POLYHYDROXYALKANOATES

1. Introduction

Polyhydroxyalkanoates (PHA) are polyesters comprising hydroxycarboxylate monomers. These monomers have carbon chains ranging from 3 to 16 carbon (Fig. 1**a**). In nature, PHA occur as water-insoluble inclusion bodies within the cytoplasm of a variety of bacteria. Biological PHA is composed of 3-hydroxy-carboxylate units, all in the (*R*) configuration, ie, biological PHA are isotactic.

Two distinct classes of PHA are synthesized by different groups of bacteria: the short-chain-length PHA (PHA_{SCL}) is composed of monomers having 3 to 5 carbon while the medium-chain-length PHA (PHA_{MCL}) is composed of monomers having 6–16 carbon. The PHA_{SCL} is typified by polyhydroxybutyrate (PHB), a homopolymer composed of four-carbon 3-hydroxybutyrate monomers (Fig. 1**b**). The other well-known PHA_{SCL} is the copolymer, poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)], which comprise of four- and five-carbon monomeric units (Fig. 1**c**). The proportion of these monomeric units can vary, and this affects the physical properties of the polymer, eg, the copolymer is less brittle with increasing proportion of 3-hydroxyvalerate units. The PHA_{MCL} are heteropolymers, no natural homopolymeric PHA_{MCL} has been documented. 4-Hydroxy and 5-hydroxy monomers (Fig. 1**d**) do not occur naturally but they could be synthesized by some bacteria when fed with related carbon substrates such as 4-hydroxybutyric acid, γ-butyrolactone or 1,4-butanediol (1,2).

2. Biosynthesis of PHA

Cells synthesize PHA usually under growth-limiting conditions other than the carbon source. Deficiencies in nitrogen, phosphorus, and oxygen are known to trigger the synthesis. Under such conditions protein synthesis slows, leading to increased levels of reduced nicotinamide adenine dinucleotide (NADH) and reduced nicotinamide adenine dinucleotide phosphate (NADPH). These inhibit citrate synthase and isocitrate dehydrogenase, which thus slow down the tricarboxylic acid (TCA) cycle. Acetyl-coenzyme A (CoA) is then directed toward P(3HB) synthesis (3): 3-ketothiolase, termed PhaA with regard to PHA biosynthesis, reversibly joins two molecules of acetyl-CoA to form one molecule of acetoacetyl-CoA. This compound is reduced to (*R*)-3-hydroxybutyryl-CoA by NADPH-dependent acetoacetyl-CoA reductase, termed PhaB. (*R*)-3-Hydroxybutyryl-CoA is added to the PHB chain by PHA synthase, termed PhaC. While this is the usual pathway, typified by *Ralstonia eutropha*, sometimes acetoacetyl-CoA is reduced to the (*S*) isomer of 3-hydroxybutyryl-CoA by NADH- dependent reductase, as occurs in *Rhodospirillum rubrum*. The (*S*)-3-hydroxybutyryl-CoA must convert to the (*R*) isomer by enoyl-CoA hydratases before it is polymerized to P(3HB) by PHA synthase.

PhaA, PhaB, and PhaC could also catalyze the synthesis of the copolymer P(3HB-*co*-3HV) when propionic acid is present in the medium. Propionic acid is first activated to propionyl-CoA, then PhaA would condense a molecule of propionyl-CoA with a molecule of acetyl-CoA to form a molecule of 3-ketovaleryl-CoA which, upon reduction to 3-hydroxyvaleryl-CoA, can be added to the polymer

chain. The 3HV portion of P(3HB-*co*-3HV) could be increased by using valeric acid as the carbon source. In *R. eutropha*, the three genes that code for the three enzymes involved in the synthesis of PHA_{SCL} are clustered on a single operon (a set of promoter, operator and structural genes) in the sequence synthase-ketothiolase-reductase (4). Figure 2 shows the biosynthetic pathways for PHA_{SCL}.

The biosynthesis of PHA_{MCL} follow different pathways from that of PHA_{SCL}, and they occur in fluorescent pseudomonads belonging to the rRNA-homology-group I, eg, *Pseudomonas oleovorans*, *P. aeruginosa*, and *P. putida*. The monomeric units in PHA_{MCL} usually have an even-number of carbon atoms and range from 6 to 16 carbon units. When the carbon substrate fed to the bacteria is a fatty acid or triacylglycerol, the monomeric units in the PHA_{MCL} are usually not longer than the longest fatty acid substrate. This shows that intermediates of fatty acid β-oxidation are converted to 3-hydroxyacyl-CoA and incorporated directly into the PHA chain. Except in *P. oleovorans*, PHA_{MCL} is also synthesized from simple short-chain carbon substrates such as glucose and acetate, and monomeric units are even-number carbon chains. This indicates that PHA_{MCL} is also formed from intermediates of denovo fatty acid synthesis (5). Figure 3 shows the biosynthetic pathways for PHA_{MCL}.

The biosynthetic pathways of PHA_{SCL} and PHA_{MCL} appear to be mutually exclusive in bacteria due largely to the substrate specificities of the PHA synthases in the different groups of bacteria. The PHA synthase of PHA_{SCL} producing bacteria polymerizes short-chain-length hydroxyalkanoyl-CoAs while the PHA synthases of PHA_{MCL} producing bacteria polymerize medium-chain-length hydroxyalkanoyl-CoAs. This characteristic holds true when the enzyme is within the wild-type bacteria in their natural physiologic environment. It had been reported (6,7) however, that when the PHA synthase from *R. eutropha* was expressed in a recombinant bacterial strain, copolymers comprising short-chain-length and medium-chain-length monomers were synthesized. In some recombinant *pseudomonas* strains (8), both PHA_{SCL} and PHA_{MCL} are accumulated together within the same cell but in separate granules, as revealed by freeze–fracture analysis. These strains produced PHA synthases with substrate-specificities for short-chain-length and medium-chain-length precursors, respectively, which indicates that the substrate specificity of the PHA synthase had altered when acting outside of its original wild-type producer.

An interesting aspect of bacterial PHA is that the carbon source fed to the bacteria greatly influences the monomer composition of the resultant PHA. An exception is in the biosynthesis of P(3HB) because the monomers, hydroxybutyric acid, are formed from acetyl-CoA, which is a common metabolite arising from the breakdown of carbohydrates, lipids, and proteins. Other monomeric units are generally synthesized from related carbon substrates, eg, hydroxyvalerate is synthesized from the condensation of propionyl-CoA with acetyl-CoA; hydroxyoctanoate is formed from octanoic acids, a fatty acid. Most of the monomeric units in PHA_{MCL} are formed from fatty acids. This property lends itself to manipulation as it is possible to design the types of monomers to be incorporated as well as the proportion of the different monomers.

3. Biodegradation of PHA

In nature, PHA accumulated in the cells is utilized as a carbon source by the bacteria when external carbon supply is depleted (9). The PHB is hydrolyzed by intracellular PHB depolymerase, which breaks up the polymer into monomers and dimers. The dimers are hydrolyzed to monomers by dimer hydrolase. The monomers, (*R*)-3-hydroxybutyric acid, are oxidized to acetoacetic acid by (*R*)-3-hydroxybutyrate dehydrogenase. Acetoacetate breaks down to acetyl-CoA, a key metabolic intermediate, by the activities of acetoacetyl-CoA synthetase and 3-ketothiolase.

While intracellular degradation of PHA is involved in the cyclic metabolism of the material in the producer cell, this biopolymer is also degraded by extracellular PHA depolymerases excreted by other bacteria and fungi in the environment. The water- insoluble PHA is hydrolyzed to water-soluble monomers and oligomers that could then be transported into the microbial cells and metabolized as nutrients. Extracellular PHB depolymerase has a substrate-binding site that recognizes PHB, and a catalytic site that hydrolyses the polymer. All purified PHA depolymerases are specific for either PHA_{SCL} or PHA_{MCL}. Some bacteria that are able to hydrolyze PHB as well as PHA_{MCL} will secrete the two respective PHA depolymerases (10). Studies using PHB depolymerase to act on a copolymer, 3-hydroxybutyrate-*co*-3-hydroxyhexanoate found that the enzyme could not hydrolyze ester bonds between 3-hydroxybutyrate and 3-hydroxyhexanoate. The PHB depolymerase has *exo*-hydrolase (cleaves ester bonds from the hydroxyl terminus of the polymer) and *endo*-hydrolase (cleaves ester bonds within the polymer) activities (11). Extracellular PHA depolymerases hydrolyze PHA into monomers, dimers, and oligomers, the latter two will be degraded to monomers by oligomer hydrolases. In nature, only poly[(*R*)-hydroxyalkanoates] are hydrolyzed by PHA depolymerases, the (*S*) form of PHA is not because the enzyme could not hydrolyze ester bonds between monomers of (*S*) configuration. The PHA biodegradation is reviewed in (12).

4. Chemical and Physical Properties

The presence of PHA in bacterial cells was known and its structure had been elucidated since the early twentieth century. While the physiological role of PHA as a carbon and energy reserve did not elicit much interest, its chemical and physical properties did, mainly because of similarities with plastics (synthetic polymers), ie, PHA is hydrophobic and has thermoplastic properties (the material can be moulded into different shapes by heat-melting it). This discovery came at a time when alternatives to synthetic plastics were actively sought because of the realization that plastic wastes had become a big environmental pollutant and that raw materials for synthetic plastics were diminishing. Some physicochemical properties of PHA and synthetic plastics are presented in Table 1. The polymer P(3HB-*co*-3HV) has more useful material properties compared to P(3HB): Increasing the HV content in the copolymer will lower the crystallinity, melting temperature, stiffness (modulus), and tensile strength while toughness

(impact strength) and oxygen permeability are increased. Melting temperature (T_m) and glass-transition temperature (T_g) for P(3HB-*co*-4HB) decrease with increasing 4HB fractions. The tensile strength of the copolymer decreases with increasing 4HB fractions up to 16 mol%, with a corresponding increase in elongation to break. However, with higher content of 4HB (64–100 mol%), tensile strength increases. Thus, P(3HB-*co*-4HB) copolymers exhibit a wide range of material properties, depending on the 4HB content of the copolymer (13).

5. Biodegradability

One of the reasons for the global interest in PHA is because in addition to having some similarities to synthetic plastics, PHA is biodegradable, being a natural product. This property is of immense pertinence to environmentalists, consumers, and plastic manufacturers. Cashing in on the trend toward using more "environmentally friendly" plastic materials, several designed-to-degrade polymers were developed. However, many are not completely degraded, ie, the intermediate degradation products that may still be large molecules remain recalcitrant in the natural environments. It therefore became important to establish standards to define different types of degradation so that the degradable material could be more suitably matched for an application or product. The American Society for Testing and Materials (ASTM) and International Standards Organization (ISO) have committees of diverse expertise to deliberate and set standards to cover many aspects of a material, eg, definitions of different types of degradation and test methods. These are used internationally as guidelines in the manufacture, application and trade of plastic products.

Physical degradation or disintegration is where the product breaks down into smaller, even powdery, pieces in which the molecular structure and chemical properties remain unchanged. While this may remove some negative impacts, eg, improve aesthetics, the disintegrated products could bring different impacts such as altering the soil properties. Plastics are generally hydrophobic, accumulation of fine particles of disintegrated plastic products would reduce the water-holding capacity of a soil, which in turn would alter the microbial community and affect the fertility and sustainability of the soil. Fine particles are more easily mobilized by air and water, and therefore may be dispersed to and impact wider areas.

Chemical degradation is where some bonds of the polymer are broken that would alter the molecular size and structure. Photodegradation, thermodegradation, and water hydrolysis are some examples of chemical degradation. Oligomers may result and these may be more soluble or they could be metabolized by microbes. On the other hand, recalcitrant intermediates of chemical degradation may be more hazardous, eg, more toxic or be biomagnified as they pass through the food chain.

Biodegradation is, by definition, the breaking of chemical bonds in the polymer molecule by enzymes. Intermediates must be metabolized and mineralized within a specified period of time.

Much studies have been conducted to test the biodegradability of PHA. Parameters taken to measure biodegradability include gross weight loss and

changes in surface morphology of the product (usually observed under electron microscopy), and changes in molecular weight, thermal properties, and infrared (IR) analysis (all of which pertain to changes in material properties). Evolution of carbon dioxide is often used as a definitive indication of biodegradation based on the understanding that CO_2 is the ultimate endproduct arising from microbial metabolism and mineralisation of the PHA. The amount of evolved CO_2 could only be accurately measured in an enclosed and controlled environment. This finding could be correlated with other measurements obtained from the same controlled environment such as total organic carbon and changes to the material properties of the PHA samples. Although biological PHA and some PHA blends were found to be biodegradable, the rate of degradation varied widely, and was dependent on environmental factors such as temperature, moisture, pH, salinity, microbial species and density, and available nutrients. Rate of degradation was also dependent on monomer composition of the PHA, crystallinity, and surface area. Biodegradation of PHA results from physical as well as biological factors, eg, surface erosion may result from physical factors and this may render the PHA more susceptible to enzymatic attack, specifically that of extracellular PHA depolymerases. These enzymes are produced by many but not all microorganisms, and the synthesis and activitiy are greatly influenced by environmental conditions. The PHA depolymerases hydrolyze the insoluble PHA into oligomers and monomers that may be water soluble, and therefore could be metabolized completely.

Any new polymer or polymer blend would have to be tested for degradability, preferably biodegradability because this trait is becoming increasingly desirable especially for single-use disposable products. These products are the source of plastic pollution, therefore if they are made of biodegradable materials, they could be composted and recycled in the natural environment.

6. Polymer Blends with PHA

P(3HB) is very crystalline and is therefore very brittle. Besides producing copolymers of PHB by feeding the bacteria with related carbon substrates, another way of improving the material properties is to blend P(3HB) with other types of synthetic or natural polymers. Polymer blends are physical mixtures of different types of polymers. A miscible blend is obtained when the different polymers can exist as a single phase. If the different polymers separate out into distinct phases, then the blend is considered immiscible. The P(3HB) could form miscible blends with poly(vinyl alcohol), atactic P(3HB), poly(lactide), and poly(ϵ-caprolactone-*co*-lactide), but forms immiscible blends with poly(ϵ-caprolactone) and other polymers. The miscible blend of bacterial isotactic P(3HB) and atactic P(3HB) has increased elongation to break but lower Young's modulus and tensile strength (14). The immiscible blend of P(3HB) and poly(ϵ-caprolactone) has lower Young's modulus and tensile strength compared to P(3HB) but no increase in elongation to break (15).

7. Applications

PHA is a high molecular weight, hydrophobic material with thermoplastic properties. It is biodegradable in environments containing microorganisms. In nature, it is made by bacteria from renewable compounds. All these properties make PHA a highly desirable material because they encompass utility with biodegradation and nondependence on petrochemicals. The PHB is too brittle for practical applications but its copolymer P(3HB-*co*-3HV) is more flexible and had been used to make shampoo bottles and disposable trays. The polymer P(3HB-*co*-4HB) is also more flexible and was found to degrade at faster rates compared to P(3HB). The polymer P(3HB-*co*-4HB) has been proposed to be used as a system for the controlled release of 4HB, known as γ-hydroxybutyrate. 4HB has anaesthetic properties, could regulate metabolism in the brain, and is used to treat alcohol addiction (16). Studies have shown that although lipase did not degrade P(3HB), it caused significant weight loss (erosion) of P(4HB). In films of P(3HB-*co*-4HB), the erosion rate by lipase was higher with higher content of 4HB in the copolymer (13).

The polymers PHA_{MCL} are amorphous and gel-like when there is unsaturation in some monomers. Due to their low-crystallinity and low melting temperatures, PHA_{MCL} could not effectively be melt processed and moulded for use as thermoplasts. They are, however, amenable to cross-linking by ultraviolet (UV) irradiation and exposure to oxygen to produce a rubber-like material. Cross-linked PHA_{MCL} remained biodegradable although the rate of biodegradation decreased with cross-link density (17). The polymers PHA_{MCL} are therefore suitable for use as impervious lining material, adhesives, paint binder (18) and as controlled-release medium. Halogens and other functional groups can be attached to the unsaturated bonds for novel applications such as flame retardants. The polymer PHA_{MCL} can be added to PHB to get a less brittle material, or it can be added to hydrophilic biodegradable thermoplasts such as starch to increase moisture resistance.

PHA has been considered as a source for obtaining enantiomerically pure (*R*)-hydroxycarboxylic acids for use as chiral building blocks in the synthesis of fine chemicals. Chemical degradation of PHB to (*R*)-3-hydroxybutanoic acid (19), and *in vivo* depolymerization of PHB to (*R*)-3-hydroxybutyric acid (20) were reported.

Although PHA has many desirable properties, the high cost of the material is the biggest deterrent to wide applications. Medical applications would be a more practical target in the current situation, and products such as tissue scaffolds (21) and surgical inserts are being developed.

8. Industrial Production

Currently, PHA is produced on a large scale by fermentation processes. These consist of several aspects, each of which warrants research and development to optimize yield as well as to minimize production costs.

1. The producer cells: The polymer PHA_{SCL} is widely studied in *R. eutropha* (formerly known as *Alcaligenes eutrophus*) in which the PHB biosynthetic genes were found to be clustered in one operon in the sequence PhaC (encodes for PHA synthase), PhaA (encodes for 3-ketothiolase), and PhaB (encodes for acetoacetyl-CoA reductase). These genes have been successfully cloned and expressed in *Escherichia coli* (22). Later, P(3HB-*co*-3HV) was also produced in recombinant *E. coli* (23). The PHA_{MCL} biosynthetic genes are elucidated in *P. oleovorans* and *P. putida*, and have also been cloned and expressed in *E. coli* (24). Although *E. coli* does not naturally produce PHA, it is a suitable cloning host because it can use many types of cheap carbon substrates, it is relatively easy to extract and purify PHA from it, and it does not produce intracellular depolymerase. Therefore, synthesized PHA could attain high molecular weight and will not be degraded intracellularly.

Large-scale fermentation invariably uses recombinant *E. coli* for several reasons:

(i) The cells carry multiple copies of the biosynthetic genes, which means increased production of PHA compared to the wild-type strains.
(ii) Recombinant cells may be able to grow at a faster rate and attain higher cell densities within the bioreactor.
(iii) Recombinant cells do not produce intracellular PHA depolymerase so they do not self-degrade their PHA.
(iv) Recombinant cells may have more fragile cell wall or produce enzymes that lyse the cells under certain stimuli, eg, elevated temperature. This removes the requirement for complicated extraction of PHA from the cells.

2. Fermentation feedstocks: The carbon source is of primary importance not only for cell growth but it has a big influence on the type of PHA formed. To be cost-effective, fermentation industries utilize agriculture products as primary source of nutrients because they are often cheaper than pure compounds (eg, starch is cheaper than glucose) and readily available. However, sucrose, starch, and vegetable oils may not be cheap enough for use as fermentation substrates because they are primarily produced as food. By comparison, agricultural by-products such as molasses and cheese whey are cheaper. However one should be aware that the nutrient and chemical composition of by-products are more variable than primary products. This would not be an issue for the biosynthesis of PHB because the biosynthetic pathway utilizes acetyl-CoA, which is a common intermediate metabolite generated from many complex carbon compounds. Variation in composition of an agricultural by-product, eg, the types and amount of different fatty acids in palm oil mill effluent, would however affect the monomer composition of a PHA_{MCL} because fatty acids (the hydroxy form) are incorporated directly into the polymer.
   A model whereby PHB production is integrated with sugar production in

Brazil had been proposed (25). In large cane sugar mills, sugar, and molasses are available relatively cheaply. The large amount of bagasse (solid residue left behind after sugar has been extracted) is burned to generate thermal and electrical energy. Therefore the basic requirements for PHB production, ie, carbon source and energy, are available within the sugar mill complex. Natural solvents that are by-products of ethanolic fermentation (another activity in the complex) can be used to purify PHB. This would reduce the negative impact on the environment as compared to using chlorinated compounds to extract the PHA. By-products from the alcohol and PHA fermentation processes, eg, spent culture media and microbial biomass can be composted and used as fertilizers in the cane fields. In a similar vein, integration of P(3HB) fermentation process in a cheese manufacturing industry has been proposed as a strategy to convert the whey into a valuable product (26).

3. The fermentation process: As PHA is an intracellular inclusion, maximizing the yield of PHA would mean maximizing cell density. By using fed-batch method to obtain high cell-density, good yields of P(3HB) were obtained with *R. eutropha, Alcaligenes latus*, and with recombinant *E. coli*. This method also produced good yields of PHA_{MCL} with *P. putida.* Table 2 shows some PHA productivities obtained by this method. The lowest production cost of P(3HB) was calculated to be U.S.$ 2.6/kg (27). In a high cell-density culture, dissolved oxygen is often a limiting factor. Dissolved oxygen could be improved by increasing mixing speed and gas-flow rate, and feeding oxygen-enriched air, all of which require pressurized vessels. All of these will increase production costs.
4. Recovery and purification of PHA: In laboratory-scale studies, cells are separated from the fermentation broth, washed and dehydrated by lyophilization or warm air-drying. The PHA is extracted out of the cells by chlorinated solvents such as chloroform and methylene chloride. Large volumes of these solvents are required to extract as much of the PHA as possible. The whole solution is filtered to remove undissolved cell biomass, then the solvent has to be concentrated and mixed with cold methanol whereby the PHA precipitate out. This method produces relatively pure PHA but it is not a suitable method for large-scale recovery and purification because chloroform is expensive as well as hazardous to the environment. Hypochlorite could also digest the cells but causes some degradation to the PHA. This effect could be reduced by pretreating the cells with surfactant before hypochlorite digestion (28). The method proposed by Nonato and coworkers (25) uses natural solvents, not chlorinated synthetic solvents, to break the cells and dissolve the PHA. After the cell debris is removed, the solvent is immersed in hot water to vapourise the solvent and precipitate the PHA. Using enzymes to lyse the cells is another method of extracting PHA out of the cells. In this case, most of the cell biomass would be solubilized by the enzymes but not the PHA (29). Instead of enzymes, it would be more economical to use inexpensive chemicals such as ammonium hydroxide (30) or sodium hydroxide (31) to digest the cells other than the PHA. This method of alkaline digestion is especially suitable for cells with

high accumulation of PHA when the walls become fragile.

A nonsolvent-based extraction process for PHA_{MCL} has been described (17), whereby the whole fermentation broth is heat-sterilized at the end of the culture period. This thermal treatment causes the following effects: ruptures the cells (intracellular PHA is released), degrades the polynucleic acids (prevents high medium viscosity), and denatures the proteins including intracellular PHA depolymerase (prevents degradation of the PHA). The denatured proteins would be more easily digested by subsequent protease treatment. The solid mass is recovered from the broth by centrifugation, after which it is treated with protease and an anionic detergent to solubilize all cell components except PHA. The PHA suspension is washed and concentrated by cross-flow microfiltration. By this method of extraction, a highly stable polymer latex is obtained because the PHA_{MCL} granules have a density close to that of water, and therefore would not settle but form a suspension in water. This latex has 96% purity and can be applied to a surface as a coating. When the water is removed, eg, by drying, the amorphous PHA readily coalesce into a film. When the film is heated or passed through UV or electron beam irradiation, the PHA_{MCL} becomes cross-linked. The PHA_{MCL} coatings are transparent and have good water-barrier properties. If a higher purity of the PHA_{MCL} is required, eg, for use in biomedical applications, the latex can be extracted with methylene chloride and subsequent precipitation in methanol.

9. Future Outlook

Although PHA has all the traits desirable for an alternative material to synthetic plastics, industrial production has not progressed greatly due largely to the high cost of production by using fermentation processes. In this context, it is felt that there must be a readjustment of expectations in all parties concerned: the manufacturers, end-users, and policy-makers. The reasons are as follows:

1. The cost of production of PHA is compared with that of synthetic plastics, which is not realistic. Raw materials for synthetic plastics are obtained very cheaply because they are by-products of the petrochemical industries. However, stockpiles of fossil fuels are not renewable in a practical frame of time. With depleting stocks, the price of petrochemicals will not only increase, but will become unavailable.
2. PHA is not a by-product, it is a primary product produced from microbial fermentation using renewable substrates.
3. The industrial process for synthetic plastics had been optimized for a much longer period compared to that for PHA.
4. The additional properties of PHA such as biodegradability and biocompatibility must be duly incorporated into the value of the product because these are important traits absent in synthetic plastics but are key elements in any new materials in order to help sustain the natural environments instead of destroying it.

5. In the current scenario, it is not economically viable to use PHA for bulk commodities such as single-use disposable products even though it is this area that contributes most to environmental deterioration. It would be more practical to use PHA for specialty products such as surgical inserts and tissue scaffoldings whereby the requirements of mechanical strength, biocompatibility, and resorption are met, and the products justify the "high" cost of production of the material.

10. In Vitro Biosynthesis of PHA

As with many aerobic fermentation processes, limitations in the yield of intracellular products come from oxygen transfer rate that in turn limits the density achievable by the producer cells. One way to overcome this is to produce PHA in cell-free *in vitro* systems, using enzymes and activated substrates. A coordinated system needs to be developed whereby the enzymes could perform efficiently outside the cell and they and other cofactors could be regenerated continuously so that reactions do not stop (32). Producing the activated substrates, eg, the CoA form of hydroxyalkanoic acids, however, is a challenge. In a study whereby PHA synthase from *R. eutropha* was used to polymerize a racemic mixture of 3-hydroxybutyl CoA *in vitro*, only the (*R*) monomer was polymerized (33). This specificity is good because only the (*R*) monomers in an atactic polymer get hydrolyzed by depolymerase enzymes, the (*S*) monomers are not (34). Another advantage of *in vitro* biosynthesis of PHA is the relative ease of recovering the polymer. This would greatly reduce the cost of production. Addition of halogens to unsaturated monomers may be more easily manipulated in an *in vitro* system. Going by the economic viability of producing steroids and vitamins by bioconversions (*in vitro* enzymatic reactions), *in vitro* biosynthesis has potential for commercial production of PHA.

11. Transgenic Plants

In comparison to producing PHA by heterotrophic microbial systems, a photosynthetic system utilizing carbon dioxide as the carbon source and light energy is believed to be more economical because the major requirements are available free. Starch and oil- producing plants are targeted for genetic engineering so that the natural biosynthetic pathways for starch and oil could be diverted to make PHB and PHA_{MCL}, respectively. Thus, P(3HB) were produced in cotton fiber cells in transgenic cotton plants (35) and PHA_{MCL} were produced in transgenic *Arabidopsis thaliana* carrying the PHA synthase gene from *P. aeruginosa* (36). Studies are underway to produce the industrially useful P(3HB-*co*-3HV) (37) and P(3HB-*co*-4HB) (38) in plants.

A pertinent criticism about devoting large hectares of agriculture land to producing materials for industrial applications is that naturally arable land should be used to produce food crops. The rationale is that while the human population is increasing, suitable land for agriculture is decreasing due to a num-

ber of factors such as desertification, depletion of fresh water supply, and urbanization. An alternative to PHA producing plants is to develop the PHA synthesizing mechanism in photosynthetic microorganisms such as cyanobacteria (39). These can then be cultured in photobioreactors. Production costs arising from organic carbon substrates and energy input required in conventional bioreactors may be significantly reduced. Some cyanobacteria produce very small amounts of PHA, ~5% cell dry weight. Studies are directed at increasing this amount and removing the requirement on other carbon substrates such as acetate, ie, to produce PHA from CO_2 as sole carbon source.

12. Concluding Remarks

Industrial production of PHA_{SCL} such as P(3HB) and P(3HB-*co*-3HV) by fermentation using recombinant bacteria and renewable carbon substrates is well developed although there is much on-going efforts to reduce the cost of production. To be economically viable, the products should be targeted for use in the biomedical and other specialty areas. Polymer blends and composite materials incorporating PHA have good potential in this respect, but the new materials should be biocompatible and resorbable. Meanwhile, there is wide anticipation for successful transgenic plants to produce PHA at volumes and costs conducive for bulk applications such as in disposable products. It is hoped that PHA_{MCL} could be mass-produced by transgenic plants because the yield by fermentation is much lower compared to that of PHA_{SCL} (intracellular accumulation of PHA_{MCL} is lower than that of PHA_{SCL}), and *in vitro* biosynthesis of PHA_{MCL} is more complex than that for PHA_{SCL} because of the many different hydroxyalkanoic acids required as substrates.

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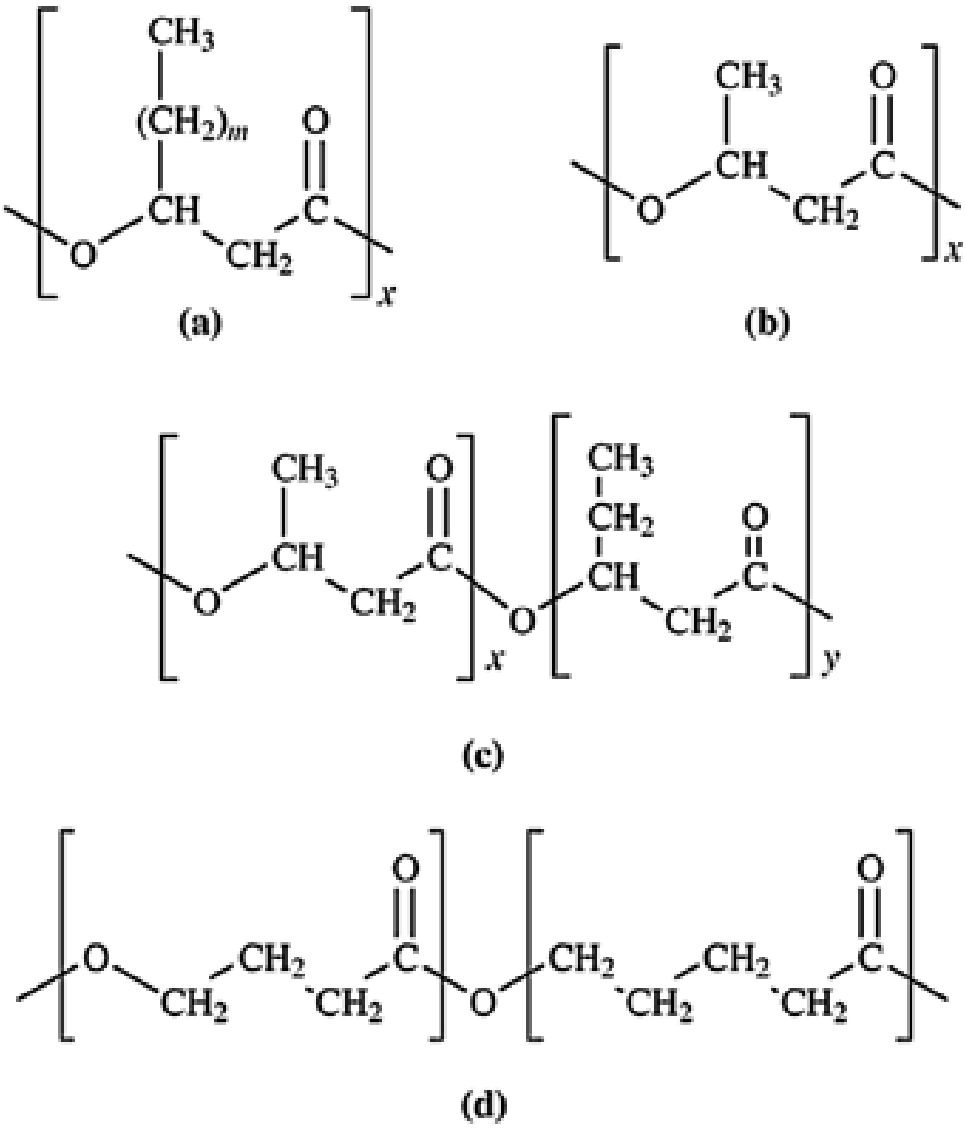

Fig. 1. Structures of PHA. (**a**) General structure of P(3HA). (**b**) P(3HB) homopolymer. (**c**) P(3HB-*co*-3HV) copolymer. (**d**) PHA showing 4-hydroxybutyrate and 5-hydroxyvalerate monomeric units.

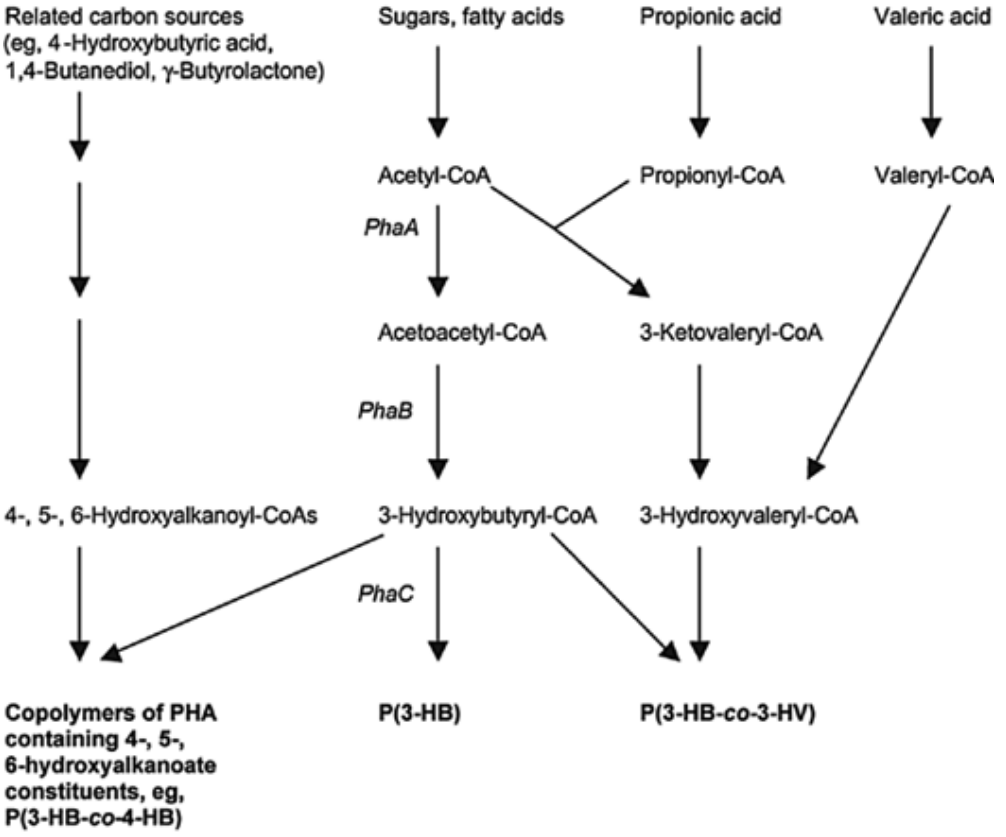

Fig. 2. Biosynthetic pathways for PHA_{SCL}. PhaA: 3-ketothiolase; PhaB: NADPH-dependent acetoacetyl-CoA reductase; PhaC: PHA synthase.

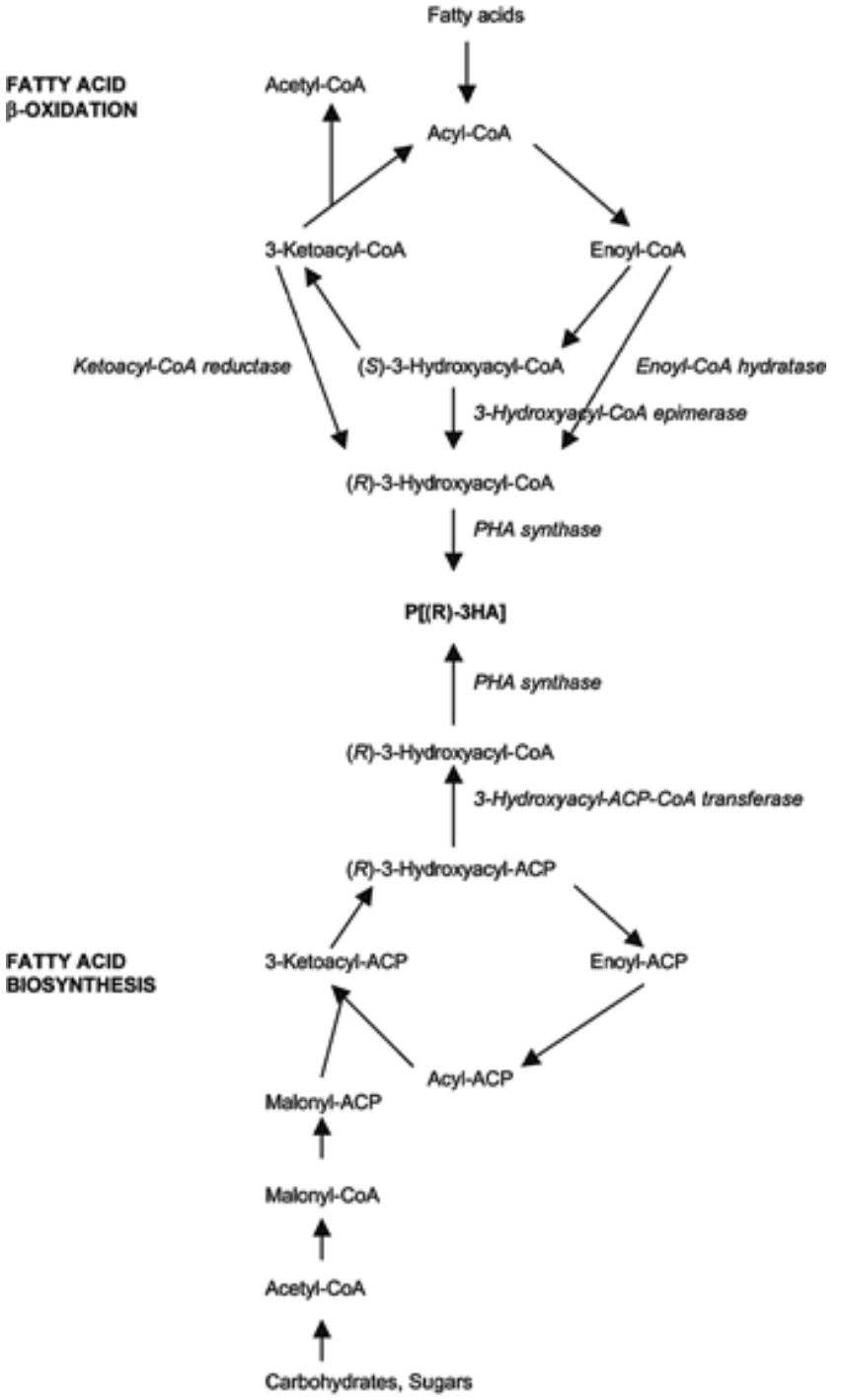

Fig. 3. Biosynthetic pathways for PHA_{MCL}.

Table 1. **Physicochemical Properties of PHA and Polypropylene**

Polymer	Molecular weight (M_w, $\times 10^5$)	Melting temperature (T_m, °C)	Glass transition temperature (T_g, °C)	Tensile strength (MPa)	Young's modulus (GPa)	Elongation to break (%)	Reference
P(3HB)	2	180	5	40	4.0	8	40
P(3HB-3HV) 25 mol% 3HV	3	137	−6	30	0.7	10	41
P(3HO)[a]	2	68	−35	6–10	2.5–9	300–450	42
polypropylene	2	180	−10	38	1.7	400	40

[a]Polyhydroxyoctanoate is P(3HO), a PHA_{MCL} with predominant hydroxyoctanoate monomers.

Table 2. **Some Productivities of PHA by Fed-Batch Culture**

PHA	Bacteria	Carbon substrate	Time (h)	Cell concentration ($g\ L^{-1}$)	PHA concentration ($g\ L^{-1}$)	PHA content (%)	PHA productivity ($g\ L^{-1}\ h^{-1}$)	Reference
P(3HB)	*Ralstonia eutropha*	glucose	74	281	232	82	3.14	43
P(3HB)	*Ralstonia eutropha*	sucrose	50	120	78	65	1.56	25
P(3HB-3HV)	*Ralstonia eutropha*	glucose + propionic acid	46	158	117	74	2.55	44
P(3HB)	*Alcaligenes latus*	sucrose	20	111.7	98.7	88	4.94	27
P(3HB)	*Alcaligenes latus*	sucrose	18	143	71.4	50	3.97	45
P(3HB)	Recombinant *Escherichia coli*	glucose	49	204.3	157.1	77	3.2	46
P(3HB)	Recombinant *Escherichia coli*	concentrated whey solution	49	87	69	79	1.4	26
PHA_{MCL}	*Pseudomonas putida*	oleic acid	38	141	72.6	51.4	1.91	47