POLYHYDROXYALKONATES: GREEN PLASTICS OF THE FUTURE

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Abstract

Plastic waste disposal is a major ecotechnological problem. One of the major approaches to solve this problem is the use of biodegradable biopolymer. Polyhydroxyalkanoates (PHAs) are biodegradable polymers of hydroxyalkonates, which are accumulated as energy storage material in various microorganisms to overcome environmental stress. Polyhydroxyalkanoates have gained major importance due to their structural diversity and close analogy to plastics and have attracted attention as biodegradable alternatives to conventional thermoplastics and as biomaterials. According to the variations in toughness and flexibility they can be used in various ways similar to many non biodegradable petro plastic which are currently in use. The need is to produce PHAs, which have better elastomeric properties suitable for its various applications. This review gives an overview on biodegradability and commercial reliability of PHA.

Keywords: Polyhydroxyalkonates, bioplastic, biodegradation, environment

1. Introduction

The research and development of polyhydroxyalkonates (PHA) has started from the beginning of the 20th century while over the last four decades significant progess has been made in our understanding, mainly motivated by the environment friendly properties of PHA. Unlike the present petrochemical- based plastics, PHAs are produced from renewable resources. Petrochemical plastics have found widespread application in our daily life and currently in wide use being regarded as a major threat of pollution of the environment. Over the years an increased use of plastic in our routine life gave rise to the accumulation of plastics materials in our environment. Synthetic plastics are resistant to degradation, and consequently their disposal is fuelling an international attraction for the development of biodegradable polymers, compatible

with our natural ecosystem. These problems have been the primary motivating factor in the research and development of PHA as a potential substitute for petrochemical – based plastics. PHAs are biocompatible as well as biodegradable, and their degradation product 3 hydroxyalkonate is a normal mammalian metabolite[1.] Polyhydroxyalkanote(s) (PHAs) are natural biopolymers and they are osmotically inert compounds which are optically active, biocompatible, biodegradable and hydrophobic[2]. Many prokaryotic organisms accumulate PHAs as reserve material when carbon (C) source is available in excess in the environment and there is a limitation of nutrients essential for growth[3]. An interesting alternative to decrease the environmental impacts of plastics is to replace conventional petroleum-derived polymers with biodegradable ones. In this context, polymers of biological origin, such as starch derivatives, polylactic acid, cellulosic polymers and polyhydroxyalkanoates, play an important role[4].

2. Types of PHA

PHAs can be characterized by chain length, type of functional group and

degree of unsaturated bonds. A higher degree of instauration increases the rubber qualities of a polymer, and different functional groups change the physical and chemical properties of a polymer. PHAs are divided into two groups, i.e. short-chain-length PHAs (SCL-PHAs) that are comprised of Polyhydroxybutyrate (PHB) and the copolymer polyhydroxy-co-valerate (PHBV); and medium-chain-length PHAs (MCL-PHAs) that consist of 3-(R)-hydroxyhexanoate/ 3-(R) hydroxytetradecanoate monomers. This difference is mainly due to the substrate specificity of the PHA synthases that can accept 3HAs of a certain range of carbon length[5]. The properties of PHAs vary with their composition. Each type of PHA generally consists of 1,000-10,000 monomers, but most are synthesized by SCL monomers. They are mainly composed of R-(-)-3-hydroxyalkanoic acid monomers[2]. The first group includes polyesters containing monomers that are C4 or C5 hydroxyalkanoic acids, such as P (3HB), poly (3-hydroxyvalerate) [P(3HV)] or the copolymer P(3HB-co-3HV). The second group, known as PHA mcl, includes those polymers formed by monomers equal to or longer than C6, usually existing as copolymers of two

to six different types of 3-hydroxyalkanoic acid units Hybrid polymers containing both short-chain and medium-chain monomer units also exist, such as poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) synthesized.This grouping is due to the substrate specificity of the PHA synthesis that only accept 3-hydroxyalkanoates(3HAs) of a certain range of carbon length. The PHA synthetases of *A. eutrophus* can only polymerize 3HAs (SCL) while that of *Pseudomonas oleovorans* only polymerize 3HAs (MCL). A lot of PHAs (MCL) containing various functional groups such as olefins, branched alkyls, halogens, aromatic and cyano has been reported[6]. SCL-PHAs are thermoplastics with a high degree of crystallinity, while MCL-PHAs are elastic or tacky materials with a low degree of crystallinity and a low melting temperature. The most attractive feature of MCLPHAs is that various MCL-PHAs bearing different functional groups in the side chains can be synthesized by some organisms, including *Pseudomonas oleovorans* and *P. putida,* when they are grown with substrates containing the corresponding chemical structures[7]. Figure 1 depicts the journey of the development of PHA

in science and technology through the twentieth century.

PHB (or poly-3-hydroxybutyrate (P (3HB))) is the most common type of PHA produced and is an example of a short chain length homopolymer produced by *A. eutrophus*. The addition of a small percentage of longer side chain subunits into PHB causes an increase in the flexibility of this copolymer, making it useful as a substitute for bulk thermoplastics. Such a copolymer is produced by the Imperial Chemical Industries (Billingham, United Kingdom) by large-scale fermentation of *Alcaligenes eutrophus*[9] . Compared to PHB, P (3HB-3HV) is less stiff, tougher, and easier to process, making it more suitable for commercial production. It is also water resistant and impermeable to oxygen, increasing its value.

P (3HB-*co*-3HHx) is also an interesting copolymer because the backbone of this polymer consists of 3HB (an SCL monomer) and 3HHx (an MCL monomer). Incorporation of small amounts of3HHx units (5 mol%) into the 3HB sequence reduces the melting point from 180 °C to less than 155°C , thus significantly improving the thermal process ability and physical properties[10]. One more type of PHA co polymer that

shows useful physical properties is poly [3-hydroxybutryate-co-4-hydroxybutryate] (P [3HB-co-4HB]). Similar to 3HB, 4HB is a normal mammalian metabolite which has been found in extracts of rat, pigeon and man[11].

3. Biosynthesis of PHA

PHA are synthesized and intracellular accumulated in most bacteria a under unfavorable growth condition such as limitation of nitrogen, phosphorus, oxygen or magnesium in the presence of excess supply of carbon source. Strategies are still being developed to simulate conditions for efficient production of PHAs. . Some bacteria such as *A. eutrophus*, A. *Latus* and mutant strain of Azotobacter *vinelandii* are known to accumulate PHA during growth in the absence of nutrient limitation[6].

An attractive feature of the microbial PHAs is the ability to produce them using renewable carbon sources. The plastic materials widely in use today are synthesized from fossil fuels such as petroleum and natural gas. PHAs on the other hand can be produced using renewable carbon sources such as sugars and plant oils, which is an indirect way of utilizing the atmospheric CO_2 as the carbon source. Various waste materials such as whey[12,13] molasses and starch[14,15] are also being considered as potential carbon sources for PHA production. The carbon source available to a microorganism is one of the factors (others being the PHA synthase substrate specificity and the types of biochemical pathways available) that determine the type of PHAs produced. For industrial scale production, the carbon source significantly contributes to the final cost. This makes the carbon source one of the most important component in the production of PHA and is therefore a prime target for potential cost reduction.

PHA synthases are the key enzymes of PHA biosynthesis. They use coenzyme A (CoA) thioesters of hydroxyalkanoic acids (HA) as substrates and catalyze the polymerization of HAs into PHA with the concomitant release of CoA[16].

PHA synthases are specific for the synthesis of either PHA (SCL) or PHA (MCL). In the *Alcaligenes eutrophus*, polyhydroxybutyrate (PHB), a thoroughly characterized PHA is synthesized from acetyl-CoA by a sequential action of three enzymes. The first enzyme, in the pathway 3-ketothiolase promotes the condensation

of two acetyl CoA moieties in a reversible manner to form acetoacetylCoA. This is followed by the action of acetoacetyl-CoA reductase which reduces acetoacetyl-CoA to R(-)-3- hydroxybutynl-CoA. The PHA synthase then polymerize the R (-)-3-hydroxybutyrl-CoA to form PHB. A. eutrophus can accumulate PHB as inclusion up to 80% of the dry weight which the bacterial are cultivated in media containing excess carbon such as glucose, but limited in one essential nutrient, such as nitrogen and phosphate[4].

4. Biodegradation of PHA

Microorganisms in nature are able to degrade PHAs by using PHA hydrolases and PHA depolymerases. The activity of these enzymes may vary and depend upon the composition of the polymer and the environmental conditions[17].

Degradation of bioplastic depends upon temperature, moisture level, pH, and nutrients supply available to the microbial community as well as the physical properties of the PHA material, such as crystallinity, composition or surface area.

There are also specialized bacteria, such as a thermophilic actinomycete that is capable of PHA degradation at high temperature and the anaerobic *Ilyobacter delafieldiithas* that can degrade PHAs to short chain esters such as acetate, propionate and butyrate, carbon dioxide and hydrogen gas in the environment where no oxygen is present[18].

The biodegradation of poly (HA) should clearly distinguish between extracellular Poly (HA) degradation and intracellular poly (HA) degradation.

4.1. Extracellular degradation is the utilization of an exogenous carbon/energy source by a not necessarily accumulating microorganism. The source of this extracellular polymer is poly (HA) released by accumulating cells after death. The ability to degrade poly (HA) is widely distributed among bacteria, and depends on the secretion of specific poly (HA) depolymerases which are carboxyesterases (EC3.1.1) and hydrolyze the water-insoluble polymer to water-soluble monomers and/ or oligomer.

4.2. Intracellular degradation is the active mobilization (hydrolysis) of an endogenous carbon/energy storage

reservoir by the accumulating bacterium itself[19].

Biodegradation rates of polymer films in seawater have been found to be higher than those of compacted pellets. As 3-PHB and 3-PHB/3-PHV are degraded and the specimens lose their mass, molecular weight of both polymers is decreased, i.e. polymer chains get destroyed[20].

Electron microscopy has revealed that degradation occurs at the surface by enzymatic hydrolysis (surface erosion). The molecular weights of PHA samples remained almost unchanged during the course of biodegradation. A number of microorganisms such as bacteria and fungi in soil, sludge, and sea water excrete extracellular PHA-degrading enzymes to hydrolyze the solid PHA into water-soluble oligomers and monomer, and subsequently utilize the resulting products as nutrients within cells.

Structural genes of extracellular PHB depolymerases have shown that all enzymes are comprised of N-terminal catalytic domain, C-terminal substrate-binding domain, and a linker region connecting the two domains. The C-terminal domain acts as a substrate-binding domain for water-insoluble P (3HB) substrate, as the PHB depolymerase without C-terminal domain lost the hydrolyzing activity toward the water-insoluble P (3HB), while it retained the activity toward water-soluble (R)-3HB oligomers[21]. The molecular weights of PHA samples remained almost unchanged during the course of biodegradation. A number of microorganisms such as bacteria and fungi in soil, sludge, and sea water excrete extracellular PHA-degrading enzymes to hydrolyze the solid PHA into water-soluble oligomers and monomer, and subsequently utilize the resulting products as nutrients within cells.

Many bacterial species are able to accumulate a water-insoluble, dense, crystalline granule which is known as lipid poly(3-hydroxybutyrate)(Poly(3HB)). P(3HB) is the most common biological polyester produced by various microorganisms in nature.The densities of crystalline and amorphous P(3HB) are 1.26 and 1.18 g/cm3, respectively. Mechanical properties like the Young's modulus (3.5 GPa) and the tensile strength (43 MPa) of P(3HB) material are close to those of isotactic polypropylene. The extension to break (5%) for P(3HB) is lower than that of polypropylene (400%). Therefore, P(3HB) appears as a stiffer

and more brittle plastic material when compared with polypropylene[22].On the other hand, the mechanical properties of P(3HB-co-3HV) is dependent on the molar ratio of 3HV as shown in Table 1. The value of Young's Modulus decreases with an increase of 3HV from 0 – 25 mol%, thus indicating that P(3HB-co-3HV) becomes more flexible[23,24].

5. Applications of PHA

PHAs are natural thermoplastic polyesters, and hence the majority of their applications are as replacements for petrochemical polymers currently in use for packaging and coating applications. The extensive range of physical properties of the PHA family of polymers and the broadened performance obtainable by compounding and blending provide a correspondingly broad range of potential end-use applications[25].

PHAs have a wide range of applications owing to their novel features. Initially, they were used in packaging films mainly in bags, containers and paper coatings. Similar applications as conventional commodity plastics include the disposable items, such as razors, utensils, diapers, feminine hygiene products, cosmetic containers—

shampoo bottles and cups. In addition to potential as a plastic material, PHAs are also useful as stereo regular compounds which can serve as chiral precursors for the chemical synthesis of optically active compounds[26,16].

Composites of bioplastics are already used in electronic products, like mobile phones (NEC Corporation and UNITIKA Ltd. 2006). Potential agricultural applications include encapsulation of seeds, encapsulation of fertilizers for slow release, biodegradable plastic films for crop protection and biodegradable containers for hothouse facilities[27]. The medical applications of PHAs have been explored extensively in recent years. PHAs have been used to develop devices, including sutures, nerve repair devices, repair patches, slings, cardiovascular patches, orthopedic pins, adhesion barriers, stents, guided tissue repair/regeneration devices, articular cartilage repair devices, nerve guides, tendon repair devices, bone-marrow scaffolds, tissue engineered cardiovascular devices and wound dressings[28].

PHAs are also potential material for applications in controlled drug release systems[29]. The biocompatibility and

biodegradability properties of PHAs make them attractive as materials for drug delivery. A wide variety of monomers can be incorporated into PHAs, resulting in various physical properties that range from highly crystalline materials to strong elastomers. PHAs with longer-side-chain hydroxyl acids have been used in pressure-sensitive adhesive formulations[30]. PHAs can be used as latex, for instance for paper-coating applications[31], or can be used to produce dairy cream substitutes[32] or flavor delivery agents in foods[30]. In addition to its range of material properties and resulting applications, PHAs promise to be a new source of small molecules.

6. Conclusion and Future Outlook

PHAs are the plastic of the future they can be injected, extruded or molded to form all sorts of products just like traditional plastics and are economically competitive. Expressing the bacterial genes for PHA synthesis specifically in plants seeds address current issues such as the degradation of plant health and PHA extraction from whole plants. Environmentally accountability is constantly increasing in consequence to both consumers and industry. As

pollution of the environment with petrochemical-based plastics continues apace, and attempts to dispose of them produce, in turn, further alternative and unacceptable pollution, biodegradable plastics produced by offer a shining hope for the amelioration of this global problem.

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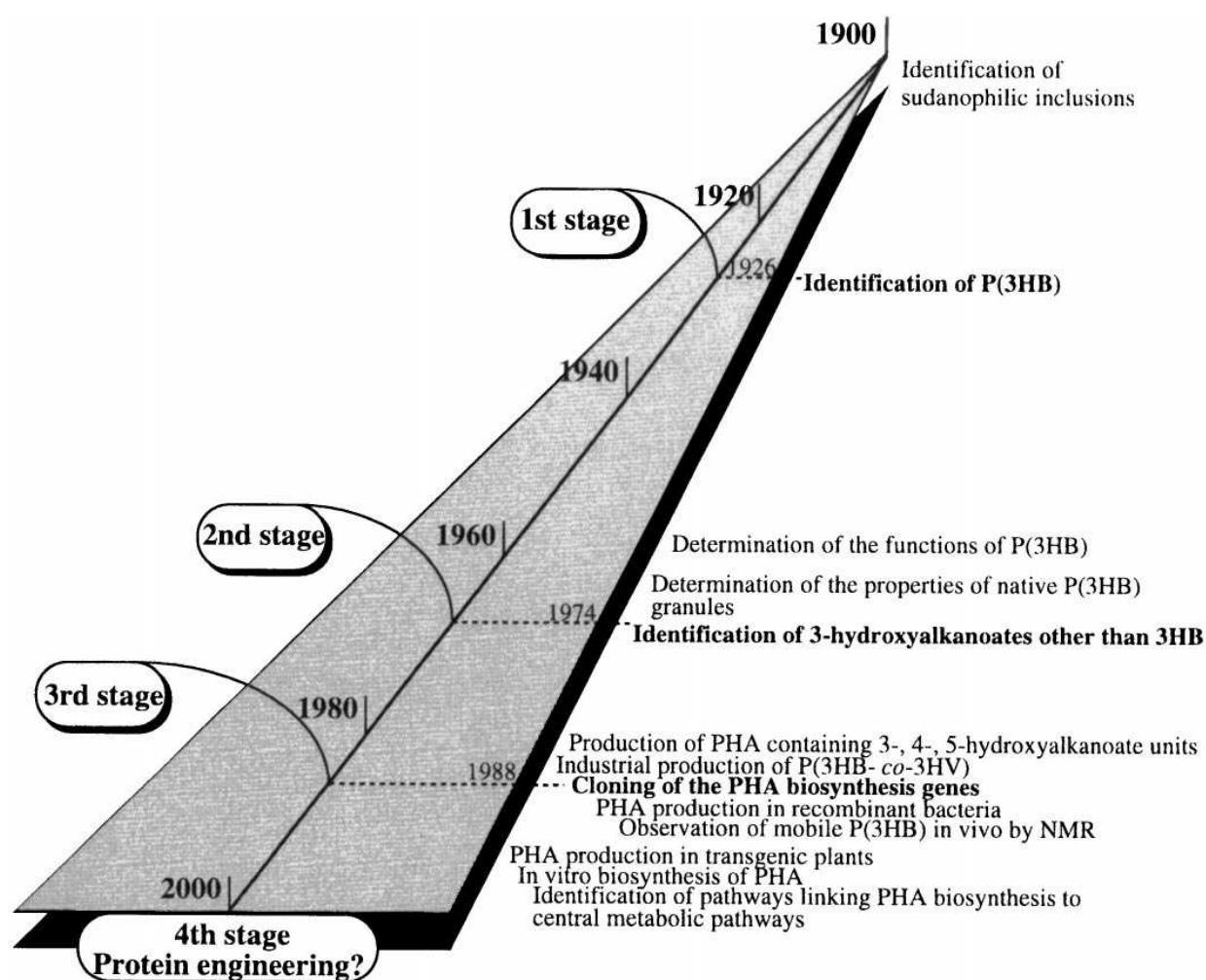
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Figure 1: The development of PHA science and technology through the twentieth century [8]

Table 1: Thermal and mechanical properties of P(3HB-*co*-3HV) copolymers [23, 24]

Properties	3HV composition (mol%)					
	0	3	9	14	20	25
Melting temperature, T_m	179	170	162	150	145	137
Glass transition temperature, T_g	10	8	6	4	− 1	− 6
Young's Modulus (GPa)	3.5	2.9	1.9	1.5	1.2	0.7
Tensile strength (MPa)	40	38	37	35	32	30