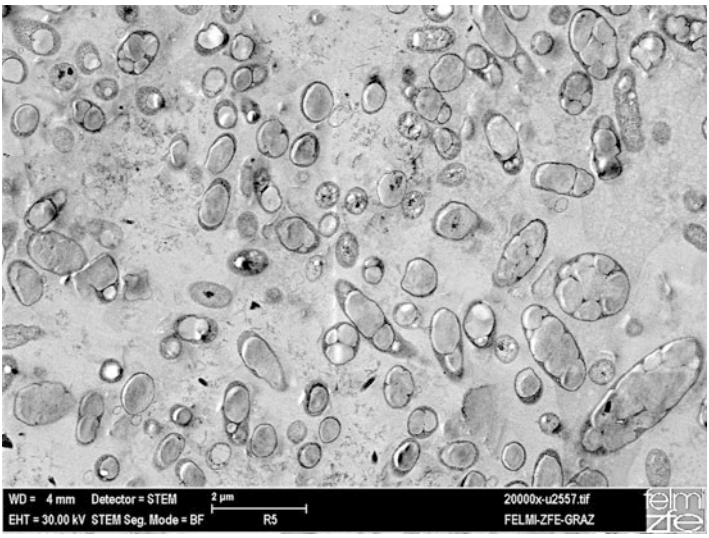


Fig. 1 PHA carbonosomes observed in *Cupriavidus necator* cells as bright inclusions. Magnification: 20,000×. (STEM picture prepared and provided with courtesy by E. Ingolić, FELMI-ZFE Graz)

biopolymers or bioplastics *sensu stricto* (Koller and Mukherjee 2020). This degeneration of definitions has created confusion among consumers, in the industry, and in academia. For example, PLA is synthetically polymerized from lactic acid, and lactic acid is a natural product. Nevertheless, PLA is not a natural material (it is not biosynthesized), nor is it a biopolymer (it does not occur in nature). PCL and PBAT are synthetic polymers (production based on fossil feedstocks), while PCL biodegrades in nature, and PBAT can be composted in industrial composters. PBS is partially renewable and biodegrades in certain environments. These subtle yet important differences between these polymers, which are often thoughtlessly and carelessly summarized as "bioplastics," and the human tendency to generalize have given rise to ambiguous definitions such as "bioplastics." Indeed, no single category that can exactly and unambiguously define PLA, PBS, PBAT, and PCL exists. Therefore, the use of the term "bioplastics" will be avoided in this chapter.

Having set the confusions with definitions straight, the revision of PHA can continue. PHA are biopolymers since they are formed inside living organisms and are therefore biodegradable; this is the most important aspect in differentiating PHA biopolyesters from PLA, PCL, PBAT, PBS, etc. In the case of PHA, microbes organize not only the conversion of renewable feedstocks to monomers (hydroxyalkanoates) but also polymerize them to synthesize the PHA biopolyesters in granular organelle-like cell components, which are about 200–500 nm in size and easily visible during microscopic observation (Fig. 1). More recently, these inclusions, having a hydrophobic PHA core and a hydrophilic proteinaceous surface layer, are termed "carbonosomes" (Jendrossek 2009). The most recognized model describing their intracellular formation, the so-called scaffold model, hypothesizes

that complexes consisting of P(3HB) synthase and the protein PhaM are linked to the bacterial nucleoid, forming the PHA granule initiation complex (Jendrossek and Pfeiffer 2014).

In 1944, Lemoigne and co-workers demonstrated that the microscopically visible lipophilic inclusions already observed more than 50 years ago by Martinus Willem Beijerinck indeed contain the polymeric materials that were extractable from the cells using chloroform (Lemoigne et al. 1944). The final proof for the nature of these inclusions was provided by Weibull in 1953, when he studied isolated PHA granules prepared by dissolving the *Bacillus megaterium* cell wall using lysozyme (Weibull 1953). Inside living cells, PHA inclusions reside in neither solid nor liquid form but rather in a mobile amorphous elastomeric state and do not resemble crystalline structures seen after extraction and having gone through a heat history; this was already assumed in the 1980s (Barnard and Sanders 1988) and early 1990s (de Koning and Lemstra 1992). Actually, there had been two hypotheses explaining the presence of P(3HB)'s thermodynamically unfavorable amorphous state in native granules. The "kinetic model" predicted the low crystallization rate of polymer in small native granules, while the "plasticizer model" relied on the presence of unknown molecules in native granules, which prevented granules from crystallization (Bonthrone et al. 1992). In 2019, it was experimentally proven that both theories are correct and that the amorphous state of intracellular PHA is kinetically (a) stabilized by the low rate of crystallization in limited volume in small PHA granules, and (b) that water present in PHA granules acts as a plasticizer, thus preventing the polymer from transition into the thermodynamically favorable crystalline state (Sedlacek et al. 2019a).

As mentioned before, polymers generally consist of many repeat units, such as 3HB in the case of P(3HB). This homopolymer is a linear (polymer chains do not entangle), waterproof, microwaveable, absolutely isotactic (stereoisomeric; chains easily crystallize) polyester with thermoplastic properties and a glass transition temperature (T_g) of around 0 °C. Hence, polymer chains keep on moving and crystallizing even at room temperature. As mentioned above, P(3HB) was the first described member of the PHA family, discovered already in 1888 by Beijerinck (reviewed by Chowdhury 1963).

Catalysts driving the conversion of raw materials to PHA are exclusively intracellular biocatalysts, namely, the enzymatic toolbox catabolizing heterotrophic or autotrophic substrates to acetyl-CoA and other PHA precursors. The enzymes, converting acetyl-CoA and related compounds to PHA, viz., 3-ketothiolase (a.k.a. acetyl-CoA-acetyltransferase, β-ketoacyl-CoA thiolase, or thiolase II, EC 2.3.1.9), catalyze the condensation of the C2-compound acetyl-CoA to the C4-compound acetoacetyl-CoA and are encoded on the *phaA* gene. Other enzymes include NADPH-dependent 3-ketoacyl reductase (EC 1.1.1.36) encoded on the *phaB* gene, which enables the "pseudofermentative" reduction of acetoacetyl-CoA to (*R*)-3-hydroxybutyryl-CoA to regenerate $NADP^+$, and PHA synthases (a.k.a. PHA polymerases, EC 2.3.1.-) encoded on *phaC* genes, which are responsible for the polycondensation of (*R*)-3-hydroxybutyryl-CoA to P(3HB). Based on their substrate specificity and structure of subunits, PHA synthases were originally grouped in three types or classes, namely, type I, II, and III. Types I and III PHA

synthases are responsible for the biosynthesis of short-chain-length PHA (*scl*-PHA) like P(3HB) or poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (P(3HB-*co*-3HV)) in most technologically relevant PHA production strains (Rehm 2003). Later, PHA synthases found in *Bacillus* sp. were recategorized as a new PHA synthase type IV due to considerable structural differences to type III synthases; such type IV synthases preferably polymerize *scl*-monomers such as 3HB and 3HV but can also utilize *mcl*-monomers as minor building blocks (Tsuge et al. 2015).

In the early 1980s, a second class of PHAs had been found by Witholt and co-workers at University of Groningen, the Netherlands (de Smet et al. 1983; Karmann et al. 2019). This so-called medium-chain-length PHA (*mcl*-PHA) consists of monomers having carbon units between 6 and 14 units. The material properties are found to range from thermoplastic elastomers to fluidoplastics but with the suitable functional fatty acid as growth substrate; also thermosets and rubber-like polymers had been produced with wild-type strains mainly belonging to *Pseudomonas* sp. Interestingly, these *mcl*-PHA-accumulating strains were also isolated from the environment (e.g., *Pseudomonas guezennei*, Simon-Colin et al. 2008) and appeared to be part of microbial mats as it is the case of P(3HB) producing bacteria as shown by the pioneering research by D.C. White at the University of Tennessee, USA (Findlay et al. 1990).

Organisms tested on lab or pilot scale for PHA production on gaseous substrates (CO_2, CO, CH_4) possess the enzymatic toolbox to convert these gases to organic building blocks, instead of or in addition to the enzymes needed for conversion of sugars (enzymes involved in glycolysis (Emden-Meyerhof-Parnas pathway), the KDPG pathway (Entner-Doudoroff pathway), or hydrolases for conversion of di- and polysaccharides to convertible monosaccharides, fats (lipases, enzymes needed for β-oxidation), etc.).

Intracellular PHA depolymerases (encoded on *phaZ* genes), in turn, degrade the stored PHA to carbon and energy substrates under conditions of starvation, thus serving for the "cyclic nature of PHA metabolism" (Doi et al. 1990). In addition, extracellular PHA depolymerases (EC 3.1.1.75 and EC 3.1.1.76), belonging to the group of carboxylesterases, are excreted by many bacteria or fungi, are less specific than their intracellular counterparts, and serve for biodegradation of spent PHA articles when composted. Hence, in the environment where microorganisms secrete PHA depolymerases, those microorganisms are basically predating on PHA originated from lysed cells (reviewed by Jendrossek and Handrick 2002).

Figure 2 visualizes the principle metabolism of PHA biosynthesis starting from model substrates, indicating the reactions catalyzed by enzymes running PHA biosynthesis and intracellular degradation.

2.3 PHA Play Multifaceted Roles in Nature

PHA are important constituents of the organisms that produce them. These carbonosomes (Jendrossek 2009) where the PHA are stored primarily act as carbon

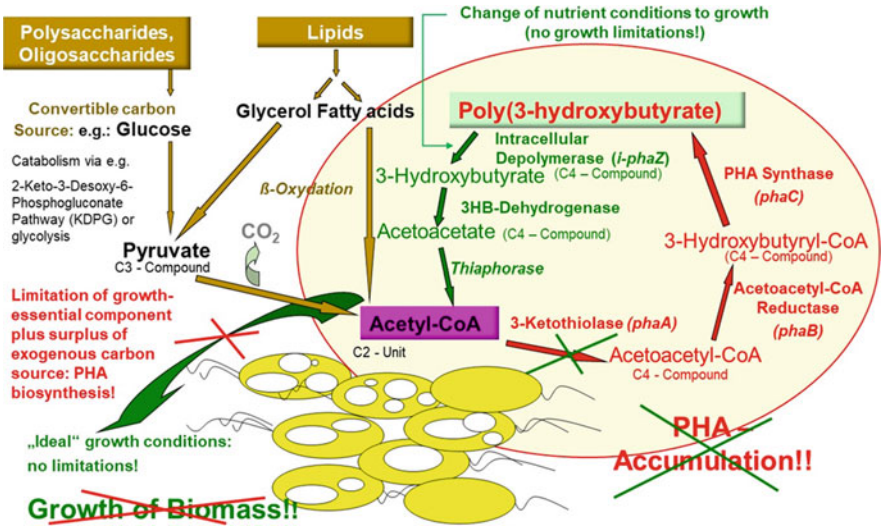


Fig. 2 Schematic of intracellular P(3HB) homopolyester biosynthesis and degradation. *Brown arrows:* catabolism of substrates to acetyl-CoA, the central metabolite for both biomass formation and P(3HB) biosynthesis. *Green arrows* indicate balanced microbial growth under not nutritionally limited (balanced) cultivation conditions and intracellular mobilization of PHA reserves, respectively; *red crosses* symbolize stop of biomass growth by the onset of limitation of growth-essential nutrient. *Red arrows* indicate P(3HB) biosynthesis under nutritional stress (e.g., limitation of nitrogen or phosphate source); *green crosses*, in turn, symbolize inhibition of P(3HB) formation when nutrients are balanced again

and energy storage compounds to support the cells during periods of starvation (Macrae and Wilkinson 1958). Moreover, in many PHA-producing sporulating organisms like *Bacillus cereus* or *Bacillus megaterium*, the onset of endospore formation depends on intracellular carbon and energy supply accomplished by the degradation of the PHA pool (Sadykov et al. 2017; Valappil et al., 2008), while in *Azotobacter* sp., PHA serve as an electron sink to regulate local oxygen levels (Dawes and Senior 1973).

In addition, PHA granules also act as important protectants by supporting the cells when they are exposed to challenging stress conditions, such as sudden osmotic imbalance, excessive UV radiation, oxidative, or thermal (both heat and frost) stress. During these challenging events, cells harboring PHA have a survival advantage in comparison to PHA-free cells, as it was demonstrated by exerting both PHA-accumulating wild-type strains and their PHA-free mutant strains to abovementioned stress factors. This complex interrelation of PHA to the SOS response of cells to challenging conditions has only recently been expansively studied, and review articles on the multifaceted roles of PHA in the survival of microorganisms, which goes far beyond avoiding starvation during lack of exogenous carbon supply, have been written during the last few years (Obruca et al. 2018, 2020, 2021; Müller-Santos et al. 2020).

In addition to their roles as carbon and energy stocks and stress protectants, the biological functions of PHA encompass their importance as an electron source to drive reductive sulfate respiration in purple and green sulfur bacteria, such as *Desulfovibrio*, *Desulfobacterium*, *Desulfococcus*, *Chromatium*, or *Amoebobacter* (today: *Thiocapsa*), which enables their anoxygenic photosynthesis (Hai et al. 2004). Moreover, in nitrogen-fixing (diazotrophic) microbes, PHA biosynthesis serves for maintaining energy production and NAD^+ recovery; hence, they perform a "pseudofermentative" reaction for the necessary oxidation of redox equivalents (Encarnación et al. 2002).

Interestingly, PHA also play pivotal roles in several symbiotic or syntrophic relationships between bacteria and other organisms. For example, PHA metabolism plays an important role between different bacterial species, as demonstrated for microbial mats by the syntrophic action of heterotrophic PHA-producing species (e.g., *Labrenzia* sp., *Halomonas* sp.), which, during the day, convert organic compounds excreted by photosynthetic cyanobacteria into PHA. These cyanobacteria, in turn, generate these organic compounds at night by converting CO_2 generated by oxidative PHA degradation in the heterotrophic bacteria, when no exogenous carbon sources are available for them. Hence, each species maintains the metabolism of the other running at different times of the day and night (Villanueva et al. 2007). Moreover, the role of PHA metabolism of the betaproteobacterium *Herbaspirillum seropedicae* in promoting the growth of the millet *Setaria viridis* was described, showing the significance of PHA biosynthesis in symbiosis between bacteria and plants (Alves et al. 2019). In addition, in 2013, Kim et al. demonstrated that PHA biosynthesis and accumulation in microbial symbiont cells are required for normal symbiotic association with and beneficial effects for the insects that symbiotically host the microbes due to the environmental conditions within the host, which are, to a certain extent, stressful for the bacteria (Kim et al. 2013).

Besides high-molecular-mass PHA, it was reviewed by Jendrossek and Pfeiffer (2014) that oligo-P(3HB), consisting of about 100–200 3HB monomers, as well as P(3HB) consisting of about 30 3HB units covalently bound to proteins ("conjungated P(3HB)", cPHB) presumably exist in all organisms, are supposed to have functions different to storage P(3HB), and might be of vital importance, but their biological functions are not fully elucidated yet (Table 1).

2.4 PHA Production Strains: Bacteria and Archaea as Cell Factories for Biopolymer Production

Natural PHA production strains are found in environments as diverse as soil in the temperate zone as well as polar regions and desserts, the rhizosphere of plants, marine water and their sediments, salt lakes, freshwater lakes, estuaries, activated sludge, the vicinity of submarine volcanoes, and many more. Many of them are mesophilic organisms concerning their optimal pH-value, temperature, halophilicity,

Table 1 The multifaceted role of high-molecular-mass PHA for microbes producing them

Role	Reference
Carbon and energy storage	Macrae and Wilkinson (1958)
Electron sink for intracellular oxygen regulation	Dawes and Senior (1973)
Electron source for sulfate respiration in purple and green sulfur bacteria	Hai et al. (2004)
Substrate for the onset of sporulation in Bacilli	Valappil et al. (2008)
Protectant against hypertonic salinity	Obruca et al. (2017)
Protectant against sudden osmotic imbalances	Sedlacek et al. (2019b)
Oxidation protectant	Obruca et al. (2010)
Heat protectant	Obruca et al. (2016a)
Freeze/thawing protectant	Obruca et al. (2016b)
UV protectant	Slaninova et al. (2018)
Enabling symbiosis between bacteria and other microbes	Villanueva et al. (2007)
Enabling symbiosis between bacteria and plants	Alves et al. (2019)
Enabling symbiosis between bacteria and insects	Kim et al. (2013)
"Pseudofermentative" effects by maintaining energy production and NAD^+ recovery of nitrogen-fixing bacteria	Encarnación et al. (2002)

and substrate concentration. The currently most used organisms for industrial-scale PHA production are, as far as disclosed by the manufacturers, mesophilic soil bacteria, namely, *Cupriavidus necator*, *Azohydromonas australica* (former *Alcaligenes latus*), *Pseudomonas* sp., *Aeromonas* sp., or *Paraburkholderia sacchari* (formerly *Burkholderia sacchari*). Only recently, transformant strains of the halophile *Halomonas* sp. are used by new companies established in PR China to produce P(3HB) and its copolyesters with 3-hydroxyvalerate (3HV), 4-hydroxybutyrate (4HB), and 3-hydroxyhexanoate (3HHx). However, the number of excellent wild-type PHA production strains well adapted to heavily challenging environmental conditions, such as salinity exceeding 5 M NaCl (Koller 2019b), temperatures around the freezing point of water (*Pseudomonas extremaustralis* thrives well at temperatures between 4 and 37 °C (López et al. 2009)) or above 50 °C (*Schlegelella thermodepolymerans* has its temperature optimum at 55 °C (Kourilova et al. 2020); *Thermus thermophilus* showed PHA biosynthesis at 75 °C (Pantazaki et al. 2003)), and pH-values below 4 (acidophiles; *Bacillus cereus* accumulates increased amounts of PHA at pH 4.5–5.8 (Valappil et al. 2008)) and above 11 (alkaliphiles; for *Spirulina platensis*, the optimum pH-value for PHA production is 9–11 (Jau et al. 2005)), is steadily growing in the scientific literature (reviewed by Karthikeyan and Mehariya 2021).

From the microbiological point of view, microbes capable of PHA biosynthesis are found in both prokaryotic domains *Bacteria* and *Archaea*. Among bacteria, PHA biosynthesis is described for both Gram-negative and Gram-positive strains; for

obligate chemoheterotrophs; for diverse oxygenic or anoxygenic photoautotrophic species like members of the cyanobacteria (prominent genera: *Spirulina*, *Nostoc*, *Synechocystis, Synechococcus*, *Anabaena*, *Chlorogloea*, *Aulosira*, etc.; Singh et al. 2017), sulfur bacteria, or purple non-sulfur bacteria (*Rhodospirilli*; Klask et al. 2015); and for some type II methanotrophs (e.g., *Methylomonas* sp.; Pérez et al. 2019). Regarding PHA-producing representatives from the *Archaea* domain, these organisms are typically extreme extremophiles, such as the salt-requiring members of the haloarchaea group, which encompasses several potent PHA-producing genera, such as *Haloferax*, *Halorubrum*, *Halogeometricum*, *Haloarcula*, or *Natrinema* (Koller 2019b; Karray et al. 2021). Besides the ample prokaryotes producing PHA, there are a limited number of reports in the literature describing PHA production by eukaryotic wild-type strains, such as yeasts (Abd-El-Haleem 2009) and microalgae (García et al. 2021); PHA production by genetically engineered plants (Poirier and Brumbley 2010) and other higher organisms is out of scope of the present text. Although PHA biosynthesis typically is an aerobic process, some reports are available describing anaerobic PHA accumulation by members of the genus *Clostridium*, which, in the future, might attract interest in such processes for the co-production of PHA and well-known clostridial metabolites like solvents and protein-rich biomass (Flüchter et al. 2019).

Table 2 provides an overview of most important PHA production strains; feedstocks used by them for production of the industrially important PHA types P(3HB), poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (P(3HB-*co*-3HV)), poly (3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P(3HB-*co*-4HB)), and poly (3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (P(3HB-*co*-3HHx)); and natural environments of their original isolation. Species of current or near-future industrial significance for PHA production are presented in bold.

2.5 Renewable Resources as Feedstocks for PHA Production

The diverse group of natural PHA production strains described above all convert renewable raw materials into biomass and PHA. Carbohydrates are by far the most popular and suitable carbon substrates for PHA biosynthesis; monosaccharides, especially glucose and fructose, are converted by the majority of heterotrophic PHA producers, while the number of strains able to convert disaccharides (lactose, sucrose, maltose, etc.) are considerably lower. For example, lactose (present in concentrations of about 4 wt% in the surplus product whey) is readily converted without the need for prior hydrolysis to PHA by strains expressing the enzyme β-galactosidase (EC 3.2.1.23) like *Hydrogenophaga pseudoflava* (Koller et al. 2007a) or *Bacillus megaterium* (Obruca et al. 2011), while for direct sucrose conversion, the enzyme invertase (EC 3.2.1.26) is needed, present in PHA producers *Hfx. mediterranei* (Koller et al. 2012), *P. sacchari* (Miranda de Sousa Dias et al. 2017), and *Azotobacter vinelandii* (Page 1992). For direct use of polysaccharides, such as starch, the production strain needs to display α-amylase (EC 3.2.1.1) activity,

Table 2 Overview of most important PHA production strains; feedstocks used by them for production of the industrially important PHA types P(3HB), poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (P(3HB-*co*-3HV)), poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P(3HB-*co*-4HB)), and poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (P(3HB-*co*-3HHx)); and natural environments of their first isolation

Type of PHA	Feedstocks	Natural microbial production strains				
		Gram-negative bacteria (selection)	Gram-positive bacteria (selection)	Cyanobacteria (selection)	Archaea (selection)	Eukaryotes
P(3HB)	Fructose, glucose, glycerol; sucrose (beet and cane; molasses, green syrup), waste cooking oil; CO_2; CH_4; biodiesel; hydrolyzed whey lactose; starch; syngas; spent coffee grounds	*Cupriavidus necator* (*former Ralstonia eutropha*); *Azohydromoas australica* (*A. lata*; former *Alcaligenes latus*); *Paraburkholderia sacchari*; *Halomonas* sp.; *Methylobacterium* sp., *Methylomonas* sp.; *Paraburkholderia fungorum*; *Hydrogenophaga pseudoflava*; *Saccharophagus degradans*; *Rhodospirillum rubrum*	*Bacillus megaterium*, *Bacillus cereus*; *Clostridium beijerinckii* ASU10	*Synechocystis* sp. PCC 6803 and MA19; *Chlorogloea fritschii*; *Gloeothece* sp. PCC 6909; *Oscillatoria limosa*; *Nostoc muscorum*; *Spirulina platensis*; *Spirulina subsalsa*; *Anabaena cylindrica*; *Aulosira fertilissima*; *Phormidium* sp. TISTR 8640	*Haloarcula marismortui*; *Haloarcula* sp. IRU1; *Halogeometricum borinquense* TN9; *Halococcus saccharolyticus*; *Haloterringena hispanica*; *Halobiforma haloterrestris*; *Natronobacterium gregoryi*; *Haloquadratum walsbyi*	*Scenedesmus* sp. (microalga)

(continued)

Table 2 (continued)

Type of PHA	Feedstocks	Natural microbial production strains				
		Gram-negative bacteria (selection)	Gram-positive bacteria (selection)	Cyanobacteria (selection)	Archaea (selection)	Eukaryotes
Location of strain isolation	Bacteria:					
	– *C. necator:* soil bacterium; first ancestor isolated as *Hydrogenomonas eutropha*; originally *isolated from a lawn soil* at the US-East Coast, probably state of NY, by researchers from the University of California (Schatz and Bovell 1952). Makkar and Casida (1987) isolated an *Alcaligenes eutrophus* strain (obtained from soil in the vicinity of University Park, PA, USA), which was later reclassified to *Cupriavidus necator* (Vandamme and Coenye 2004)					
	– *Azohydromonas australica*: isolated as "two strains (H-1 and H-4; today DSM 1122 and 1123) of the new organism . . . were isolated from soil samples collected from two widely separated sites of the Berkeley campus of the University of California" (Palleroni and Palleroni 1978); termed as *Alcaligenes latus* in this publication, today *Azomonas lata* in DSM strain collection. These strains showed high similarity with a strain isolated from a soil sample collected at Orara Reserve, Manly Vale, Australia (DSM 1124; basis for new species name *Azomonas australica*). Today, it is assumed that these organisms belong to the same species (Xie and Yokota 2005). Strains DSM 1123 and 1124 were comprehensively compared many years ago for their PHA accumulation performance on the inexpensive agricultural raw materials starch hydrolysate (maltose), green syrup (intermediate of sucrose production), and beet molasses (Braunegg et al. 1999)					
	– *Paraburkholderia sacchari* (DSM 17165): isolated as *Burkholderia sacchari* IPT 101 by Gomez et al. (1996) (researchers from IPT—Instituto de Pesquisas Tecnológicas) in Brazil from soil of sugar cane plantation					
	Halomonas sp. (ancestor of the currently utilized engineered *Halomonas bluephagenesis* strains developed by the group pf George Chen): isolated as *Halomonas* TD01 from solid and water samples collected from Aydingkol salt lake in Xinjiang, PR China (Tan et al. 2011; team of George Chen)					
	– *Bacillus megaterium*: isolated as soil bacterium with various subspecies (e.g., DSM 90, DSM 333, etc.) and from other environments (e.g., petroleum effluents); strain *Bacillus megaterium* uyuni S20 (well-described PHA producer) was originally isolated from a Bolivian salt lake (Rodriguez-Contreras et al. 2013). Origin of other important *Bacillus megaterium* ssp. (e.g., *B. megaterium* KM or Maurice Lemoigne's strain, by which PHA was discovered) remains unclear					
	Bacillus cereus: isolated from soil samples, milk or whey (Pirttijärvi et al. 1998)					
	Cyanobacteria:					
	– *Synechocystis* sp. MA19: isolated from a wet volcanic rock in Japan (Miyake et al. 1996). *Synechocystis* sp. PCC 6803: isolated from "natural samples". Origin of other *Synechocystis* sp. according to DSMZ: "sediments", "water column", etc.					
	– *Chlorogloea fritschii*: isolated from Indian soil samples (Mitra 1950)					

	– *Nostoc muscorum* ("inhabits both terrestrial and freshwater aquatic environments" (Cameron 1960))					
	– *Spirulina platensis*: natural water organism (according to DSMZ)					
	– *Spirulina subsalsa* (according to DSMZ: "Isolated from sediments from the Netherlands Schiermonnikoog")					
	Archaea (reviewed by Koller 2019b):					
	– *Haloarcula marismortui*: isolated from Dead Sea					
	– *Haloarcula* sp. IRU1: isolated from hypersaline Lake Urmia, Iran					
	– *Halogeometricum borinquense*: isolated from solar salterns of Marakkanam in Tamil Nadu, India					
	– *Halococcus saccharolyticus*: isolated from salt in Cadiz, Spain					
	– *Halobiforma haloterrestris*: isolated from the surface of hypersaline soil collected in Aswan (Egypt)					
	– *Natronobacterium gregoryi*: isolated from soda salt lake liquors from the East African Magadi soda lake					
	– *Haloquadratum walsbyi*: isolated at the Egyptian Sinai Peninsula and from saltern crystallizers in Australia and Spain					
P (3HB-*co*-3HV)	Fructose, glucose, glycerol; sucrose (beet and cane; molasses); hydrolyzed whey lactose; plus 3HV-precursor for all bacterial strains	*Cupriavidus necator*; *Azohydromonas australica*; *Halomonas* sp.; *Paraburkholderia fungorum*; *Hydrogenophaga pseudoflava*		*Oscillatoria limosa*; *Anabaena cylindrica*; *Phormidium* sp. TISTR 8640; *Aulosira fertilissima*; *Anabaena spiroides* TISTR 8075 (no 3HV-precursors needed; copolyester production from CO_2 as sole carbon source; Tarawat et al. 2020)	*Hfx. mediterranei*; *Haloarcula hispanica*; *Halogeometricum borinquense* E3; *Halobacterium noricense*; *Halococcus dombrowskii*; *Hcc. hamelinensis*; *Hcc. morrhuae*; *Hcc. qingdaonensis*; *Hcc. salifodinae*; *Halorubrum chaviator*; *Hrr. coriense*; *Natrinema ajinwuensis* (=*altunense*); *Nnm. palladium*; *Natronococcus occultus*	*Rhodotorula minuta* Y4 (yeast)
	Note: Archaea listed in this row: copolyester production without 3HV-precursor addition on biodiesel; hydrolyzed whey lactose; extruded rice bran; stillage; hydrolyzed sugarcane bagasse, hydrolyzed cassava bagasse					

(continued)

Table 2 (continued)

Type of PHA	Feedstocks	Natural microbial production strains				
		Gram-negative bacteria (selection)	Gram-positive bacteria (selection)	Cyanobacteria (selection)	Archaea (selection)	Eukaryotes
Location of strain isolation	– *Paraburkholderia fungorum* (DSM 1749): before known as *Pseudomonas hydrogenovora*; isolated as "strain 9–5" by Kodama et al. (1975) at Department of Agricultural Chemistry, University of Tokyo from "samples of soil, river water or sewage" (1 of 100 strains isolated from about 200 natural samples). Other subspecies: *P. fungorum* DSM 17061 was isolated from fungus *Phanerochaete chrysosporium* (according to DSMZ; country unknown)					
	Archaea (reviewed by Koller 2019b):					
	– *Haloferax mediterranei*: isolated from evaporation ponds of solar salterns at the Spanish coast near Alicante as "strain Q4" by Rodríguez-Valera et al. (1980)					
	– *Halogeometricum borinquense* E3: isolated from solar salterns of Marakkanam in Tamil Nadu, India					
	Halobacterium noricense: isolated from a bore core of an Austrian Permian salt deposit					
	– *Halococcus dombrowskii*: isolated from dry rock salt from Austrian alpine salt mine					
	– *Hcc. hamelinensis:* isolated from stromatolites from the Hamelin Pool in the Australian Shark Bay					
	– *Hcc. morrhuae*: Dead Sea isolate					
	– *Hcc. qingdaonensis:* isolated from a crude sea-salt sample collected near Qingdao, PR China					
	– *Hcc. salifodinae*: isolated from alpine rock salt in Austria					
	– *Halorubrum chaviator*: isolated from sea salt in Baja California, Mexico, Western Australia, and Greece					
	– *Hrr. coriense*: isolated from the Dead Sea; *Natrinema ajinwuensis* (=*altunense*): isolated from salt production pans, India					
	– *Natrinema palladium*: isolated from Kayacik saltern, Turkey					
	– *Ncc. occultus*: isolated from the Lake Magadi, Kenya					
P (3HB-*co*-4HB)	Sucrose + 4HB-precursor; glucose + precursor; glycerol + precursor	*Cupriavidus necator*; *Paraburkholderia sacchari* (former *Burkholderia sacchari*); *Delftia acidovorans*; *Haloferax mediterranei*; *Aneurinibacillus* sp. H1	–	–	*Hfx. mediterranei*	–

Location of strain isolation	– *Delftia acidovorans*: isolated by Y. Doi's group as *Comamonas acidovorans* DS-17 from "activated sludge from the municipal wastewater treatment plant of Narashino-shi, Japan" (Saito and Doi 1994). Renamed to *D. acidovorans* by Wen et al. (1999)					
	– *Aneurinibacillus* sp. H1: isolated by the team of one of the co-authors of this chapter (S.O.) from compost in Brno, Czech Republic, by using an osmoselection protocol (Pernicova et al. 2020)					
P (3HB-*co*-3HHx)	Inexpensive vegetable oils (canola or soy); waste cooking oil	*Aeromonas caviae*; *Aeromonas hydrophila*	–	–	–	–
Location of strain isolation	– *Aeromonas caviae*: isolated from epizootic of young guinea pigs (DSM 7323), USA; influent of a municipal wastewater treatment plant in Bangkok (DSM 29415), minced meat (DSM 30025; country unknown), or used oil emulsions (DSM 30188; according to DSMZ, country unknown). *Aeromonas hydrophila*: isolated from surface water (according to DSMZ; country unknown; DSM 30016), tin of milk with a fishy odor (DSM 30187; country unknown) (all information from DSMZ strain collection). The PHA production strain of Lee et al. (2000a) was isolated from "raw sewage samples." Both *Aeromonas hydrophila* and *A. caviae* were also isolated from grocery's products ("leave parts of vegetables"; Callister and Agger 1987)					

which is the case, e.g., in *Hfx. mediterranei* (Huang et al. 2006). Reports on direct cellulose utilization for PHA biosynthesis based on the catalytic action of the endohydrolase cellulase (EC 3.2.1.4) activity are scarce in literature. However, similar to starch, cellulose can be hydrolyzed during upstream processing to its monomer (glucose), which is then converted by most industrially relevant PHA producers.

Apart from easily water-miscible hydrophilic heterotrophic substrates, many lipophilic substrates are also converted by microbes for PHA biosynthesis (Walsh et al. 2015). This encompasses long-chain fatty acids (Chee et al. 2010), triacylglycerides (Basnett et al. 2018), or fatty acid methyl esters (Koller and Braunegg 2015a). In the case of such lipophilic substrates, we often observe higher substrate-to-PHA yields than when starting from carbohydrates. This is caused by the stepwise degradation of the fatty acid chains to a pool of acetyl-CoA during the β-oxidation pathway. Acetyl-CoA, in turn, acts as the direct precursor of PHA under conditions favoring PHA biosynthesis (typically ample supply with an exogenous carbon source, combined with limited availability of other growth-essential nutrients, such as nitrogen or phosphate). Technologically, lipophilic substrates pose challenges for the cultivation process concerning the necessary fine distribution of the substrates in aqueous cultivation media. Substrate droplets must be as tiny as possible to realize a maximum lipid-to-water surface area. Often, emulsifiers are needed for this purpose (Muhr et al. 2013). However, some organisms like *Pseudomonas putida*, which are excellent PHA producers from lipophilic substrates, produce emulsifiers (rhamnolipids) that improve the availability of the lipophilic substrates during the cultivation (Bonilla et al. 2005).

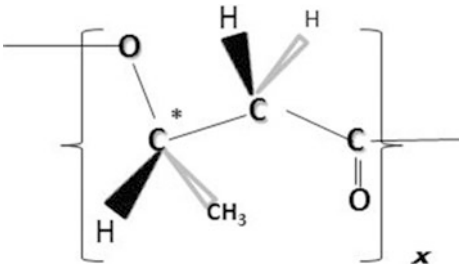
A raw material of increasing interest for PHA production is methane, the major component of biogas and of natural gas (Khosravi-Darani et al. 2019). Biogas, in turn, can be generated by anaerobic decomposition of various organic waste and surplus materials, such as kitchen and gastro-waste, agro-waste, waste of animal-rearing (Achinas et al. 2017), and even spent items made of fossil plastics and biopolymers like PHA, which ultimately closes the carbon cycle of PHA (Vu et al. 2020). Biogas formation provides a biotechnological substrate of high homogeneity and purity in comparison to the direct use of heterogenic organic waste streams for PHA production, which are generally characterized by a fluctuation in their chemical composition (Saratale et al. 2021). Constant composition of the feedstock, in turn, facilitates the reproduction of PHA quality in subsequent cultivation setups. In principle, microbes are able to convert methane to PHA need, in addition to the PHA synthesis enzymes and the enzymatic machinery of the serine pathway, which allows the assimilation of methane to cellular molecules (Strong et al. 2016).

Another fascinating approach is the photoautotrophic conversion of CO_2 via the Calvin cycle to biomass and PHA by cyanobacteria and eukaryotic microalgae. In addition to PHA, cyanobacteria convert CO_2 to produce other marketable bioproducts, such as bioactives or phycobiliproteins (a class of pigments used as food colorants, immunofluorescence markers, etc.), which makes these organisms versatile cellular factories. Here, robust cyanobacterial wild-type strains are available, which can directly utilize CO_2 from abundantly available industrial effluent gas. This strategy combines mitigation of a greenhouse gas (CO_2) with the

generation of value-added bioproducts. If one develops this thought further, it is easily imaginable to use effluent gas directly stemming from fossil plastic incineration for PHA bioplastic manufacturing, thus turning a petrochemical product into a biopolymer (Kamravamanesh et al. 2018).

Another waste-derived feedstock for PHA production of tomorrow is syngas, a mixture of CO_2, CO, and H_2 (Amstutz and Zinn 2020). Technically, syngas can be generated by gasification of a variety of organic waste and surplus materials, such as switchgrass, lignocelluloses, and other organic materials. *Rhodospirillum rubrum*, a non-sulfur purple bacterium, is currently best described as a producer of P(3HB) homopolyester from syngas. Such organisms not only assimilate CO_2 as carbon feedstock but also convert CO, which is toxic for most organisms even in low concentrations via the so-called water-shift reaction to CO_2; the entire metabolic pathway needed is the so-called Wood-Ljungdahl pathway (reductive acetyl-CoA pathway), which serves microbes to generate energy and in autotrophic carbon assimilation. In the methyl branch of this pathway, CO_2 is reduced to formic acid, which, under the consumption of ATP, generates a methyl molecule bound on the corrinoid iron-sulfur protein. H_2 present in syngas serves as hydrogen donor, while CO_2 acts as electron acceptor and biomass building block. The reaction between CO_2 (converted to a methyl group in the methyl branch of the pathway) and CO (generated in the carbonyl branch or consumed directly from syngas), catalyzed by the acetyl-CoA synthase complex, generates acetyl-CoA, which finally acts as a precursor for P(3HB) biosynthesis (Karmann et al. 2019). Similar to biogas, syngas is a homogenous carbon source, which facilitates the reproducible biosynthesis of homogenous PHA. However, safety precautions need to be observed due to the risks that come along with working with syngas. This challenge was addressed by Karmann et al. (2017) who developed a process analytical technology (PAT) platform for safe handling of syngas for biotechnological purposes in general and explicitly for PHA production.

3 P(3HB) Homopolyester



3.1 P(3HB)'s History

As disclosed above, P(3HB) is the homopolyoxoester of the monomer 3HB. P(3HB) is a polymer with thermoplastic properties and a glass transition temperature (T_g) around 0 °C (above that temperature, polymer chains keep on moving and crystallizing, even at room temperature). P(3HB) is for sure the best-studied and most frequently referred PHA biopolymer from this family of natural biopolymers. Despite being easily produced, P(3HB) homopolyester reveals drawbacks during melt processing. Its remarkably high degree of crystallinity, high melting point, and the overlap of its melting range and onset of degradation impede its processing in its pristine form (Modi et al. 2011). Moreover, P(3HB) is thermosensitive. When processing P(3HB), it is often observed that crystallization occurs very slowly. Biodegradation of highly crystalline P(3HB) occurs typically at lower rates compared to PHA copolyester specimens of similar molecular mass and shape when subjected to the same environmental conditions. However, this high crystallinity gives P(3HB) excellent creep resistance. It is reported that such specimens retain stable properties for 5 years when stored in a temperature range between −40 and +60 °C. They also have excellent UV stability over time, outperforming PP in this aspect (Hänggi 2018).

3.2 P(3HB) Properties

Despite being easily produced (no precursor substrates needed for incorporation of building blocks other than 3HB), P(3HB) homopolyester has drawbacks in processing it as a thermoplastic:

- Its remarkably high degree of crystallinity (X_c; about 60–70%), high melting point (T_m; about 170–180 °C), and the overlap of its melting range and the onset of degradation temperature (T_d; typically below 200 °C, often about only 180 °C) impede its processing as a sole component without additives and/or blending materials (Modi et al. 2011).
- P(3HB) is thermosensitive; at melting temperature, splitting of P(3HB) chains can easily occur via intermediate formation of hexa-bonds by the β-ester bonds and subsequent random chain scission, thus leading to reduced molecular mass. This process is catalyzed especially by the presence of small quantities of oxides of bivalent metals (Csomorová et al. 1994).
- When processing P(3HB), it is often observed that crystallization occurs too slowly, which is needed for the production of hard and creep-resistant specimens. This process can take a very long time: at room temperature as long as about 400 days, in comparison to up to 200 days for P(3HB-*co*-3HV) copolyester (Hänggi 2018). Only after this time, the properties of injection molded specimens change from ductile to tough and even to brittle. Importantly, crystallization time can be considerably decreased by reducing the size of polymer crystals, also

referred to as spherulites. Without additives, nucleation occurs spontaneously, resulting in large spherulite sizes of up to 2 mm. Barham and colleagues recognized in 1984 these large spherulite formations during the crystallization of highly pure P(3HB) and stated: "When foreign particles are added the nucleation rate is modified." (Barham et al. 1984). Indeed, crystalline boron nitride or other nucleating agents like nano-clay or natural fibers dramatically reduce the size of the crystalline spherulites to about 20 μm, and this, in turn, reduced the crystallization time to about 3 days, and the microstructure of the P3HB became more homogenous (Hänggi 2018). Barham et al. (1984) also noticed that crystallization temperature is decisive for the crystallization kinetics; for P(3HB), molten specimens should rapidly be cooled down to 90 °C, but not below, which would just freeze the material, making it unstable for months until the thermodynamic optimum is reached. In addition, friction between P(3HB) chains occurs when cooling down the material; this can be avoided by adding plasticizers (e.g., sorbitol, glycerol, etc.), which are molecules compatible with both the crystalline and the amorphous domains of the polymers.

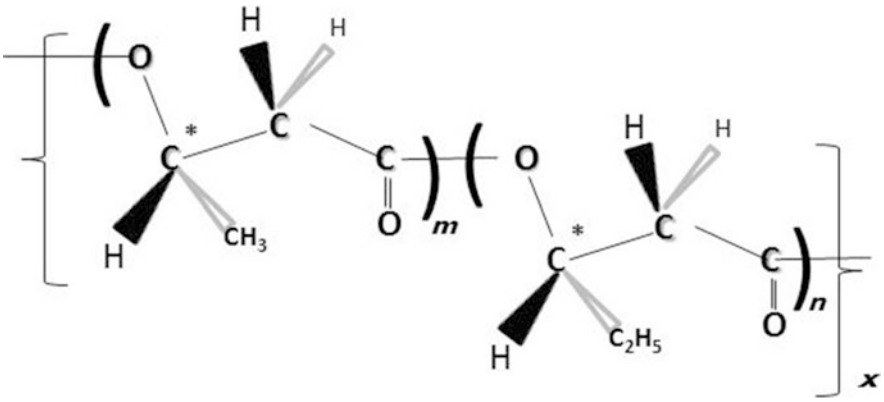
- Moreover, biodegradation of highly crystalline P(3HB), despite being in principle biodegradable and compostable, occurs typically at lower rates compared to PHA copolyester specimens of similar molecular mass and shape when subjected to the same environmental degradation conditions.
- Brittleness of P(3HB) is a typical issue, even when compounding and processing the material appropriately. This is due to the transformation from the amorphous to the crystalline state, which is accompanied by a change in density: crystals are of higher density than the amorphous domains. The polymer molecules leave the amorphous domains toward the more dense crystalline part; now, voids are generated between the spherulites, occurring as cracks through the parts. This issue can be overcome by adding plasticizers or compatible polymers to fill the voids between the crystals or to link the crystals (spherulites) with molecules (Hänggi 2018).

However, there are also notable advantages in using P(3HB):

- P(3HB) is beneficial for producing hard, creep-resistant items. This is due to the fact that P(3HB) does not stop crystallizing until the thermodynamic optimum is reached, that is, until all polymer molecules are fixed in crystals. As soon as this stable status is reached, the specimens made of P(3HB) remain there (no more amorphous domains to post-crystallize) and do not alter their properties anymore for a long time. It is reported that such specimens keep their stable properties for 5 years when stored in a wide temperature range between −40 and +60 °C (Hänggi 2018).
- P(3HB) has the beneficial property that, due to the linearity of the polymer chains unable to entangle, its melt viscosity can be adjusted as required for specific processing. In the molten state, the polymer chains are highly mobile, while when cooling down they get a degree of viscosity according to the current temperature. As a rule of thumb, a change of 10 °C alters viscosity by about 40 times. This allows the fine-tuning the viscosity directly at the processing machine according

to specific requirements: higher speed molding requires lower temperature because crystallization onset occurs earlier. In contrast, when aiming at filling the finest cavities or complex structures in a given mold, the temperature needs to be increased, and crystallization of P(3HB) only starts after having filled all mold cavities (Hänggi 2018).
– With respect to mechanical and UV stability over time, P(3HB) is reported to even outperform fossil plastics like PP (Hänggi 2018).

4 P(3HB-*co*-3HV) Copolyester, the Best Researched PHA Heteropolyester



4.1 The First Discovery of PHA Heteropolyesters

While the chemical structure of P(3HB) homopolyester was elucidated in the 1920s by Lemoigne, it wasn't until the 1970s that P(3HB-*co*-3HV) was discovered when Wallen and Davis isolated PHA from dried activated sludge by chloroform extraction and then precipitating the material by ether addition and cooling. Prepared PHA samples were treated with hot ethanol, and surprisingly the ethanol-insoluble fraction exhibited the infrared spectrum published for P(3HB) homopolyester and featured a high melting point of about 170 °C, also typical for P(3HB). In contrast, the ethanol-soluble fraction precipitated when cooling the solution; the obtained material had a T_m of only about 100 °C. Wallen and Davis correctly hypothesized that this polymer contained 3HB building blocks but had a different structure than P(3HB) homopolyester (Wallen and Davis 1972). Wallen and Rohwedder, after further research, revealed in 1974 the isolation of new microbial strains accumulating PHA from carbon sources present in effluent water. Based on GC-MS analyses, it was confirmed that the polymer contained, besides 3HB, also 3HV and some 3HHx units. This was the first unambiguous proof that PHA monomers other than 3HB existed (Wallen and Rohwedder 1974). In 1981, Morikawa and Marchessault

discovered that pyrolysis of such 3HB- and 3HV-containing microbial PHA generates unsaturated compounds (crotonic acid and pentenoic acid, respectively), which were recognized as valuable chemical synthons (Morikawa and Marchessault 1981). Using GC-MS, Findlay and White (1983) discovered a total of 11 different 3-hydroxyalkanoates (3HAs) in polymers extracted from marine sediments and 6 different 3HAs in PHA accumulated in *Bacillus megaterium*.

4.2 Biosynthesis of P(3HB-co-3HV)

The more popular PHA producers like *C. necator* or *A. lata* biosynthesize P(3HB) homopolyester from simple carbon sources like sugars, while P(3HB-*co*-3HV) copolyester biosynthesis requires feeding them co-substrates chemically related to 3HV. Typically, fatty acids with an odd number of carbon atoms, such as propionic or valeric acid, can be used as 3HV-precursors. They are metabolized by the strains to propionyl-CoA, which undergoes condensation with acetyl-CoA, yielding 3-hydroxyvaleryl-CoA (3HV-CoA). 3HV-CoA, in turn, undergoes PHA synthase-catalyzed polymerization reaction to prolong the growing PHA chains (leading to the incorporation of 3HV monomers), analogous to 3-hydroxybutyryl-CoA, which leads to 3HB production. This principle of producing P(3HB-*co*-3HV) copolyesters by feeding appropriate 3HV-precursor feeding was patented by the inventors Holmes, Wright, and Collins for ICI at the very beginning of the 1980s (EP0052459A1), where it is claimed that "The copolymers are made microbiologically: for part of the cultivation the micro-organism is under conditions of limitation of a nutrient, e.g., nitrogen source, required for growth but not polyester accumulation. For at least part of this period of growth limitation the substrate is an acid or a salt thereof that tives the comonomer units." They concluded that "propionic acid was the preferred acid".

Additionally, there exists a number of PHA producers from the haloarchaea branch, which produce P(3HB-*co*-3HV) from simple, 3HV-structurally unrelated substrates like sugars or glycerol (see Table 2; reviewed by Koller 2019a, b). Among these haloarchaea, P(3HB-*co*-3HV) production from unrelated substrates is best described for *Hfx. mediterranei*, where it was elucidated that multiple constitutively active propionyl-CoA-generating pathways are responsible for the permanent availability of the 3HV-precursor propionyl-CoA (Han et al. 2013).

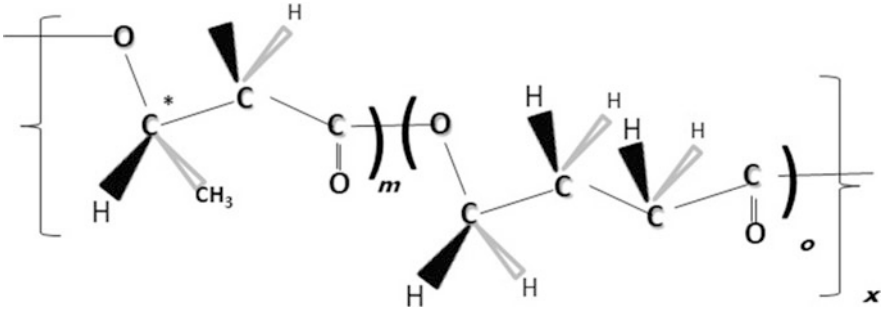
4.3 Properties of P(3HB-co-3HV)

In 1992, Luzier summarized for the first time the advantageous material features of P(3HB-*co*-3HV) copolyesters in comparison to P(3HB) homopolyester in properties like melting temperature, crystallinity, tensile strength, flexural modulus, elongation at break, and impact strength for different grades of PHA commercially produced at that time by Imperial Chemical Industries (ICI), UK. In this study, Luzier

investigated P(3HB) homopolyester and P(3HB-*co*-3HV) with 10 or 20 mol% 3HV. With increasing 3HV content, melting point T_m decreased from 170 °C for the homopolyester P(3HB) to 140 and 130 °C for the two P(3HB-*co*-3HV) copolyesters; degree of crystallinity (X_c) decreased from 80% to 60% and 35%, respectively. Tensile strength (40, 25, and 20 MPa) and flexural modulus (3.5, 1.2, and 0.8 GPa) also decreased, while elongation at break (8, 20, and 50%) and impact strength (60, 110 and 350 J/m) increased with increasing 3HV content in PHA.

The utilization of a continuous cultivation technique with dual carbon (butyric and valeric acids) and nitrogen (ammonium) limited growth, and *C. necator* produced P(3HB-*co*-3HV) with 52 mol% 3HV content resulting in a very low T_m of around 75 °C (Zinn et al. 2003). Hence, P(3HB-*co*-3HV) copolyesters exhibited better processability due to a broader processing temperature window between melting point T_m and onset of thermal decomposition T_d, which is about the same (~180 °C) for P(3HB-*co*-3HV) copolyester and its P(3HB) homopolyester counterpart. The P(3HB-*co*-3HV) copolyesters being more flexible and tougher than P(3HB) allowed them to be processed via extrusion, injection, and blow molding, thus enabled them to be used for manufacturing bottles, extruded sheets, films, fibers, and P(3HB-*co*-3HV)-coated paper (Luzier 1992).

5 P(3HB-*co*-4HB) Copolyester



4-hydroxybutyrate (4HB) is the only well-studied achiral monomer found in natural PHA. 4HB was first described as a PHA building block by the team of Yoshiharu Doi in Japan, who discovered this novel monomer in PHA samples produced by two strains of *C. necator* when being supplied with butyric acid and 4HB-precursors such as 4-hydroxybutyric acid or 4-chlorobutyric acid. Feeding these organisms with butyric acid alone resulted in accumulation of pure P(3HB) homopolyester. Depending on the ratio between butyric acid and 4-hydroxybutyric acid (or 4-chlorobutyric acid) fed to the organisms and the type of strain selected, up to 49 mol% 4HB was incorporated into the poly(3HB-*co*-4HB) copolyesters. The presence of 4HB in PHA was confirmed by NMR. A significant decrease in crystallinity at increasing 4HB fraction in PHA was reported, with the copolyester

with 49 mol% 4HB being almost completely amorphous without any detectable crystalline regions (Doi et al. 1988). Since then, as comprehensively reviewed by Utsunomia et al. (2020a), these copolyesters resemble, depending on the 4HB fraction, thermoplastic polymers at low 4HB fractions or elastomers at high 4HB fractions.

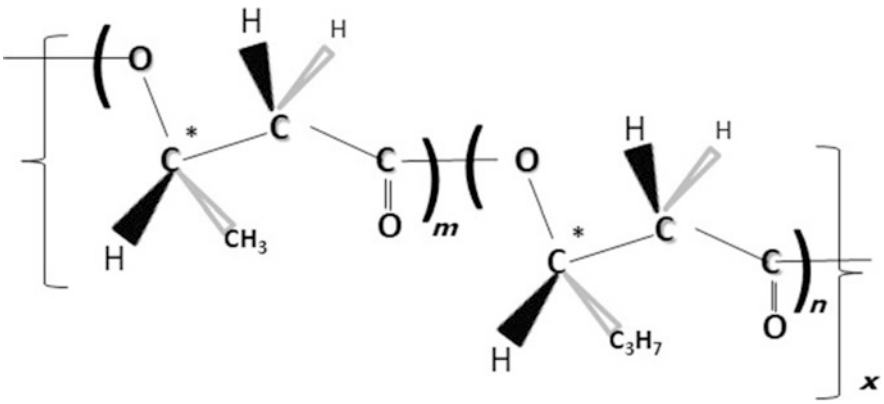
Other strain, carbon source, and 4HB-precursor combinations have been described in the literature for the production of 4HB-containing PHA heteropolyesters. Miranda de Sousa Dias et al. (2017) carried out fed-batch cultivation of the excellent sucrose converter *P. sacchari* (in the original article, the old name *Burkholderia sacchari* was used) for high-productivity (almost 2 g/(L h)) P(3HB-*co*-4HB) copolyester biosynthesis on sugar cane sucrose as the main carbon source, where γ-butyrolactone (GBL) was used as the 4HB precursor (Miranda de Sousa Dias et al. (2017)). Kucera et al. (2019) used the strain *Cupriavidus malaysiensis* USMAA2-4 (DSM 19379), isolated from the Malaysian Sg. Pinang River, for production of poly(3HB-*co*-4HB) copolyesters from different 4HB-related precursors like GBL, 1,4-butanediol, ε-caprolactone, and 1,6-hexandiol; highest 4HB shares in P(3HB-*co*-4HB) were reported for the use of GBL and 1,4-butandiol. Co-feeding of GBL or 1,4-butandiol and 3HV-related precursors (propionic or valeric acid) resulted in biosynthesis of P(3HB-*co*-3HV-*co*-4HB) terpolyesters. Another important P(3HB-*co*-4HB) copolyester producer is the bacterium *Delftia acidovorans*. This strain was used by Mothes and Ackermann (2005) to produce P(3HB-*co*-4HB) copolyesters with tailored 4HB content in a two-stage continuous chemostat cultivation setup when feeding defined mixtures of acetic acid and GBL. Surprising findings were made in 2007 by Lee et al., who noticed that increasing magnesium concentrations in cultivation medium drastically increases the 4HB in P(3HB-*co*-4HB) copolyesters when supplied with mixtures of glucose and 1,4-butandiol as the 4HB precursor. Using a glucose/precursor mix of 4/1, only 10 mol% 4HB were found in the copolyester when using a low $MgSO_4*7H_2O$ concentration of 0.05 mM. This 4HB content was boosted to 52 mol% when increasing the $MgSO_4*7H_2O$ concentration to 0.30 mM. Authors explained this effect by magnesium ions negatively impacting the uptake of the main carbon source glucose, probably due to the disturbance of the cellular transmembrane transport caused by bivalent cations like Mg^{2+}(Lee et al. 2007).

Apart from the use of bacteria as biocatalysts, Hermann-Krauss et al. (2013) described the production of P(3HB-*co*-3HV-*co*-4HB) terpolyester by the haloarchaeon *Hfx. mediterranei* when feeding inexpensive crude glycerol phase from biodiesel production and GBL as 4HB-precursor. These experiments were based on previous findings by Koller et al. (2007b), who used the same strain for P(3HB-*co*-3HV-*co*-4HB) production on hydrolyzed whey lactose, another abundantly available raw material along with GBL.

Regarding the precursors needed for incorporation of 4HB building blocks into respective P(3HB-*co*-4HB) copolyesters, one might argue that these 4HB precursors are not natural products; therefore, can P(3HB-*co*-4HB) copolyesters be deemed "natural"? Indeed, 4-hydroxybutyric acid is typically produced as its sodium salt by alkaline saponification of γ-butyrolactone, which, in turn, is a typical petrochemical

product. It is industrially produced via dehydrocyclization of 1,4-butanediol at a temperature of 180–300 °C, catalyzed by copper. This means that the three major 4HB-precursors for biosynthesis of P(3HB-*co*-4HB) copolyesters can be chemically converted into each other (1,4-butanediol → GBL → 4-hydroxybutyrate). 1,4-butandiol, in turn, is industrially produced from the alkane butane, again a fossil product, via oxidation with molecular oxygen plus tellurium oxide-containing catalysts. However, during the last decade, a biosynthetic route for production of 1,4-butanediol by recombinant bacteria was developed, in addition to the ample reports on 2,3-butandiole biosynthesis. Burgard et al. (2016) has suggested that their systems biology approach could enable the development of recombinant *Escherichia coli* (*E. coli*) expressing a cascade of heterologous enzymes, and this recombinant strain is able to convert glucose via succinyl-CoA (citric acid cycle intermediate), succinate semialdehyde, 4-hydroxybutyrate, 4-hydroxybutyryl-CoA, and 4-hydroxybutanal to 1,4-butanediol. Besides glucose, this recombinant strain was also able to convert other hexoses, pentoses, and sucrose to 1,4-butanediol. In addition, it should be emphasized that the 4HB-precursor 4-hydroxybutric acid is not exclusively produced chemically in the above-described sequence (1,4-butanediol → GBL → 4-hydroxybutyrate) but also occurs in living organisms such as in the human brain, where it acts as an important neurotransmitter (Cash 1994).

6 P(3HB-*co*-3HHx) Copolyester



Hybrid short-chain-length-medium-chain-length PHA (*scl-mcl*-PHA) copolyesters consisting of 3HB and a relatively small amount of *mcl*-PHA building blocks such as 3-hydroxyhexanoate (3HHx), 3-hydroxyoctanoate (3HO), or 3-hydroxydecanoate (3HD) were originally developed and patented by Isao Noda and co-workers. These PHA copolymers were shown to overcome problems associated with well-established P(3HB-*co*-3HV) copolyesters called "isodimorphism," whereby the 3HV units in the copolyester poly(3HB-*co*-3HV) are easily incorporated into the

crystal 3HB-lattice and vice versa, thus preventing efficient disruption of the highly crystalline matrix of P(3HB) homopolymers, therefore requiring the presence of very high percentage of the 3HV comonomer in the respective copolymer to improve their processability and subsequent commercial application. In contrast, *mcl* building blocks described above disturb the 3HB matrix even more efficiently than the achiral building block 4HB does (Noda et al. 2010). Especially poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (P(3HB-*co*-3HHx)) having 10–17 mol% 3HHx feature excellent flexibility, expressed by exceptionally high elongation at break of up to 850%, which outperforms commercially available BIOPOL™ P(3HB-*co*-3HV) with 20 mol% 3HV (Chen et al. 2001).

The first report on the production of such *scl-mcl*-hybrid-type P(3HB-*co*-3HHx) copolyesters was provided by Kobayashi et al. (1994), who demonstrated production of P(3HB-*co*-3HHx) by natural *Aeromonas* spp. on fats and oils. This was a scientific breakthrough at that time, as prior to this, it was set in stone that microbes produce either *scl*-PHA or *mcl*-PHA, simply depending on the type of their PHA synthase. These novel findings resulted in the first patent on such types of PHA by Shiotani and Kobayashi for Kanegafuchi Chemical Industry Co Ltd. (US Patent 5,292,860, 1994), which already claimed the use of *Aeromonas caviae* as production strain. Later, patents for production of this type of PHA were filed by Kaneka and Procter & Gamble for the inventions in this field by several researchers including Isao Noda (US Patent 5,498,692, 1996; US Patent 5,990,271, 1999), in addition to a patent on halogen-free process for recovery for such *scl-mcl*-PHA hybrid copolyesters (US Patent 5,942,597).

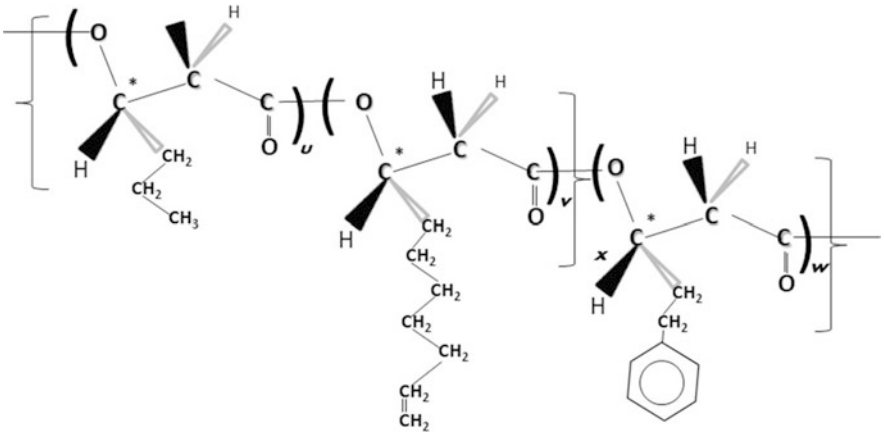
On a bioreactor scale (2 L working volume), such materials were for the first time produced by the wild-type strain *Aeromonas hydrophila* cultivated on glucose during the phase of balanced growth, followed by feeding lauric acid under phosphate limitation during the PHA accumulation phase. This strain was isolated from raw sewage samples by Lee et al. (2000a) and shown to produce P(3HB-*co*-3HHx) from long-chain fatty acids lauric acid and oleic acid in high cell density (96 g/L) cultivations; intracellular P(3HB-*co*-3HHx) fractions in cell mass amounted to 45 wt %, with 17 mol% 3HHx found in P(3HB-*co*-3HHx). Volumetric productivity for poly(3HB-*co*-3HHx) was calculated to amount to slightly more than 1 g/(L h). With glucose or sodium gluconate as substrates, P(3HB) homopolyester was produced instead of P(3HB-*co*-3HHx) copolyester by the same organisms (Lee et al. 2000a).

The industrial-scale process was described by Chen et al., who achieved a volumetric productivity of 0.54 g/(L h) for P(3HB-*co*-3HHx) with 11 mol% randomly distributed 3HHx in a two-stage batch process (glucose for growth phase, lauric acid for phosphate-limited PHA accumulation phase) on a 20 m^3 scale (working volume 10 m^3), corresponding to the production of 100 g/L biomass containing 50% P(3HB-*co*-3HHx) within 46 h of cultivation. Importantly, the authors underlined in this study that the batch cultivation mode was applied due to complications observed when running this process with *A. hydrophila* 4AK4 in fed-batch mode (Chen et al. 2001).

Besides wild-type *A. caviae*, genetically modified *C. necator* (in publication: *Ralstonia eutropha*) expressing the *Pseudomonas fluorescens* GK-13 synthase gene

is also reported to accumulate *scl-mcl*-PHA hybrid copolyesters. Dependent on the substrate provided (salts of different fatty acids), the generated copolyesters contained, besides 3HB, *mcl*-PHA building blocks such as 3HHx, 3HO, 3HD, or 3-hydroxydodecanoate (3HDD) (Noda et al. 2005). Later, Riedel et al. (2015) developed a process for *scl-mcl*-PHA hybrid copolyester production by rec. *R. eutropha* from industrial rendered animal waste fats of high melting temperature and high free fatty acid content; on a 5-L bioreactor scale, a cell dry mass of 45 g/L, containing 60 wt% P(3HB-*co*-3HHx) with 19 mol% 3HHx, was obtained. For recovery of P(3HB-*co*-3HHx) produced in this process from biomass, the authors only recently presented a two-stage extraction process based on the biogenic, nontoxic solvents acetone (solvent for P(3HB-*co*-3HHx)), and isopropanol (precipitant of P(3HB-*co*-3HHx) from the acetone solution) (Bartels et al. 2020).

7 Other PHA Copolyesters



Besides the abovementioned PHA copolymers that have 4 distinct comonomers (building blocks; 3HB, 3HV, 4HB, and 3HHx), to date some 150 different comonomers for PHA have been discovered (Hazer and Steinbüchel 2007; Chen 2009), and comprehensive reviews on these building blocks have been composed (Zinn et al. 2001, 2016; Koller 2018a). Generally, such copolymers are either *scl*-PHA (building blocks with no more than five carbon atoms; polymerization catalyzed by Class I or Class III PHA synthases) or *mcl*-PHA (building blocks with 6–14 carbon atoms, e.g., 3HHx, 3-hydroxyheptanoate (3HHp), 3HO, 3-hydroxynonanoate (3HN), 3HD, 3HDD, etc.). Exceptions in this context are the above-described "Nodax™-type" *scl-mcl*-hybrid PHAs like P(3HB-*co*-3HHx). For *mcl*-PHA, copolyesters with saturated (unbranched and branched) and unsaturated (unbranched only) and side chain-functionalized monomers are reported, typically characterized by low glass transition temperature T_g, low crystallinity, and low melting temperature T_m (Zinn 2010). Functionalized monomers include halogenated

(bromo-, chloro-, and fluoro-3-hydroxyalkanoates) representatives, cyano-hydroxyalkanoates, and aromatic building blocks (e.g., 3-hydroxy-5-phenylvalerate, 3-hydroxy-5*p*-methylphenylvalerate) (Hany et al. 2009; Hanik et al. 2019; reviewed by Zinn et al. 2001; Koller 2018a).

Post-synthetic chemical modification of *mcl*-PHA with terminally unsaturated monomers (obtained by feeding ω-unsaturated fatty acids as precursors to *Pseudomonas putida*) can generate PHA bearing epoxide groups, which allows chemical cross-linking of the materials for enhancing the elastomeric properties by reducing crystallinity, increasing water resistance, and, at the same time, not compromising the material's biodegradability (Park et al. 1998). Alternatively, such pendant vinyl groups in PHA building blocks can be chemically oxidized to terminal diol groups, which leads to highly hydrophilic PHA (Lee et al. 2000b). By oxidation of poly (3-hydroxyoctanoate-*co*-3-hydroxyundec-10-enoate) with $KMnO_4$, Lee and Park (2000) succeeded for the first time in producing PHA with pendant carboxylate groups (about half of the vinyl groups were converted to carboxylate groups). These materials had hydrophilicity high enough to make them even water-soluble, while they were no more soluble in the typical hydrophobic PHA solvent chloroform. As an unintended side effect, these harsh oxidation conditions degraded the backbone of the PHA biopolymer, resulting in substantially reduced molecular mass. This problem was overcome in 2003 by Stigers and Tew, who used OsO_4 and ozone and obtained carboxylated poly(3-hydroxyoctanoate-*co*-3-hydroxyundec-10-enoate) with 100% conversion of the pendant vinyl groups. These materials showed only little molecular mass degradation and high solubility in polar solvents, such as acetone and acetone/water and THF/water mixtures (Stigers and Tew 2003). Olefinic PHA was further modified with amino acids rendering them to interesting hybrid polymers for medical applications (Bassas-Galià et al. 2015).

Table 3 provides an overview on material characteristics of the "bulk PHA" polyesters P(3HB), P(3HB-*co*-3HV), P(3HB-*co*-4HB), P(4HB), P(3HB-*co*-3HHx), and selected types of *mcl*-PHA.

8 Bioreactors, Cultivation Regimes, and Product Formation Conditions for PHA

8.1 Principle Aspects of PHA Cultivations

PHA bioproduction typically occurs under controlled conditions such as temperature, pH value, and dissolved oxygen tension (DOT) in aerated bioreactors. These bioreactors are typically stirred tank reactors (STRs) developed for some of the well-established aerobic cultivation processes known in biotechnology, namely, production of yeast biomass (autocatalytic process), penicillin (*Penicillium chrysogenum*), or citric acid (*Aspergillus niger*). In 2005, Lenz and Marchessault illustratively

Table 3 Typical values reported for the material properties of different types of PHA

Type of PHA	Melting point T_m [°C]	Glass transition temperature T_d [°C]	Degree of crystallinity X_c [%]	Elongation at break ε [%]	Tensile strength σ [MPa]
P(3HB)	170–180[a,b,c]	~3–5[b,d]	55–80[a,e]	3[f], 4[c], 8[b]	36[f], 40–50[b, g,h]
P(3HB-*co*-10%-3HV)	~140[b]	~0–1[i,g]	60[b]	20[b]	25[b]
P(3HB-*co*-20%-3HV)	~130[b]	~−15	35[b]	50[b]	20[b]
P(3HB-*co*-4HB)	160[j] (11% 4HB)	−2 (7% 4HB; [h])	~20[k] to 32[j]	242 (10% 4HB)[h]	24[h] (11% 4HB)
	152 (17% 4HB)[j]	−7 (16% 4HB[h])	~13[k] to 30 (17% 4HB[a,b,k])	444 (16% 4HB)[h]	26[h] (20% 4HB)
P(4HB)	60[f,g]	~−50[f,g,h]	34[h]	1000[g,f]	50[f]
P(3HB-*co*-3HHx)	~170 (for 12–68% 3HHx)[l]; 164–156 (for 4–12% 3HHx)[d]	−1 to −12 (for 4–12% 3HHx)[d]	56–21 (for 12–68% 3HHx)[l]	4–177 (for 12–68% 3HHx)[l]	18–7 (for 12–68% 3HHx)[l]
	151 (for 12% 3HHx)[m, p]	−1 (for 12% 3HHx)[m, p]	29 (for 12% 3HHx)[m, p]	67.3 (for 12% 3HHx)[m, p]	11.3 (for 12% 3HHx)[m, p]
mcl-PHA	~ 40 to 60[n,o]	~−30 to −60[i,n,o]	~10 to 20[n,o]	Some 100%[i,o]	Typically below 10
	53 and 65 (*mcl*-PHA with 3HHx, 3HO 3HD, and 15% or 39% 3HDD)[m, p]	−44 and −43 (*mcl*-PHA with 3HHx, 3HO 3HD, and 15% or 39% 3HDD)[m, p]	12 and 19 (*mcl*-PHA with 3HHx, 3HO 3HD, and 15% or 39% 3HDD)[m, p]	189 and 125 (*mcl*-PHA with 3HHx, 3HO 3HD, and 15% or 39% 3HDD)[m, p]	8.7 and 11.3 (*mcl*-PHA with 3HHx, 3HO 3HD, and 15% or 39% 3HDD)[m, p]
	58 (*mcl*-PHA with 3% 3HHx, 19% 3HO, 27% 3HD, 20% 3HDD, and 31% or 49% 3HTD)[m, p]	−40 (*mcl*-PHA with 3% 3HHx, 19% 3HO, 27% 3HD, 20% 3HDD, and 31% or 49% 3HTD)[m, p]	17.5 (*mcl*-PHA with 3% 3HHx, 19% 3HO, 27% 3HD, 20% 3HDD, and 31% or 49% 3HTD)[m, p]	275 (*mcl*-PHA with 3% 3HHx, 19% 3HO, 27% 3HD, 20% 3HDD, and 31% or 49% 3HTD)[m, p]	7.6 (*mcl*-PHA with 3% 3HHx, 19% 3HO, 27% 3HD, 20% 3HDD, and 31% or 49% 3HTD)[m, p]
	67 (*mcl*-PHA with 2% 3HHx, 11% 3HO, 22% 3HD, 16% 3HDD, and 49% 3HTD)[m]	−40 (*mcl*-PHA with 2% 3HHx, 11% 3HO, 22% 3HD, 16% 3HDD, and 49% 3HTD)[m, p]	17 (*mcl*-PHA with 2% 3HHx, 11% 3HO, 22% 3HD, 16% 3HDD, and 49% 3HTD)[m, p]	108 (*mcl*-PHA with 2% 3HHx, 11% 3HO, 22% 3HD, 16% 3HDD, and 49% 3HTD)[m, p]	3.2 (*mcl*-PHA with 2% 3HHx, 11% 3HO, 22% 3HD, 16% 3HDD, and 49% 3HTD)[m, p]

(continued)

Table 3 (continued)

Type of PHA	Melting point T_m [°C]	Glass transition temperature T_d [°C]	Degree of crystallinity X_c [%]	Elongation at break ε [%]	Tensile strength σ [MPa]
	Poly(HP-*co*-HA-*co*-HE): No crystalline melting endotherm[c]	−40 to −6: poly(HP-*co*-HA-*co*-HE) with 0–60% aromatic building blocks[c]			

3HB: 3-hydroxybutyrate; 3HV: 3-hydroxyvalerate; 3HHx: 3-hydroxyhexanoate; 3HO: 3-hydroxyoctanoate; 3HD: 3-hydroxydecanoate; 3HDD: 3-hydroxydodecanoate; 3HTD: 3-hydroxytetradecanoate; 4HB: 3-hydroxybutyrate
Poly(HP-*co*-HA-*co*-HE): *mcl*-PHA with saturated (3HHx, 3HO), unsaturated (3-hydroxyheptenoate, 3-hydroxynonenoate, 3-hydroxyundecenoate) and aromatic (3-hydroxyphenylvalerate) building blocks
[a]Depending on degree on polymerization (molecular mass)
[b]Luzier (1992)
[c]Hartmann et al. (2004)
[d]Murugan et al. (2017)
[e]Mitomo et al. (2001)
[f]Martin and Williams (2003)
[g]Reviewed by Utsunomia et al. (2020a)
[h]Saito et al. (1996)
[i]Reviewed by Koller (2018a)
[j]Kunioka and Doi (1990)
[k]Cong et al. (2008)
[l]Volova et al. (2016)
[m]Liu and Chen (2007)
[n]Muhr et al. (2013)
[o]Strongly dependent on exact monomeric composition (saturated, unsaturated, and aromatic building blocks)
[p]Zinn (2010)

remarked that PHA production is performed "by a largescale fermentation process not unlike the brewing of beer" (Lenz and Marchessault 2005).

Indeed, especially the processes for penicillin and citric acid production are regarded as templates for PHA production: these two bioproducts are produced in a similar two-stage process as PHA is as well. In the first stage, a nutritionally balanced cultivation medium is used to generate a high concentration of catalytically active biomass. After that, a switch in the cultivation conditions toward unbalanced growth conditions provokes changes in the metabolism, characterized by the stopping of the biomass formation (no more cell propagation) and the formation of the secondary bioproducts penicillin, citric acid, or PHA, respectively. In the case of penicillin, this switch from mycelium formation to predominant antibiotic excretion

occurs below a critical oxygen level and depletion of complex nutrients (e.g., corn steep liquor) (Zangirolami et al. 1997), while citric acid formation is typically initiated at the time point when accessible iron ions are no more available for the fungal cells in sufficient quantities; now, *A. niger* cells excrete and "send out" citric acid to scavenge remaining iron ions by forming citrate-iron complexes, which can be metabolized by the cells for building important cellular components, such as cytochromes, and expression of the lyase enzyme aconitase (EC 4.2.1.3), which is pivotal to keep the citric acid cycle (TCC) running (Max et al. 2010). In the case of PHA biopolyesters, the switch from the phase of balanced microbial growth to product formation is typically accomplished by an extreme increase of the ratio of carbon to nitrogen source. Hence, nitrogen limitation stops the formation of active prokaryotic biomass but shifts the carbon flux toward PHA accumulation (Macrae and Wilkinson 1958). For some industrially important PHA production strains, a certain amount of PHA is already synthesized during the logarithmic (exponential) phase of bacterial growth ("partially growth-associated product formation"; observed, e.g., for *Cupriavidus necator*). However, high productivity for PHA is not observed before the onset of nutritionally challenging conditions (Braunegg et al. 1998). Besides nitrogen limitation, also phosphate limitation was described as a factor provoking PHA biosynthesis, because it prevents further biomass growth due to the impossibility to generate important phosphate-containing cell components like nucleic acids, cofactors, or adenosine phosphates. However, phosphate limitation is rather scarcely used on an industrial scale due to the decreased pH buffer capacity of phosphate-free medium. Oxygen limitation also provokes PHA biosynthesis due to the interruption of the TCC, which is based on the impossibility of regeneration of the oxidized form of redox equivalents (NAD^+, $NADP^+$) when molecular oxygen as terminal electron acceptor is missing. However, such oxygen-limited bioprocesses drastically reduce volumetric product formation rates, which impedes industrial implementation. This makes nitrogen limitation still the method of choice for high-throughput PHA production processes on a larger scale. On a lab scale, other growth-limiting factors have also been described to favor PHA production rates when present in suboptimal quantities, such as potassium (potassium limitation was described to result in PHA of extraordinary high molecular mass by a methane-utilizing mixed culture), sulfur, or iron (Helm et al. 2008).

On the industrial scale, PHA production in said bioreactors typically occurs in fed-batch mode; hence, the substrate is added according to its conversion by the microbes. This is analogous to other established processes: during yeast production, substrate pulses need to be added carefully to avoid switching the metabolism from biomass formation to ethanolic fermentation, despite the ample availability of oxygen ("crabtree effect"); excessive carbon source concentrations during yeast propagation need to be avoided (Pfeiffer and Morley 2014). During penicillin and citric acid production, too, carbon source needs to be supplied well in accordance with the consumption rate by the cells in order to avoid the formation of conidia and to favor mycelium growth instead of premature onset of product formation. Especially in the case of penicillin production, excessive loads of carbon sources would stop fungal biomass growth too early and provoke the start of penicillin excretion at

biomass concentration too low to reach sufficient volumetric productivity for the antibiotic (Birol et al. 2002).

8.2 Continuous Cultivation

The future of production of PHA of predefined composition and reproducible molecular mass and polydispersity might be the switch to continuous cultivation processes. This is characterized by the productivities of continuous cultivations that outperform discontinuous batch and fed-batch processes, thereby drastically increasing operation periods per cultivation batch at maximum productivity, minimizing the number of interruption of operation for starting up, shutting down, and cleaning the equipment, and reduced personal involvement during the operation. In contrast to autocatalytic processes like yeast propagation or production of primary metabolites like lactic acid, one-stage continuous biotechnological processes are only to some extent suitable for high-throughput production of secondary metabolites like PHA. Nevertheless, single-stage cultivations were successfully implemented to tailor the monomeric unit compositions of P(3HB-*co*-3HV) (Zinn et al. 2003) and *mcl*-PHA (Amstutz et al. 2019; Hartmann et al. 2004, 2006). This was possible due to the fact that the chemostat continuous operation mode allows nutrient limitations in contrast to nutrient starvations as it is the case in batch and fed-batch cultivations. All nutrients are efficiently used converted, and thus a tailored composition can be achieved (Zinn et al. 2003).

Higher volumetric productivities than in single-stage chemostats can be achieved when two continuously operated bioreactors are linked in series. The active biomass is formed under continuous supply of a nutritionally balanced medium, while in a second vessel, only traces of nitrogen source from the first bioreactor are present. Carbon source is supplied at a feed rate according to the consumption rate by the cells. This enables considerably higher intracellular PHA fractions compared to single-stage continuous setups. Moreover, two-stage continuous cultures better address problems associated with different conversion rates for different substrates needed to produce copolyesters (reviews by Koller and Muhr 2014; Koller and Braunegg 2015b). Such two-stage continuous PHA production processes were successfully demonstrated in the past, e.g., for tailored P(3HB-*co*-4HB) production by *Delftia acidovorans* cultivated on acetic acid and GBL (Mothes and Ackermann 2005). Recently, a modified two-stage approach has been demonstrated, where the culture harvest of the first chemostat was fed to four chemostats at the second stage. This allowed the study of different growth conditions in the second stage to control the content of the 3-hydroxyphenylvalerate in the *mcl*-PHA accumulated in a recombinant *P. putida* strain (Hanik et al. 2019).

Beyond this two-stage process engineering setup, a combination of a continuously operated STR for autocatalytic biomass propagation and a tubular plug flow reactor for PHA accumulation even better matches the fundamentally different kinetic characteristics of biomass formation (autocatalytic) on the one hand, and

Fig. 3 Multistage bioreactor cascade for high-throughput PHA biosynthesis, financed by BASF SE, in operation at Graz University of Technology, Austria. (Own picture M. Koller)

PHA accumulation in not multiplying cells (first-order kinetics) on the other hand. Based on theoretical considerations, this was already postulated by Braunegg and associates in 1995 (Braunegg et al. 1995), while it took far more than one decade until the proof of concept for these ideas was provided by Atlić et al. (2011), who produced high cell concentrations of *C. necator* biomass on glucose in a continuous STR, linked to a cascade of four additional continuously operated STRs, which acted as a process engineering substitute for the mentioned tubular reactor and were supplied with carbon source only. This approach can be understood by the fact that such STR cascades, with an increasing number of STRs, approach the plug flow characteristics of a tubular reactor. Indeed, high productivity of P(3HB) homopolyester were obtained in this setup, which was operated for many days under steady-state conditions; moreover, P(3HB) quality (molecular mass and polydispersity) were highly uniform and reproducible. A photograph of this cascade setup is provided in Fig. 3.

In the future, such multistage continuous PHA production processes might enable the formation of PHA of tailored microstructure, e.g., blocky structured PHA copolyesters with alternating segments of 3HB oligomers and other building blocks such as 3HV 4HB, or 33HHx (Utsunomia et al. 2020b). Such blocky structured heteropolyesters enable more stringent control of polymer properties by increasing pliability, at the same time maintaining the structural rigidity provided by certain block regions (Ashby et al. 2018). This would be possible by fine-tuned supply of the individual cascade bioreactors with specific carbon sources acting as precursors

of individual PHA building blocks: while supply with a main carbon source (e.g., sugars) generates blocks of 3HB-oligomers, supply of short fatty acids with an odd number of carbon atoms (propionic acid, valeric acid) generates segments with high 3HV content; supply of compounds like γ-butyrolactone, 1,4-butanediol, or sodium 4-hydroxybutyrate results in 4HB blocks. This might enable the high-throughput production of high-quality PHA heteropolyesters of a microstructure of unprecedented reproducibility, which is not accessible by established fed-batch cultivation regimes. On the downside, continuous bioprocesses, especially on multistage, are more prone to disturbations and contaminations by alien microflora than discontinuous setups. This could partly be overcome by the application of robust, extremophile production strains which can be cultivated monoseptically even under strongly reduced sterility precautions, as recently postulated by the concept of "next-generation industrial biotechnology" (NGIB; Chen and Jiang 2018). Such concepts would further contribute to the reduction of PHA production costs.

9 PHA Recovery

As an important difference between the above-described three bioprocesses, citric acid and penicillin are excreted as extracellular products, while PHA gets stored intracellularly as granular inclusion bodies. This poses additional challenges in PHA recovery after the cultivation process compared to the recovery of the extracellular bioproducts like citric acid (precipitation as calcium citrate at elevated temperature) or penicillin (extraction of the easily soluble sodium salt). For the release of PHA granules stored in the interior of microbial cells, the cells need to be disrupted to set PHA granules free (reviewed by Koller 2020).

Remarkably, PHA-producing wild-type strains are characterized by rather high robustness of their cell wall, which in turn challenges PHA recovery. This is in contrast to recombinant PHA producers, derived from wild-type strains that are not naturally equipped to carry excessive loads of storage materials, as demonstrated for recombinant *E. coli*, a nonnatural PHA synthesizing strain, transformed with PHA biosynthesis genes. These recombinant organisms were indeed able to store excessive loads of PHA, which makes the cells fragile; stirring this biomass in diluted NaOH solution resulted in cell disintegration and release of PHA granules (Choi and Lee 1999). Mechanic cell disintegration for PHA recovery was demonstrated by high-pressure homogenization as it is also used for the recovery of other intracellular bioproducts like many enzymes. After mechanical break up of cells, released PHA granules can be separated from cell debris in the liquid phase by dissolved air floatation (Koller et al. 2013).

More frequently, extraction methods are used which, on the one hand, weaken the cell membrane and, on the other hand, solubilize the PHA biopolyesters. Best established PHA extraction solvents like chloroform, although giving high product purity, extraction yields, and extraction efficiency, are halogenated and toxic and stem from petroleum chemistry. The use of such solvents in biotechnology,

especially for large-scale use, should be avoided. Current trends in PHA recovery, therefore, aim to replace these traditional methods by "greener" approaches, such as biogenic solvents (alcohols, lactic acid esters, acetone, etc. (reviewed by Koller 2020)), digestion of non-PHA cell mass by enzyme cocktails (Yasotha et al. 2006), or even in the intestine of insects (Chee et al. 2019), use of supercritical solvents (Hejazi et al. 2003), nontoxic surfactants like soaps (Pospisilova et al. 2021), or ionic liquids (Kobayashi et al. 2015).

10 Commercialization of PHA

It has to be emphasized that current PHA production on an industrial scale is embryonic compared to the global fossil plastic production, which amounts to about 400 million tons per year, excluding fibers for textiles such as polyesters and polyamides and elastomers, such as for car tires. Including those take the total synthetic polymer production to over 550 million tons per year. PHA production did not exceed 10 kt in 2020 although companies claim production capacity estimated to approximately 20–30 kt per year. In 2020, the total production volume of renewable polymers was about 4.2 million tons, or about 1% of the total fossil plastics production. Several PHA-producing companies have announced significant volume projections and capacity announcements exceeding one million tons over the next 5 years to 2025 (Koller and Mukherjee 2022). One primary driver behind such large-scale volume announcements is the acceptance of the financial markets on the viability of PHA as a material that can replace single-use plastics packaging (Jost 2018). This is reflected by the recent listing of Danimer Scientific on NASDAQ, one of the US stock exchanges. However, the European Union is about to announce their Single-Use Plastics Directive, which bans fossil plastics as well as renewable polymers such as PLA, PBS, and PHA. However, legislation in PR China and in the United States mostly favor the use of compostable and biodegradable materials which should help the cause of large-scale production and acceptance PHA as an alternate to single-use petrol-based plastics.

Indeed, we currently witness annual growth rates for biopolymers of about 8%, with PHA being part of this upward trend. In any case, the global PHA market is projected to grow from US-$57 mio. in 2019 to US-$98 mio. in only 5 years, which corresponds to a compound annual growth rate (CAGR) of 11.2%. It is expected that PR China will keep the lead in global PHA production also during the next years, while Europe currently produces about 25% of all PHA. However, European PHA production is expected to increase by 27% from 2019 to 2023 (reported by Kourmentza et al. 2020).

Industrial-scale PHA biopolymers currently being produced are *scl*-PHA including P(3HB), P(3HB-*co*-3HV), and P(3HB-*co*-4HB) and one *scl*-*mcl*-PHA, namely, P(3HB-*co*-3HHx) (Koller and Mukherjee 2022). There is one company commercializing medium-chain-length PHA (*mcl*-PHA) where the medium-chain-length part is higher than six carbon atoms on a reasonable scale: PolyFerm Canada sells

VersaMer® PHA as "irregular pieces, pellets, latex" consisting, *inter alia*, of P(3HO-*co*-3HHx), P(3HN-*co*-3HHp), and *mcl*-PHA containing also unsaturated building blocks. These materials have outstanding low T_g values (−45 to −35 °C), low T_m (45–65 °C), low molecular mass typical for *mcl*-PHA (100–150 kDa), and very high elongation at break of 1200–1400% (online resource 2 n.d.). In 2016, PolyFerm Canada has licensed its technology to TerraVerdae Bioproducts, an industrial biotechnology company developing advanced bioplastics and other biomaterials from C1-feedstocks (methanol), which are scaling up (online resource 2 n.d. and personal communication Bruce Ramsay).

The majority of companies commercializing PHA, however, is focusing on the "top-selling" *scl*-PHA bulk products, namely, brand names of commercialized polyesters in square brackets below:

- P(3HB): PHB Industrial S.A, Brazil [Biocycle®], Bio-On, Italy [Minerv-PHA®] (currently not producing), Biomer, Germany [Biomer®], Mango Materials, USA [YOPP®], COFCO, PR China, Newlight Technologies LLC, USA [AirCarbon® PHA], NAFIGATE Corporation [HYDAL® PHA]; in the past: Imperial Chemical Industries (ICI), UK, until end of 1990s [BIOPOL®]; Chemie Linz/PCD Polymere GmbH, Austria, in the late 1980s
- P(3HB-*co*-3HV): TianAn Biologic Materials Co. [ENMAT®], PHB Industrial S.A, Brazil [Biocycle®], Bio-On, Italy [Minerv-PHA®], PhaBuilder, PR China; in the past: Telles (joint-venture Metabolix & ADM from 2009 to 2012 [Mirel®], Imperial Chemical Industries, UK [BIOPOL®])
- P(3HB-*co*-4HB): Tianjin GreenBio Materials Co., Ltd., PR China [SoGreen®], Shenzhen Ecomann Biotechnology Co., Ltd., PR China [AmBio®], Tepha Medical Devices, Inc., USA [TephaELAST®], CJ, Republic of Korea (technology sold from Metabolix) [Yield10®], PhaBuilder, PR China [copolyester "mP34HB 10"], Medpha, PR China [Medpha®], in addition to Tepha's surgically important homopolyester P(4HB) [TephaFLEX®]
- P(3HB-*co*-3HHx): Danimer Scientific, USA (former Meredian Holdings Group Inc. and MHG; technology originally from Proctor & Gamble) [Nodax®], Kanegafuchi Chemical Industry Co Ltd. (Kaneka), Japan [PHBM®], Bluepha Co., Ltd., PR China [Bluepha PHA], RWDC Industries, Ltd., USA [Solon®]

11 Spent PHA Is Naturally Degraded

Although PLA undergoes composting to CO_2, water, and biomass in industrial composting facilities under elevated temperature, it is not compostable under home-composting conditions and does not biodegrade in the environment. This high recalcitrance of PLA was demonstrated a long time ago, e.g., by Hyon et al. (1984), who showed that PLA fibers remained intact for half a year in phosphate buffer saline at 37 °C and pH value 7.4; only when elevating the temperature to 100 °C, 50% mass loss was observed. This is in contrast to PHA types that are being commercialized today and discussed above (Ong et al. 2017). These PHA grades are

all biodegradable and compostable, both under industrial and home-composting conditions, which is confirmed by the strict certification processes many commercially available types of PHA were subjected to (Koller and Mukherjee 2020). Subsequent paragraphs provide examples for degradability of different types of commercial PHA.

11.1 P(3HB)

Even the highly crystalline P(3HB) homopolyester is biodegradable and compostable. In aerobic environments, the end products of biodegradation are carbon dioxide, water, and humus, while under anaerobic conditions, CH_4 is also produced, with no harmful intermediates or by-products being generated. In this context, the company Biomer claims that their products (P(3HB)) are "fully biodegradable" and compostable (online resource 3 n.d.). A study by Kim et al. compared biodegradability of P(3HB) homopolyester from ICI with the thermoplastic starch-based composite Mater-Bi® from Novamont and Sky-Green®, a chemosynthetic biodegradable polymer consisting of succinic acid, adipic acid, butanediol, and ethylene glycol. Biodegradation experiments were carried out in forest soil, sandy soil, activated sludge soil, and in farm soil at different temperatures (28, 37, and 60 °C) over a period of 28 days. P(3HB) homopolyester showed complete degradation (90% conversion to gases and water, the rest being humus is defined as complete biodegradation) in all conditions, including activated sludge soil at 37 °C. None of the aforementioned polymers tested showed complete biodegradation at any conditions and soil. Some showed modest degradation in sandy soil and forest soil. In farm soil as well, P(3HB) again outperformed the other polymers at 37 °C (Kim et al. 2000). It was shown long time ago by Kumagai et al. (1992) that degradability of P(3HB) strongly depends on the polymer's crystallinity; when incubated in solution of *Alcaligenes faecalis* depolymerase at 37 °C and pH value 7.4, faster degradation was observed for polymer samples (produced either by solvent casting or by crystallization from the melt) of lower crystallinity, and molecular mass remained mainly unchanged during degradation. The authors concluded that the depolymerase enzyme first hydrolyzes P(3HB) chains in an amorphous state on the film surface and subsequently degrades P(3HB) chains in the crystalline state. Importantly, this study also showed that spherulite size has hardly any impact on the degradation rate. In 1992, Luzier noticed that no degradation of P(3HB) was observed in environments free of biocatalytic activity, such as in dry or humid air, which in turns makes products made of P(3HB) stable in non-biotic environments, e.g., during the use of packaging, as a formed product or article where they do not come in contact with microorganisms that can consume them as food or substrate.

11.2 P(3HB-co-3HV)

Luzier (1992) studied the biodegradability of P(3HB-*co*-3HV) copolyesters in the early 1990s. He demonstrated that ICI BIOPOL® products (made from P(3HB-*co*-3HV)) exhibited the slowest degradation in seawater (350 weeks for complete degradation of standard injection-molded specimens), while the highest degradation rates were reported in anaerobic sewage (only 6 weeks for complete degradation). Degradation rates in estuarine sediments, aerobic sewage, and soil were in between these two extremes. Similar to P(3HB) homopolyester, no degradation was observed in humid or dry air without the presence of microflora. In their biodegradability tests, Rosa et al. (2004) demonstrated that P(3HB) having 72% degree of crystallinity and poly(3HB-*co*-3HV) having 50% degree of crystallinity (3HV fraction not disclosed) from PHB/ISA demonstrated weight loss over 10 months. Both polymers showed similar biodegradation in soil composting medium at 46 and at 24 °C in a soil simulator containing *inter alia* manure.

ENMAT's Y1000P P(3HB-*co*-3HV) has been certified as "compostable" by the US-Biodegradable Products Institute (BPI) in 2008 and is listed as a Food Contact Material ("FCM") substance No. 744 in Table 1 of Annex I of the Plastics Regulation of the EU and EU REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) compliant since 2008 (online resource 4 n.d.).

P(3HB-*co*-3HV) has been demonstrated to hold high promise for biomedical application due to its high biocompatibility. In contrast to competing materials used in biomedical applications such as PLA and PLGA, P(3HB-*co*-3HV)'s biodegradation products are less bioactive in vivo and result in less tissue acidification. Moreover, in vivo degradation of P(3HB-*co*-3HV) occurs at slower rates than for PLA, which makes the material more useful for implant manufacturing for bone repair and bone regeneration. Especially when produced by Gram-positive microbes like representatives of the genera *Bacillus*, *Streptomyces*, or *Rhodococcus*, P(3HB-*co*-3HV) is reported to have biocompatibility superior to PHA from Gram-negatives due to the lacking lipopolysaccharides, a group of endotoxins from the cell wall of Gram-negatives, which are typically co-extracted with PHA during the recovery process and cause inflammatory reactions (Zinn et al. 2001; Koller 2018b; Bucsella et al. 2020). The expedient biocompatibility of P(3HB-*co*-3HV) was only recently substantiated again by Mohandas et al. (2021), who used cast films made of P(3HB-*co*-3HV) from the marine bacterium *Bacillus cereus* MCCB 281 to test attachment, viability, and proliferation of fibroblast cells on them; high compatibility for these cells on the films was shown. Moreover, by hemolysis, in vitro platelet adhesion, and coagulation assays, the excellent blood compatibility of these P(3HB-*co*-3HV) films for use as blood contact graft materials was shown.

11.3 P(3HB-co-4HB)

Already in 1989, Kunioka et al. noticed that rate of biodegradation of P(3HB-*co*-4HB) film samples with 0–37 mol% 4HB in soil and activated sludge was enhanced by the presence of 4HB units (Kunioka et al. 1989). In a study by Nakamura et al. (1992), the impact of 4HB fraction in P(3HB-*co*-4HB) films on biodegradability was studied in more details; in the aqueous solution of an extracellular *A. faecalis* P (3HB) depolymerase, films with 0, 6, 10, 28, 85, and 94% 4HB were subjected toward enzymatic degradation at 37 °C and pH value of 7.5. The rate of degradation, expressed by mass loss, strongly increased with increasing 4HB fraction op to 28%; already the copolyester with 6% 4HB was degraded much faster than the P(3HB) homopolyester, which correlates well with decreasing crystallinity. However, samples with very high 4HB content were degraded considerably slower than P(3HB) due to the hard susceptibility of the depolymerase enzyme to the 4HB moieties (Nakamura et al. 1992). In marine environment, degradability of P(3HB), P(3HB-*co*-3HV) (4, 21 and 61% 3HV) and poly(3HB-*co*-4HB) (10% 4HB) samples (films obtained by solvent casting) was compared during an incubation period of 1 year. It was shown that all samples were degraded via surface erosion, while surface erosion was most pronounced for the P(3HB-*co*-3HV) sample with 21% 3HV. Interestingly, the speed of surface erosion was not too much dependent on PHA's composition but highly on the temperature of seawater (Doi et al. 1992). Similar to the results for P (3HB) described by Kumagai et al. (1992), no change in molecular mass during degradation was observed for any of the samples (Doi et al. 1992).

For currently commercially available P(3HB-*co*-4HB), GreenBio's product "Sogreen-00X" can be "completely degraded into carbon dioxide and water at the environment of soil, rivers, sewage and marine water in 3–6 months" (manufacturer information; online resource 5 n.d.), while Shenzhen Ecomann's Ambio® P (3HB-*co*-4HB) is TÜV Austria certified for industrial and home composting and also FDA approved (online resource 6 n.d.).

11.4 P(4HB)

P(4HB) homopolyester is not accessible by wild-type organisms due to the fact that the microbial metabolism of 4HB precursors intrinsically always yields, besides 4HB, also some 3HB. However, its in vivo degradation yields 4HB as the sole product, which is a naturally occurring, biocompatible compound in human tissue, including various organs, muscle, and brown fat. Therefore, P(4HB) is FDA approved for medical application as a suture material since 2007 and still the only PHA with FDA clearances for clinical usage. Currently, Tepha Inc. is the globally only company producing P(4HB) on a commercial scale. They produce the material from inexpensive feedstocks by recombinant *E. coli*, based on a patent originally filed by Metabolix (reviewed by Utsunomia et al. 2020a). Regarding in vivo

degradability of TephaFLEX® poly(4HB), its absorption rate is reported with only 8–52 weeks, which is considerably faster than reported for P(3HB). With an in vivo half-life of only about 27 min, 4HB is metabolized very quickly in the human body to CO_2 and water (online resource 7 n.d.).

11.5 P(3HB-co-3HHx)

PHBH®, the "100% plant-derived biopolymer developed by Kaneka, is certified to biodegrade in seawater," and received the label "OK Biodegradable MARINE" by TÜV Austria (formerly Vinçotte). This means that "the degree of biodegradation should be 90% or more within 6 months in seawater (30 °C), and disintegration to less than 10% remnants in a 2 mm sieve after 12 weeks at 30 °C. Acquired certification in September 2017 from Vinçotte (now TÜV Austria Belgium), an international certification organization based in Belgium." This degradation under standardized marine conditions (23 °C) was successfully demonstrated for prototype PHBH®specimens like drinking straws, bottles, and knives. According to comparative experiments, PHBH®was faster degraded under marine conditions than PCL, PHSA, PBAT, PBS, and PLA (not degraded in seawater at all after 28 days). Toxicity tests were also carried out to assess the biocompatibility of PHBM®for marine organisms; no acute toxicity was observed for fish, plankton, and shellfish. Further labels for Kaneka's PHBH®are "OK Compost Industrial," "OK Compost Home," "OK Biodegradable Soil" (certification in progress), and "OK Biobased" according to TÜV Austria, the "Biobased" certification for Japan, and the "Industrial Compostable" certification for Japan and USA. Eric Lepoudre, Business Manager Biopolymers at the Green Polymer Division at Kaneka, reported that according to ISO 14855 tests, PHBH®shows even better aerobic biodegradability in compost than cellulose and almost identical anaerobic biodegradation in aqueous phase according to ISO 14853 than cellulose (note: PLA was not degraded at all under these conditions during a 28 days test span), while under anaerobic conditions in solid phase according to ISO 15985, degradation was only insignificantly slower than for cellulose and much faster than for PLA (Lepoudre 2018) [online resource 8]).

Solon® P(3HB-*co*-3HHx) is thoroughly tested for its biodegradability, compostability, and natural origin; the materials are certified for biodegradability in soil ("OK biodegradable SOIL", TÜV Austria), fresh water ("OK biodegradable WATER"), and marine water ("OK biodegradable MARINE"), home and industrial composting ("OK compost HOME", "OK compost"), and with "OK biobased (online resource 9).

The same biodegradability was ascertained for Danimer Scientific's Nodax® P (3HB-*co*-3HHx), which is certificated, besides its "biobased" nature (ASTM D 6866) 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,

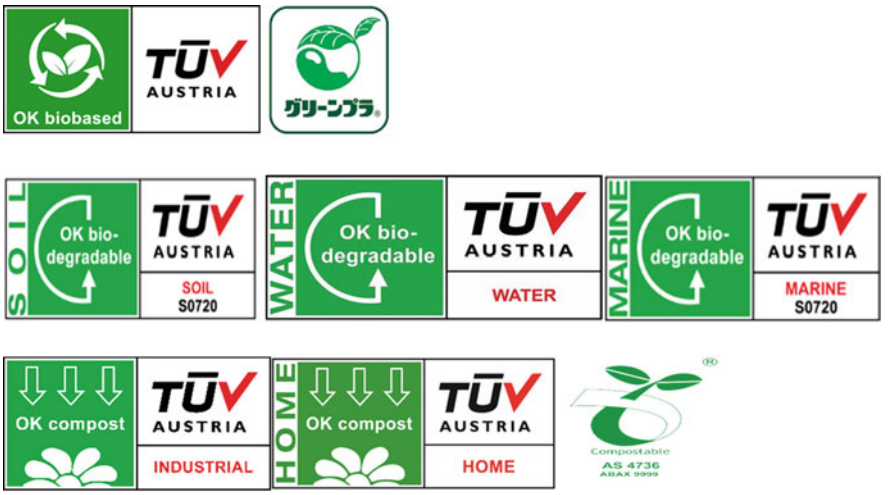


Fig. 4 Selected certification labels awarded to different commercially available PHA products. Upper row: "OK biobased" by TÜV Austria and Japanese "biobased" label; middle row: "OK biodegradables SOIL," "OK biodegradable Water" (for degradation in freshwater), and "OK biodegradable MARINE" by TÜV Austria. Third raw: TÜV Austria labels for industrial and home composting and "compostable" certification according to Australian Standard (AS) 4736-2006

ASTM D6400, EN 13432) and home composting (TÜV Austria, ASTM D6400, EN 13432) (online resource 10 n.d.).

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 "nonhazardous waste" after disposal (online resource 11 n.d.). Figure 4 shows the most important certificates for currently commercialized PHA.

12 Conclusions

Fossil plastics have exhibited their beneficial role in improving our quality of life in food and medical applications. Plastics in automobiles have allowed for many new technological and safety improvements. Hence, there is little doubt that plastics with their ubiquitous presence in our lives have made our society better. However, the end-of-life issues with plastics, especially their lack of appropriate collection systems and leakage into the environment, may have already risen to epidemic propositions. A UN study concluded that microplastics are the next major environmental epidemic that is yet to be appropriately measured. These and issue of greenhouse gas emissions due to their manufacture has raised significant awareness among consumers and to a degree among policy makers and the industry. While regulations are

being put in place to reduce plastics use, not all of these measures are necessarily beneficial to the environment and practical from a convenience standpoint. The benefits that plastics provide do not need to be sacrificed due to their negative environmental impacts. PHA is the perfect example of alternatives that can bridge the benefits of plastics without the environmental damage they cause.

PHA have been established as macromolecules occurring in nature, being produced by microorganisms and therefore being biodegraded by them. Moreover, they originate from natural substrates. They play a leading and important role in the metabolism of numerous microorganisms. Their manufacture on large scale requires the use of the same microorganisms as found in nature. PHA are nontoxic and biocompatible to humans and other living organisms; therefore, they are metabolized if ingested by living organisms. The benefits exhibited by fossil plastics are also available through the use of PHA. The limitations of fossil plastics have opened the door for PHA to play a leading role to continue to benefit humanity while maintaining nature's cycle of circularity and sustainability due to nature's ability to not only produce them but also to biodegrade them.

As shown, we currently witness considerable activities in different global regions toward commercialization of the most important "bulk PHA" such as P(3HB), P(3HB-*co*-3HV), P(3HB-*co*-4HB), P(3HB-*co*-3HHx), and, to a minor extent, P(4HB) and *mcl*-PHA copolyesters. The next years will draw a clearer picture about which concepts of marketing of PHA are indeed future-fit, be it in terms of market requirements, customer acceptance, sustainability, or economic feasibility. However, it is quite clear that long-term success of commercial PHA needs to be grounded on some defined solid fundaments: robust and powerful microbial production strains, optimized and simple cultivation facilities, amply available renewable feedstocks, and sustainable and inexpensive downstream processing technologies.

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