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# Preface

Polyhydroxyalkanoates (PHAs) are very interesting polyesters synthesized by many types of bacteria. Numerous researchers from all over the world have carried out various studies on PHAs. There is already a wealth of knowledge about all aspects of PHAs in the literatures.

In this book, the focus is on the relatively recent efforts to use vegetable oils, especially palm oil and its by-products to synthesize PHAs. Palm oil is the world's most efficiently produced vegetable oil and Malaysia has been the pioneer in developing palm oil as a sustainable source of edible oil. Because of the high productivity of palm oil it costs less than other commercial vegetable oils. Therefore, it has been the preferred oil by most people from the low-income group. The production of palm oil is expected to increase to fulfill the growing demand. Besides Malaysia, Indonesia is also now a major producer of palm oil.

The palm oil industry generates large quantities of by-products and wastes rich in fatty acids that can be developed into potential feedstock for biotechnological applications such as for the production of PHA by microbial fermentation. Studies have also shown that the yields of PHAs from vegetable oils are generally better than those from sugars or other feedstock. Besides the yield of PHAs, there are many other factors that one will have to consider for large-scale production of PHAs. Of particular importance is the sustainability of the entire process of converting palm oil-based feedstock to PHA.

Malaysia is committed to the production of palm oil in a sustainable manner. The majority of Malaysians are also environmentally conscious and know the importance of biodiversity and forest conservation. Therefore, the pros and cons of developing palm oil-based feedstock for PHA production are being carefully scrutinized. This book is an attempt to provide a holistic view of the challenges involved in using palm oil and its by-products for the production of PHAs. In addition, several new applications for PHAs are also described.

This book was prepared in the midst of many other equally demanding tasks and therefore the help of many of my laboratory members was crucial. I am especially grateful to Dr. Sridewi Nanthini for compiling all the information necessary for this book. Mr. Yoga S. Salim had painstakingly drawn all the chemical structures of PHA monomers and Ms. Rathi Devi Nair did all the corrections based on inputs from all my graduate students. I am very grateful to all of them for their help in preparing this book.

Kumar Sudesh

# Contents

# Chapter 1
# Introduction

**Abstract** Polyhydroxyalkanoate (PHA) is a plastic-like material synthesized by many bacteria. PHA serves as an energy and carbon storage compound for the bacteria. PHA can be extracted and purified from the bacterial cells and the resulting product resembles some commodity plastics such as polypropylene. Because PHA is a microbial product, there are natural enzymes that can degrade and decompose PHA. Therefore, PHA is an attractive material that can be developed as a bio-based and biodegradable plastic. In addition, PHA is also known to be biocompatible and can be used in medical devices and also as bioresorbable tissue engineering scaffolds. In this chapter, a brief introduction about PHA and the fermentation feedstock for its production are given.

**Keywords** Bio-based • Biodegradable • Microorganism • Palm oil • PHA Plastics • Polyhydroxyalkanoate • Polymer

Petroleum-derived plastics have contributed significantly to our modern lifestyle due to their favorable stability, durability, and suitable mechanical and thermal properties. Plastic materials have now become an integral part of our daily life. In fact, it is almost impossible to lead a normal life without plastics. From the clothes that we wear to the cars that we drive there are numerous components that are made of plastics. The food that we eat comes in plastic packages or containers. We pay for the food and almost everything else using plastic credit cards. When we fall sick and visit the doctor, we are prescribed medicines that come in plastic bottles or plastic zip lock bags. Many medical devices are made of plastics. Almost all electronic devices contain plastic components. Our children play with plastic toys and spend the first few years of their life with plastic diapers. We might also need similar diapers during the last few years of our life. Our dependence on plastics shows that plastics are preferred because of their safety, versatility, durability, and affordability. However, synthetic plastics are difficult to be disposed due to the lack of natural enzymes and biological processes that can efficiently degrade synthetic plastics. Incineration of plastics releases hazardous gases into the environment. Harmful chemical such as hydrogen cyanide can be formed from acrylonitrile-based plastics during the combustion of these plastics (Johnstone 1990;

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Atlas 1993). On the other hand, recycling might be a better alternative but it is a labor-intensive process. Categorization of a wide variety of plastics is a time-consuming process. This process becomes worse with the presence of additives such as pigment, coating, and fillers (Fletcher 1993).

The ever-growing need to curb plastic waste management problem has resulted in the search for an alternative to petroleum-based synthetic plastics. In addition, the fact that petroleum is a non-renewable resource, which would be depleted sooner or later, has also motivated the search for bio-based plastics from renewable resources. Bio-based plastics that are also biodegradable may offer a solution to the plastic waste management problem. Therefore, biodegradable polymers are investigated to replace common plastics (Song et al. 1999). Biodegradable plastics can be classified into three categories which are; chemically synthesized polymer, starch-based biodegradable plastics, and polyhydroxyalkanoates (PHAs) (Khanna and Srivastava 2005a). PHA is a microbial storage polyester, synthesized naturally by many types of bacteria. PHA is being considered as a potential renewable alternative to some petrochemical plastics. This is because the properties of PHA resemble the properties of some commercially available plastics (Sudesh et al. 2000). In addition, PHA is completely biodegradable in nature. The bio-based and biodegradable nature of PHA would have the long-term benefits of reducing plastic waste accumulation, global warming, pollution, and dependence on fossil fuels. The availability of cheap and renewable carbon feedstock, preferably bio-based, for efficient conversion into PHA would make the PHA products' prices competitive with their petroleum counterparts. For this purpose, plant oils have been investigated and were found to be very attractive carbon sources for large-scale PHA production. Plant oils yield higher PHA content in comparison with other tested substrates such as sugars, because of their complex mixture of triglycerides (Akiyama et al. 2003). Among the various plant oils, palm oil is the world's most efficiently produced oil. Malaysia and Indonesia are both major producers and exporters of palm oil in the world. The versatility of palm oil suggests its usage as edible oils as well as for the production of oleochemicals. The palm oil industry generates large quantities of by-products composed of triglycerides and fatty acids which are suitable for microbial utilization. As is the case for almost all new technological innovations, there are pros and cons in using plant oils for the commercial production of PHA. Numerous concerns have been raised about the merits of diverting food grade oil for PHA production at the expense of food supply on a global scale. In addition, an increase in the demand for plant oils may result in further expansion of oil palm plantations into forests and subsequently threatening wild life habitats and destroying precious biodiversities. This book reviews the use of palm oil and its by-products as renewable feedstock and to provide a future outlook on the sustainability of palm oil for PHA production. The production of PHA from other plant oils is also described. Finally, discussions on the production and characteristics of the various types of PHA produced from palm oil products and some new applications of the resulting polymers are also included in this book.

# Chapter 2
# Bio-Based and Biodegradable Polymers

**Abstract** Many types of biodegradable plastics are being developed in response to the concerns over the accumulation of commodity plastics in the environment. Polyhydroxyalkanoate (PHA) stands out as an attractive material because it can be produced from renewable feedstock and subsequently it can be degraded completely by microorganisms. Approximately, 150 structurally different monomers can be polymerized into PHAs by bacteria, giving rise to polymers with diverse properties. Careful manipulation of the bacterial culture conditions and the carbon feedstock allows the design and synthesis of tailor-made polymers for various applications. This chapter focuses on the genetics and biochemistry of PHA biosynthesis in bacteria and recombinant organisms.

**Keywords** Degradation • Metabolic pathways • PHA synthase • PHA granule • Renewable feedstock • Sugars • Transgenic plants • Triglycerides

Bio-based polymers are defined as polymers produced from biological renewable resources and polymerized by chemical and/or biological methods. Bio-based polymers can be categorized into three groups: bio-chemosynthetic polymers [e.g., poly(lactic acid) (PLA), poly(butylene succinate), polyvinyl alcohol and polyglycolic acid], biosynthetic polymers (bioplastics or naturally occurring polymers [e.g., Polyhydroxyalkanoate (PHAs)]), and modified natural polymers (e.g., starch polymers and cellulose derivatives) (Sudesh and Iwata 2008) (Table 2.1). For bio-chemosynthetic polymers, the monomers are synthesized biologically and polymerized chemically. An example of bio-chemosynthetic polymer is poly(lactic acid). The lactic acid monomers which are produced by microbial fermentation are then chemically polymerized into PLA (a process catalyzed by metal catalyst) (Jem et al. 2010). In contrast to PLA, PHA is completely produced by biological process. The entire process of PHA biosynthesis right from the production of monomers and the subsequent polymerization processes occurs in the bacterial cells. Various types of bacterial enzymes act as the biological catalyst in the biosynthesis of PHA. Unlike bio-chemosynthetic polymers and biosynthetic polymers, the natural polymers such as starch need to be modified chemically and/or physically to enhance the polymer structure and improve the thermal and mechanical properties (Hoover et al. 2010).

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**Table 2.1** Some examples of bio-based and biodegradable polymers (Flieger et al. 2003; Nair and Laurencin 2007; Sudesh and Iwata 2008)

| Category | Processes involved | Example | Biodegradability |
|---|---|---|---|
| Bio-chemosynthetic polymers | Biological synthesis of monomers and chemical polymerization | Poly(lactic acid)<br>Poly(butylene succinate)<br>Polyvinyl alcohol<br>Polyglycolic acid<br>Polythioesters | Hydrolytically degradable except crystalline poly(lactic acid) and polythioesters |
| Biosynthetic polymers | Biosynthesis of polymer by microorganisms | Poly(3-hydroxybutyrate) | Enzymatically and/or hydrolytically degradable |
| Modified natural polymers | Chemical modification of natural polymer | Starch polymer<br>Cellulose derivatives<br>Proteins | Enzymatically degradable |

However, not all bio-based polymers are biodegradable. Some of the bio-based polymers such as crystalline PLA, cellulose derivatives, and polythioesters are not biodegradable (Steinbüchel 2005; Sudesh and Iwata 2008). Biodegradable polymers can be degraded hydrolytically and/or enzymatically, which involves the breakage of bonds that hold the monomers together in the polymer (Nair and Laurencin 2007). The natural polymers such as starch and proteins can be enzymatically degraded by various enzymes such as amylases and proteases, while the polymers possessing functional groups such as esters, anhydrides, carbonates, amides, and urea can be hydrolyzed (Flieger et al. 2003; Nair and Laurencin 2007).

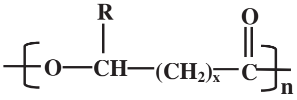
Most naturally occurring polymers such as PHA undergo hydrolysis of ester bonds, which are catalyzed by intracellular or extracellular PHA depolymerase enzymes (Guérin et al. 2010). The extracellular depolymerase enzymes are secreted by various bacteria and fungi in order to break down the PHA into low molecular weight products that can be assimilated and metabolized by the microorganisms. The final products of PHA degradation under aerobic conditions are carbon dioxide and water while methane is produced under anaerobic conditions. Thin solvent-cast PHA films can be biodegraded completely on soil surface in less than 2 months under tropical conditions (Sudesh and Iwata 2008). If the films are buried in the soil, the degradation rate will be much faster because of greater exposure to soil microorganisms. Therefore, PHAs are good candidates for biodegradable plastics applications due to their similar properties to those of conventional plastics but with high biodegradability. The common homopolymer, poly(3-hydroxybutyrate) [P(3HB)], can be hydrolytically degraded to a normal blood constituent in human body; thus, it has a potential application as biomaterial (Nair and Laurencin 2007). However, because of the high crystallinity and the absence of PHA depolymerase in humans, this polymer degrades at a very slow rate. Another type of PHA, poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)] with lower crystallinity than P(3HB) and higher rate of degradation is suggested as a better temporary substrate for tissue engineering such as bone tissue and epithelial tissue (Chen and Wu 2005; Köse et al. 2003). The PHA containing 4-hydroxybutyrate (4HB) monomer is probably the best to be used as biomaterials for tissue engineering because it can be hydrolyzed by the lipases of eukaryotes (Sudesh 2004).

## 2.1 Overview of Polyhydroxyalkanoate

PHAs are biopolyesters of various hydroxyalkanoates (HAs) synthesized as intracellular carbon and energy storage compounds by numerous bacteria (Lee and Choi 1999; Tang et al. 2008; Sudesh et al. 2011). Gram-positive and Gram-negative bacteria from at least 75 different genera are known to synthesize PHA (Reddy et al. 2003). These intracellular reserve polymers can be accumulated by some bacteria up to a maximum of 90 wt% of the cell dry weight (Madison and Huisman 1999a) under laboratory conditions such as during nutrient stress, which includes limitation of nitrogen, phosphorus, magnesium, or oxygen but in the presence of excess carbon supply (Anderson and Dawes 1990; Doi 1990; Kato et al. 1996; Madison and Huisman 1999). Under conditions that are not favorable for growth, the bacterial cells will assimilate the excess carbon sources for storage purposes. The assimilated carbon sources are then biochemically processed into 3-hydroxyalkanoic acid (3HA) monomer units and polymerized into high molecular weight water-insoluble PHA molecules that are stored in the form of inclusions or granules in the microbial cell cytoplasm.

The high refractivity of PHA granules allows it to be observed under phase-contrast light microscope as discrete inclusions of 0.2–0.5 $\mu$m in diameter (Sudesh et al. 2000; Sathesh and Murugesan 2010). The PHA molecules synthesized by bacteria have adequately high molecular weights to match the polymer qualities of conventional plastics such as polyethylene (PE) and polypropylene (PP) (Qin et al. 2007; Madison and Huisman 1999). This has drawn increasing attention on PHA as a substitute for petrochemical-based synthetic plastics as it can be thermally processed into various forms. PHAs are also advantageous over conventional plastics due to their biodegradability in natural environments (Sudesh and Iwata 2008). In addition, the use of renewable resources such as sucrose, starch, cellulose, triacylglycerols, palm oil, and activated sludge to supply microorganisms with various carbon substrates for the synthesis of PHA is also very attractive (Reddy et al. 2003; Ojumu et al. 2004).

Since the identification of P(3HB) in *Bacillus megaterium* by (Lemoigne 1926), 3-hydroxybutyrate (3HB) was regarded as the sole PHA monomeric unit. The presence of other monomer units such as 3-hydroxyvalerate (3HV) and 3-hydroxyhexanoate (3HHx) was only discovered almost 50 years later by (Wallen and Rohwedder 1974). To date, more than 150 different monomer constituents of PHA have been identified (Steinbüchel and Lütke-Eversloh 2003). Bacterial PHA can be divided into three main types depending on the number of carbon atoms in the monomeric units: short-chain-length (scl), medium-chain-length (mcl) and



$$-\!\!\left[ O-\overset{R}{\overset{|}{CH}}-(CH_2)_x-\overset{O}{\overset{\|}{C}} \right]_n$$

| Number of repeating units, x | Alkyl group, R | Polymer type |
|---|---|---|
| 1 | Hydrogen | Poly(3-hydroxypropionate) |
| | Methyl | Poly(3-hydroxybutyrate) |
| | Ethyl | Poly(3-hydroxyvalerate) |
| | Propyl | Poly(3-hydroxyhexanoate) |
| | Pentyl | Poly(3-hydroxyoctanoate) |
| | Nonyl | Poly(3-hydroxydodecanoate) |
| 2 | Hydrogen | Poly(4-hydroxybutyrate) |
| 3 | Hydrogen | Poly(5-hydroxyvalerate) |

**Fig. 2.1** General structures of polyhydroxyalkanoates (Lee 1996a)

a combination of scl-mcl. The scl-PHAs consist of 3–5 carbon atoms, mcl-PHAs have 6–14 carbon atoms whereas the number of carbon atoms in scl-mcl-PHAs can range from 3 to 14 per monomer (Li et al. 2007). While the homopolymer P(3HB) is the most widely studied scl-PHA, its copolymers containing 3HV, 3HHx, or 4HB monomers can also be synthesized (Fig. 2.1). In nature, scl-PHAs containing mainly 3HB units or mcl-PHAs containing 3-hydroxyoctanoate (3HO) and 3-hydroxydecanoate (3HD) are produced as the predominant monomers by most of the microbes (Anderson and Dawes 1990; Steinbüchel and Füchtenbusch 1998). The copolymers can be a more flexible and tougher plastics compared to the relatively stiff and brittle P(3HB). The usually elastomeric and sticky mcl-PHAs can even be modified to make rubbers (Suriyamongkol et al. 2007). Figure 2.2 shows the structures of HA constituents isolated from various microorganisms.

## 2.2 PHA Biosynthesis

PHA synthase (PhaC) is the key enzyme responsible for the polymerization of 3HA monomers (Qin et al. 2007; Pantazaki et al. 2009). Owing to the stereospecificity of this enzyme, all the 3HA monomer units are in the *R* configuration (Dawes and Senior 1973; Sudesh et al. 2000). PHA synthases are differentiated