Microbiological and biotechnological aspects of biodegradable plastics:
Poly(hydroxyalkanoates)

Nehal Thakor, Ujjval Trivedi and K C Patel*
Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar 388 120, India

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Poly(3-hydroxyalkanoates) (PHAs) have been drawing much attention as biodegradable substitutes for conventional non-biodegradable plastics. Intensive research on the physiology, biochemistry and molecular genetics of the metabolism of PHAs during last two decades has increased our knowledge on the biosynthesis of these polyesters in bacteria and also showed various applications of these polymers. Prokaryotes synthesize a wide range of different PHAs and accumulate them as insoluble inclusions in the cytoplasm for storage of carbon and energy. Naturally, PHAs are synthesized from coenzyme A thioesters of the hydroxyalkanoic acids, which are synthesized during fatty acid metabolism. Due to similarities of physical and material properties with conventional plastics, PHAs can be recommended for application in various areas like industries, medicine, pharmacy and agriculture. They are thermoplastic and/or elastomeric, insoluble in water, enantiomerically pure, non toxic, biocompatible, piezoelectric and exhibit a high degree of polymerization. For economical production of PHAs, various bacterial strains have been exploited with new fermentation strategies and cheap renewable carbon sources. Transgenic plants have been studied for production of PHAs to compete with production cost of petroleum based bulk plastics. Metabolic engineering approaches have been used to expand the spectrum of utilisable substrate and also to improve PHAs production.

Keywords: biodegradable plastics, biodegradation, bio polyesters, PHAs, PHB

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Introduction
Petroleum based plastics have been produced by chemical industries since early 1930s. They constitute an important group of materials because of their high molecular weight and low reactivity, which make them especially suited for applications where durable, inert materials are required. However, the very same properties are recognized as major drawbacks of plastic materials. Most disposed plastic materials remain at the site of disposal for their resistance to biological breakdown and result in environmental pollution. The reorganization of environmental pollution problem caused by synthetic plastics has led to search for alternative materials—the biodegradable plastics. Having similarities of physical and material properties with conventional plastics, poly(3-hydroxyalkanoates) (PHAs) can fulfill this requirement and can be recommended for application in various areas1,2.

Lemoigne from the Pasteur Institute, France for the first time reported accumulation of PHAs as cytoplasmic inclusions in Gram-positive bacterium Bacillus megaterium3. This water-insoluble material was identified as homopolymer of 3-hydroxybutyrate (3HB), poly(3-hydroxybutyrate) (PHB). Since this first report, PHB accumulation has been found in many microorganisms, such as representatives of Gram-positive and Gram-negative bacteria and also archaeabacteria, as insoluble inclusions in cytoplasm4. This occurs mainly if the cells are cultivated in the presence of an excess carbon source and growth is impaired or restricted by the lack of another nutrient, such as nitrogen, phosphorus and sulphur, or also dissolved oxygen5-10. When the supply of the limiting nutrient is restored, the PHA can be degraded by intracellular depolymerases and subsequently metabolized as a carbon and energy source6.

PHAs can be classified into two groups depending on the number of carbon atoms in the monomer unit: short-chain-length (scl-PHAs), 3-5 carbon atom containing monomers, and medium-chain-length
mcl-PHAs), 6-14 carbon atom containing monomers. The general structure of PHAs is shown in Fig. 1. The discovery of a polyester in 1983 by de Smet et al was the first report of accumulation of mcl-PHAs, having constituents of 6 to 14 carbon atoms in axenic culture. Currently, more than 140 hydroxyalkanoic acids have been identified as constituents of PHAs representing a versatile class of microbial polymer. Besides linear and branched 3-, 4-, 5- and 6-hydroxyalkanoates, various functionalized PHAs constituents, such as PHAs containing halogenated or aromatic side chains, have been described.

The physical properties of the homopolymer of 3-hydroxybutyrate, PHB, are similar to those of polypropylene, for example regarding melting point, crystallinity, glass transition temperatures, etc. and represents a stiff and brittle material. The properties are improved in copolymers containing up to 25 mol% 3-hydroxyvalerate (3HV), where toughness and flexibility are increased, and the decrease in crystallinity and melting point advance the melt-processing of the polymer without being degraded. Therefore, the poly(3HB-co-3HV) copolymer gained industrial interest and, in the late 1980s, commercialization under the trade name Biopol was initiated. Biopol® can be processed to useful materials by various processes, such as extrusion, injection molding, fiber spinning, coating or foaming. Because of the versatile applications as thermoplastic biopolymers, PHAs can also be used for natural fiber composites, or as binder in paints, and for various applications in medicine and pharmacy, such as tissue engineering.

Physiological Functions of PHAs

Generally, PHAs are accumulated as cytoplasmic inclusions in the presence of excess carbon source and limitation in another essential nutrient like nitrogen. When supply of limiting nutrient is restored, PHAs can be degraded by intracellular membrane bound depolymerases and subsequently mobilized as carbon and energy source. The major role of PHAs is that of beneficial high molecular weight reserve material, considering the ability to store large quantities of highly reduced carbon without significant effects on the osmotic pressure of the cell. The model organism in PHAs research, Wautersia eutropha (formerly known as Ralstonia eutropha) can accumulate PHB in form of multiple granules of 0.2 to 0.5 μm size, having the polyester content more than 90% of the cell dry matter (CDM). However, possession of PHAs retards the degradation of cellular components, such as RNA and protein, during nutrient starvation. PHB enhances the survival and serves as carbon and energy source for spore formation in some Bacillus species, although its accumulation is not a prerequisite for sporulation process. Putative function of PHB was attributed to nitrogen fixation in species of Azotobacter and Rhizobium, suggesting respiratory protection of nitrogenase by PHB as oxidizable substrate. One of the interesting developments in PHB biochemistry has been the discovery of PHB in the plasma membrane of bacteria and also in eukaryotic membrane fraction, with highest concentrations in the mitochondria and microsomes. Furthermore, PHB is found in association with inorganic polyphosphate in membranes; it is referred to as complexed-PHB (cPHB), and they play role in the regulation of intracellular calcium concentrations and in calcium signaling. This cPHB may also provide genetic transformability for transport of exogenous DNA through the membrane.

Physiology and Biosynthesis of PHAs

**Short-chain-length PHAs (scl-PHAs)**

The biochemistry of PHB biosynthesis is well studied. In most bacteria, such as W. eutropha, PHB is synthesized in a three-step reaction starting with acetyl-CoA (Fig. 2a).

![Fig. 1—General structure of poly(3-hydroxyalkanoates) (PHAs): R = CH₃ to C₄H₉, short-chain-length (scl-) PHAs; R = C₅H₁₁ to C₁₁H₂₃, medium-chain-length (mcl-) PHAs](image-url)
oxygen, the concentration of NADH is observed. This results in inhibition of the early enzymes of the tricarboxylic acid cycle, leading to accumulation of acetyl-CoA and PHB biosynthesis\(^\text{30,31}\). Whereas in *Rhodospirillum rubrum*, two additional enzymes are involved in PHB biosynthesis (Fig. 2b), because the reduction of acetoacetyl-CoA yields S-(+)-hydroxybutyryl-CoA, which is converted to the R-isomer by two enoyl-CoA hydratases and finally polymerized by the PHA synthase\(^\text{29}\).

A co-polymer, poly(3HB-co-3HV) can be synthesized by *W. eutropha* and many other microorganisms from either a mixed substrate of glucose and propionic acid or feeding with valeric acid. If propionate is fed, the same biochemical pathway of PHB synthesis is used, but propionyl-CoA and acetyl-CoA are condensed by the \(\beta\)-ketothiolase to give 3-ketovaryl-CoA, which leads to incorporation of 3-hydroxyvalerate monomers in the polymer, with 3-hydroxybutyrate arising in normal way\(^\text{32}\). When valeric acid is fed, propionyl-CoA is derived from the \(\beta\)-oxidation of the fatty acid and condensed with acetyl-CoA to give 3-ketovaryl-CoA\(^\text{6,18}\).

**Medium-chain-length PHA (mcl-PHAs)**

For the synthesis of mcl-PHAs in bacteria, three main pathways are involved in the generation of mcl-PHAs precursors. Firstly, chain elongation, in which acyl-CoA is extended with acetyl-CoA; secondly, \(\beta\)-oxidation of fatty acids, the main pathway when fatty acids are used as substrate; and thirdly, de novo biosynthesis, which is the main route during growth on simple compounds\(^\text{3}\).

In most mcl-PHA accumulating bacteria, fatty acid de novo biosynthesis pathway and/or fatty acid \(\beta\)-oxidation pathways are linked with PHAs biosynthesis and also several metabolic links between fatty acid metabolism and mcl-PHAs biosynthesis have been revealed (Fig. 3)\(^\text{12,33}\).

When *Pseudomonas putida* and *P. aeruginosa* synthesize co-polyester with 3-hydroxydecanoic acid as their main constituent and some other mcl-3HAs as minor constituents from glucose or gluconic acid, they utilize fatty acid de novo biosynthesis pathway, where R(-)-mcl-3HA-CoA thioesters are derived from intermediates of this pathway by a transferase, namely transacylase, that converts the ACP-thioesters into CoA-thioesters, which can be polymerized by the PHA synthase enzyme\(^\text{4}\).

Fatty acids, when used as sole source of carbon/energy and also for PHAs accumulations, are oxidized to acetyl-CoA by \(\beta\)-oxidation pathway. Under physiological conditions permissive for synthesis and accumulation of mcl-PHAs, the fatty acids are not completely degraded to acetyl-CoA, and intermediates of the \(\beta\)-oxidation pathway are partially or completely withdrawn and converted to R-3-hydroxyacyl-CoA. Enoyl-CoA hydratases, such as pha J encoded (R)-specific enol-CoA hydratase, have been identified from *Aeromonas caviae* and *P. aeruginosa*, which derived 3-hydroxyacyl-CoA from

![Fig. 3—Metabolic links of fatty acid metabolism with mcl-PHAs biosynthesis](image)

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tans-2-enoyl-CoA. In recombinant E. coli, pha B encoded 3-ketoacyl-CoA reductase has been reported to derive 3-hydroxyacyl-CoA from 3-ketoacyl-CoA32.

Huisman et al in 1989 reported that some Pseudomonas spp. synthesize mcl-PHAs. They also showed that those Pseudomonads which cannot synthesize PHB can accumulate mcl-PHAs during growth on various 3-hydroxyalkanoic acids and also on free fatty acids34. However, several recent findings have reported simultaneous production of scl- and mcl-PHAs by P. oleovorans, P. resinovorans, other Pseudomonads and A. caviae35-40.

PHA synthase can be classified into three different classes according to their structure and substrate range. Class I, PHA synthases comprise of only one type of subunit with molecular weight ranging from 60-65 kDa. Class I predominantly polymerize hydroxyalkanoic acids with three, four or five carbon atoms33,41. This class is represented by the PHA synthase of W. eutropha, which occurs in photoheterotrophic nonsulfur purple bacteria and in most of the heterotrophic bacteria, except the Pseudomonads belonging to rRNA homology group I33. Class II, PHA synthases also comprise only one type of subunit and with a similar molecular weight range as Class I; however, the substrate specificity of class II is different. These PHA synthases incorporate medium-chain-length 3-hydroxyalkanoates into PHAs. The chain length characteristically comprises 6-14 carbons with high freedom for the structure of R-pendant group. This class is represented by the PHA synthase of P. oleovorans and seems to occur exclusively in Pseudomonads belonging to rRNA homology group I31. Class III, PHA synthases comprise of two different types of subunits, each with molecular weight of approximately 40 kDa. One subunit exhibits significant homology to the class I and class II PHA synthases with all highly conserved amino acids present, but lacking extended region of the amino terminal and the carboxy terminal regions. PHA synthases of this class occur in cyanobacteria and in all sulfur purple bacteria but not in non-sulfur purple bacteria33. Due to unusual substrate range and unusual structure, class III PHA synthases from Chromatium vinosum and Thiococcus pfennigii have been studied in greatest detail33.

Till recently, only one PHA synthase has been partially studied for in vitro substrate specificity42. Specificity of PHA synthase is generally determined in vivo from cultivation experiments on precursor substrates provided as carbon source to the cell and subsequent analysis of chemical composition of the accumulated PHAs43. However, the information obtained from these results is limited for three reasons. First, several bacteria, like P. oleovorans and P. aeruginosa, harbour more than one PHA synthase genes. In vivo experiments employing the genes of native host do not allow any distinction to be made between the possibilities of two or even three PHA synthases. Second, physiological background in which PHA synthases are expressed and, particularly, the capability to provide hydroxyacyl-CoA thioesters, derived from the carbon source as substrate for the enzyme, may also vary considerably. Third, synthetic (nonphysiological) CoA thioesters of hydroxyfatty acids cannot be tested in any way43.

PHA synthase showed broad range of substrate specificity in recombinant E. coli, when various 3-HA-CoA thioesters were provided in vivo by metabolic engineering44-48. Our studies showed that Comamonas testosteroni can degrade/utilize naphthalene via salicylic acid, which is subsequently converted to pyruvate. Acetyl CoA derived from pyruvate enters to the PHB biosynthesis pathway49. Interestingly, same organism is synthesizing heteropolymers containing 3-hydroxyalkanoic acids with chain length ranging from C6 to C14, when grown on the substrates having variety of long chain fatty acids50. The class I PHA synthase of W. eutropha can accept mcl-3HA-CoA thioesters44,45. It has been reported, recently, that provision of 3-mercapto-propionate as carbon source for W. eutropha resulted in co-polymer biosynthesis, which contained 3-hydroxybutyrate (3HB) and 3-mercaptopropionate (3MP) linked via thioester bonds51. Furthermore, class III PHA synthase from T. pfennigii accepted 3-mercaptoalkanoates, i.e. 3-mercaptobutyrate (3MB), 3-mercaptopropionate (3MP) and 3-mercaptovalerate (3HV), resulting in biosynthesis of homopolymers of the different mercaptoalkanoates, now referred to as polythioesters (PTEs). These results emphasize that PHA synthases have wide range of substrate specificity52,53. The molecular biology and biochemistry of the key enzyme for PHAs biosynthesis, and specificity have been extensively reviewed elsewhere33,41,43,54.

Properties of PHAs

Generally, PHAs are water-insoluble, thermoplastic and/or elastomeric, enantiomerically pure, non-toxic, biocompatible and in particular biodegradable33. The
PHB homopolymer is completely stereo regular polyester, with all asymmetric carbon atoms in the (R)-configuration, and therefore, it is highly crystalline. The high crystallinity makes it relatively stiff and brittle. The glass transition temperature ($T_g$) of PHB lies between 5 and 9°C, and the melting point ($T_m$) lies between 173 and 180°C (Table 1). PHB decomposes at 200°C, which is close to its melting temperature. In its solid, crystalline state, the polyester is right-handed helix. The mechanical properties of PHB including Young’s modulus (3.5 GPa) and the tensile strength (40 MPa) are similar to those of polypropylene (Table 1). However, the elongation to break for PHB is about 3%, which is significantly lower than that of polypropylene (400%). The material also differs in chemical properties; PHB is having a lower solvent resistance but much better natural resistance to ultraviolet weathering than polypropylene.

The flexibility of the material is greatly improved when 3-hydroxyvalerate units are incorporated into the polymer, resulting in a decrease of Young’s modulus around 0.8 and a decrease of tensile strength below 30 MPa. The elongation to break also increases as co-monomer fraction increases. The melt temperature is greatly depressed, down to 130°C, depending on the 3-hydroxyvalerate content (Table 1). These properties generate a relatively wide window of conditions that allow thermal processing as a melt without degradation of the material. The Tg also decreases as 3-hydroxyvalerate units increase, allowing use of these materials at lower temperatures without embrittlement behaviour. The steady decrease in Young’s modulus indicates improved flexibility and toughness, producing more desirable properties for commercial applications. Having been made by bacteria as a carbon reserve, these materials can also be degraded by microbes, putting them amongst the few fully biodegradable, thermoplastic materials. PHAs show optical activity, i.e. films or solutions of PHAs rotate the plane of polarized light passing through them. The β-carbon in every monomer chain has an R absolute configuration, so the polymer is chiral which leads to high level of crystallinity when appropriate casting or molding conditions are used. Since the crystal has no centre of symmetry it also shows piezoelectric property.

The properties of co-polyester p(3HB-co-3HV), a polyester consisting mainly of 3-hydroxyoctanoic acid (3HO) and a few other PHAs, resemble in some respect those of PHB, but are also quite different in other respect, such as flexibility, crystallinity and biodegradability. Owing to their enzymatic synthesis, as is the case for PHB, mcl-PHAs have exceptional stereochmical regularity and, therefore, are fully isotactic. This allows mcl-PHAs to achieve some crystallinity, which can be as high as 25%.

The molecular weight is of the order of $2 \times 10^5$ g/mol. The glass transition temperature ($T_g$) is well below room temperature, ranging from -43 to -25°C. Melting temperatures depend on both the thermal history and the nature of the pendant chain, and varies between 39 and 61°C for mcl-PHAs containing 3HO as main constituent. Depending on the thermal history and the test conditions, the tensile modulus varies between 2.5 and 9 MPa, the tensile strength between 6 and 10 MPa, and the maximum elongation between 300 and 450%.

**PHAs Production by Bacterial Fermentation**

The suitability of a bacterium for PHAs production at large scale depends on many different factors, such as stability and safety of the organism, growth and accumulation rates, achievable cell densities and PHAs contents, extractability of the polymer, molecular weights of accumulated PHA, range of utilizable carbon sources, cost of the carbon sources and the other components of the medium, and occurrence of by-products. Even though many different PHAs have been described in the literature, only a few of them have been produced in large quantity, characterized and used to develop applications. These are PHB, P(3HV), P(3HB-co-3HV), poly(3-hydroxyhexanoate-co-3-hydroxyoctanoate) P(3HHx-co-3HO) poly(4-hydroxybutyrate) P(4HB).

Among many different microorganisms that are known to synthesize PHAs, only a few bacteria have been employed for their production. These include *W. eutropha*, *Alcaligenes latus*, *E. coli*, *P. pastoris*.
A. vinelandii, several strains of methylotrophs, P. oleovorans, P. putida KT2442 and recombinant E. coli. Each bacterium requires different conditions for growth and PHAs accumulation. Except Al. latus, mutant of A. vinelandii and recombinant E. coli require the limitation of an essential nutrient such as N, P, Mg, K, O or S. W. eutropha, the bacterium employed for the commercial production of PHB and P(3HB-co-3HV), Biopol® by Zeneca, accumulates a large amount of PHAs, up to 80% of CDM, when nitrogen or phosphate is completely depleted.

Various high cell density culture (HCD) techniques for culturing wild type bacteria, such as W. eutropha, P. oleovorans, P. putida and also recombinant strains of E. coli, have been established for production of PHAs. Table 2 summarizes different bacteria, various substrates used, type of polymer, maximum cell densities obtained and PHAs contents. PHB production in various wild type and recombinant bacteria and fermentation economics has been reviewed elsewhere. Production of PHAs using cheap renewable carbon sources, like tallow, oil remains from biotechnological rhamnose production, whey and triglycerides, have been reported along with other inexpensive carbon substrates.

Metabolic Engineering

Metabolic engineering approaches have been used to expand the spectrum of utilizable substrate and to improve PHAs production from structurally unrelated carbon sources, and also for production of PHAs having short and medium chain length 3-hydroxy-alkanoates. This requires transfer of a PHA synthase structural gene, expression of an enzymatically active PHA synthase protein and also engineering of pathways that provide substrates of PHA synthase in required concentration. Studies carried out on poly(3HB-co-3HV), using propionic acid, other aliphatic fatty acids, levulinic acid, pentanol, amino acids and citric acid cycle intermediates, and poly(3HA mcl) production, using alkanes, acyl alcohols, fatty acids and glucose, and other substrates, in different organisms are useful in understanding the pathways leading to supply of the required monomers and metabolic engineering and pathway construction for bacteriological production of these PHAs. Since the cloning of PHAs biosynthesis genes from W. eutropha, PHAs synthase genes (phaC) of more than 60 organisms have been cloned which allowed to construct several recombinant strains that produce PHAs more efficiently. When a broad-host-range plasmid containing W. eutropha PHAs biosynthetic genes was introduced to the same W. eutropha strain, the PHAs production genes could be increased up to 1.24 times than the parent strain. Heterologous expression of PHAs biosynthetic genes of W. eutropha in Pseudomonads, which were unable to synthesize PHB, allowed the production of PHB. Production of poly(4HB) by a genetically engineered W. eutropha is a good example of producing a novel

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Substrate</th>
<th>Polymer</th>
<th>Cell density (g/L)</th>
<th>PHAs content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azotobacter vinelandii</td>
<td>Glucose + fish peptone</td>
<td>poly(3HB)</td>
<td>30</td>
<td>85</td>
</tr>
<tr>
<td>Alcaligenes latus</td>
<td>Sucrose</td>
<td>poly(3HB)</td>
<td>112</td>
<td>88</td>
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<tr>
<td>Pseudomonas strain K</td>
<td>Methanol</td>
<td>poly(3HB)</td>
<td>233</td>
<td>64</td>
</tr>
<tr>
<td>Recombinant Escherichia coli</td>
<td>LB + Glucose</td>
<td>poly(3HB)</td>
<td>117</td>
<td>76</td>
</tr>
<tr>
<td>Wautersia eutropha</td>
<td>Glucose</td>
<td>poly(3HB)</td>
<td>164</td>
<td>74</td>
</tr>
<tr>
<td>Wautersia eutropha</td>
<td>Glucose + Proponic acid</td>
<td>poly(3HB-co-3HV)</td>
<td>&gt;100</td>
<td>70-80</td>
</tr>
<tr>
<td>Chromobacterium violaceum</td>
<td>Valeric acid</td>
<td>poly(3HV)</td>
<td>41</td>
<td>65-70</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>Oleic acid</td>
<td>poly(3HHx-co-3HO-co-3HDCo-3HDD-co-3HDTD)</td>
<td>93</td>
<td>45</td>
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<tr>
<td>Pseudomonas oleovorans</td>
<td>Octane</td>
<td>poly(3HHx-co-3HO)</td>
<td>40</td>
<td>35-40</td>
</tr>
<tr>
<td>Pseudomonas oleovorans</td>
<td>Gluconic acid + Octanoic acid</td>
<td>poly(3HHx-co-3HO-co-3HDCo-3HDD-co-3HDTD)</td>
<td>54</td>
<td>66</td>
</tr>
<tr>
<td>Comamonas testosteroni</td>
<td>Naphthene</td>
<td>Poly (3HB)</td>
<td>ND</td>
<td>85</td>
</tr>
<tr>
<td>Comamonas testosteroni</td>
<td>Vegetable oils</td>
<td>Polyester containing C6-C12 3-HAs</td>
<td>ND</td>
<td>87</td>
</tr>
</tbody>
</table>

3HB, 3-hydroxybutyric acid; 3HD, 3-hydroxydecanoic acid; 3HDD, 3-hydroxydodecanoic acid; 3HTD, 3-hydroxytetradecanoic acid; 3HHx, 3-hydroxyhexanoic acid; 3HV, 3-hydroxyvaleric acid; 3HO, 3-hydroxyoctanoic acid; 4HV, 4-hydroxyvaleric acid; LB, Luria-Bertani broth; PHAs, poly(3-hydroxyalkanoates); 3-HAs, 3-hydroxyalkanoic acids; ND, Not detected.
polyester by metabolic engineering. Varieties of PHAs can also be produced by heterologous expression of PHAs biosynthetic genes. Recombinant P. putida harbouring PHAs biosynthetic genes of T. pfennigii was able to accumulate a ter-polyester consisting of 3-hydroxybutyrate, 3-hydroxyhexanoate and 3-hydroxyoctanoate when cultivated on octanoate. Recently, a non-natural pathway has been constructed in E. coli for PHAs synthesis. Using this pathway, polythioesters (PTEs), new class of biopolymers, can also be synthesized and large scale production of poly(3-mercaptopropionate) [poly(3MP)] has been established. Metabolically engineered transgenic plants have also been exploited for production of PHAs to compete against petrochemically produced low cost bulk plastics.

Applications of PHAs

For their biodegradability, water resistance and oxygen impermeability, PHAs can be used for all sorts of biodegradable packaging materials, including composting bags and food packagings. Also the use of PHAs in single-use sanitary articles, like diapers, is considered as being economically feasible. In marine environments where fishing nets and other discarded objects that cause severe damage when made from non-biodegradable materials, construction materials, such as adhesives, laminates, foams and rubbers, and agricultural industries, a promising market potential for new biodegradable materials is available.

So far, commercial applications have been developed only for poly(3HB-co-3HV) (Biopol®). This material has been processed into bottles for hair care products and biodegradable motor oil. Various containers, disposable razors, and food trays for holding portions of fish and meat in the refrigerator section of supermarkets were also manufactured from Biopol®. In these cases, the rather expensive Biopol® is used solely for its “green” image in order to increase product sales and to open up the market for other applications, which require biodegradability for functional reasons.

The cross-linked PHAs can also be used as biodegradable rubbers. Further studies are focused on the application of mcl-PHAs latex in totally organic solvent-free paints. Gouda cheese coated with mcl-PHAs latex is mechanically and hygienically protected without effects on ripening and storage. A more promising field of PHAs applications, with respect to negligible production costs, are biomedical devices and related products, because PHAs are generally biocompatible with mammalian tissue and are resorbed at a slow rate. PHAs are used to develop scaffolds for tissue engineering. Many more applications are established in field of medicine and pharmacy.

Biodegradation of PHAs

Intracellular degradation of PHAs by depolymerase has not been studied in depth as compared to extracellular PHAs degradation. Intracellular degradation comprises the hydrolysis of the endogenous storage material, mobilizing the carbon/energy reservoir by the PHAs accumulating cells themself. The key enzymes of intracellular mobilization of PHAs are intracellularly located PHA depolymerases, which are able to hydrolyze native PHA. These enzymes differ from extracellular depolymerases in their inability to hydrolyze PHAs extracted from the cells on which surface layer containing proteins and phospholipids is damaged.

Extracellular PHAs degradation is a prerequisite for utilization of PHAs as carbon/energy source, independently from synthesizing organisms. Generally, PHAs are released in the environment by the accumulating cells after cell lyses. The ability to degrade PHA is widely distributed among bacteria and fungi, and depends on the secretion of specific extracellular PHA depolymerases and on the physicochemical nature of polymer itself. The factors affecting the degradation are: (i) stereoregularity of the polymer, (ii) crystallinity of the polymer, (iii) composition of the PHA, and (iv) accessibility of the PHA surface. Most importantly, monomeric composition determines the physical properties of a given PHA, e.g. changing the monomeric composition will also change the crystallinity of the polymer.

Sixteen extracellular, bacterial PHAs depolymerase genes (phaZ) have been cloned and analyzed since 1989. Fifteen genes code for depolymerases with specificity for scl-PHAs, especially PHB. Some of these depolymerase proteins have significant activity with poly(3HV) homopolyester, but none of the protein has significant activity on mcl-PHAs, and also depolymerases showing activity on mcl-PHAs do not show activity on scl-PHAs. This indicates that depolymerases are highly specific with respect to the length of the carbon side chain of the PHAs substrate. The ability to degrade PHAs is not restricted to bacteria, at least one species from 95 genera of fungi have also been identified as scl-PHAs or mcl-PHAs degrading fungi. The most, if not
all, extracellular PHA depolymerases have endo- and exo-hydrolase activity. Depending on depolymerase, the hydrolysis products are monomers and/or oligomers. All PHA depolymerases studied so far showed stereo-specificity for (R)-configuration\textsuperscript{101,102}. As biodegradability is one of the most important characteristics of PHAs, their biotechnological exploitation should always be accompanied by a thorough investigation of their biodegradation. The degradation could be performed either by growing the microorganisms in the presence of the polymer or by incubation of the polymer with the depolymerase enzyme alone. Standard methods for evaluation of biodegradation should always be accompanied by a thorough investigation of their biodegradation. The degradation could be performed either by growing the microorganisms in the presence of the polymer or by incubation of the polymer with the depolymerase enzyme alone. Standard methods for evaluation of biodegradability have been studied for PHAs\textsuperscript{103}. With the development of variety of PHAs and their increased use because of environment concerns, more detailed investigations on the structure and function of bacterial PHA depolymerase will be necessary in future.

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References

18 Jawed A & Gruys K J, Biodegradable Polymers (Biomol\textsuperscript{8}), in Biopolymers, vol 4, edited by Y Doi & A Steinbüchel (Wiley-VCH, Weinheim) 2002, 53-68.


