Bacterially Produced Polyhydroxyalkanoate (PHA): Converting Renewable Resources into Bioplastics

Jiun-Yee Chee¹, Sugama-Salim Yoga¹, Nyok-Sean Lau¹, Siew-Chen Ling¹, Raeid M. M. Abed² and Kumar Sudesh¹,*

¹ Ecobiomaterial Research Laboratory, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia
² Biology Department, College of Science, Sultan Qaboos University, P. O. Box: 36, PC 123, Al Khouz, Sultanate of Oman
*Corresponding author: Mailing address: School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia; Tel: +60 4-6534367, Fax: +60 4-6565125, E-mail: ksudesh@usm.my

Dependence on conventional plastics and their boundless usage have resulted in waste accumulation and greenhouse gas emissions. Recent technologies are directed towards the development of bio-green materials that exert negligible side-effects on the environment. A biologically-synthesized plastic, polyhydroxyalkanoate (PHA), has been attracting major interests due to its similar physical properties to synthetic plastics. Unlike synthetic plastics, PHA is produced from renewable resources and is degraded aerobically by microorganisms to CO₂ and H₂O upon disposal. The selections of suitable bacterial strains, inexpensive carbon sources, efficient fermentation and recovery processes are important aspects that should be taken into consideration for the commercialization of PHA. This chapter discusses economical strategies to reduce production costs of PHA as well as its applications in various fields.

Keywords: PHA; renewable resources; bioplastics; PHA-producing bacteria; downstream processing

1. Introduction

1.1 Development of polyhydroxyalkanoate (PHA) and its importance

Synthetic plastics are one of the greatest inventions of mankind and have been developed into a major industry and indispensable commodity in human’s life [1]. They are designed in a way to suit the constant performance and trustable qualities that are used for long life span, therefore causing them to be inert to natural and chemical breakdown. The durability of these disposed plastics contributes to the environmental problems when they go into the waste stream. Snell and Peoples [2] predicted an increase of 25 million tonnes of synthetic plastics accounted to 230 million tonnes from year 2006 to 2009. As the natural environment is continuously polluted by these hazardous plastics, the development and production of environmentally-conserved biodegradable plastics are rapidly expanding in order to trim down our reliance on synthetic plastics.

Bio-based materials such as polynucleotides, polyamides, polysaccharides, polyoxoesters, polythioesters, polyanhydrides, polisoprenoids and polyphenols are potential candidates for substitution of synthetic plastics [3]. Among these, polyhydroxyalkanoate (PHA), which belongs to the group of polyoxoesters has received intensive attention because it possesses biodegradable thermoplastic properties [4]. PHA is synthesized by bacteria under unbalanced growth conditions. Some bacteria have been reported capable to produce PHA as much as 90% (w/w) of dry cells during depletion of essential nutrients such as nitrogen, phosphorus or magnesium [5]. Not only PHA serves as storage compounds of carbon and energy sources, but also as a sink for reducing equivalents for some microorganisms [6]. PHA acts as an ideal storage compound due to its insolubility inside bacterial cytoplasm, which exerts negligible increase in osmotic pressure [7]. It was shown that the bacteria containing PHA storage materials would be able to survive during starvation period compared to those without PHA as this energy-reserve material slows down the cell autolysis and subsequently its mortality [8].

1.2 Discovery and historical development of PHA

Poly(3-hydroxybutyrate) [P(3HB)] is the most common PHA and was first described by Lemoigne, a French scientist in year 1925 [9]. Since then, various bacterial strains among archaea bacteria [9], Gram positive [10, 11] and Gram negative bacteria [12] and photosynthetic bacteria [13-16] including cyanobacteria [17, 18] have been identified to accumulate P(3HB) both aerobically and anaerobically. The recognition of the role of P(3HB) as a bacterial storage polymer that possesses a function almost similar to starch and glycogen was accepted by the year 1973 [19]. Macrae and Wilkinson noticed that Bacillus megaterium initiated the accumulation of P(3HB) homopolymer when the ratio of glucose to nitrogen in the culture medium was high [20] and the subsequent intracellular degradation (also referred to as mobilization) of P(3HB) occurred in the absence of carbon and energy sources [8]. The opinion of 3HB monomer as the only constituent of this polymer changed after a year of its acceptance as bacterial storage material when other types of monomers were discovered [21, 22].
In 1974, Wallen and Rohwedder reported the discovery of other monomer constituents beside 3HB monomer from activated sewage sludge [23]. Among the polymers extracted from the sludge, 3-hydroxyvalerate (3HV), 3-hydroxyhexanoate (3HHx) and 3-hydroxyheptanoate (3HHp) monomers existed as the major and minor constituents, respectively. In the year of 1983, 3HHp was identified in B. megaterium [10]. In the same year, De Smet and coworkers identified a new monomer, 3-hydroxycetanoate (3HO) with trace amount of 3HHx from Pseudomonas oleovorans when fed with n-octane [24]. The investigation revealed that the production of various PHA monomers was dependent on the substrate fed. To date, about 150 different monomer constituents of PHA have been found [3, 21]. Witholt and Kessler have compiled the large variety of PHA monomers with straight, branched, saturated, unsaturated and also aromatic structures [25].

PHA can be classified according to the monomer size. There are two major groups of PHA; short-chain-length (SCL) PHA with five or less carbon atoms in a monomer, and medium-chain-length (MCL) PHA with six to fourteen carbon atoms in a monomer. Cupriavidus necator (formerly known as Alcaligenes eutrophus or Wautersia eutropha) is a well studied bacterium capable of producing SCL-PHA and it has been identified to produce PHA polymers consisting of 3HB, 3HV and 4HB monomers [9, 26, 27]. P. oleovorans and Pseudomonas putida are known to synthesize MCL-PHA consisting of 3HO and 3-hydroxydecanoate (3HD) monomers as major components. SCL-PHA has been commercially produced by several companies such as Monsanto [28]. MCL-PHA has yet to be produced in large scale because the yield is relatively low compared to SCL-PHA [29].

2. Biosynthesis of PHA

2.1 Bacterial Strains

The production of various PHA using natural isolates and recombinant bacteria from renewable carbon sources is presented in Table 1. Among the more than 250 different natural PHA-producers, only a few bacteria have been employed for the biosynthesis of PHA. These include Alcaligenes latus, B. megaterium, C. necator and P. oleovorans, which are capable of utilizing various carbon sources including plant oils or wastes to produce PHA. C. necator has been the most extensively studied and commonly used bacterium for PHA production. In the 1980s, a glucose-utilizing mutant of C. necator was employed by Imperial Chemical Industries (UK) for the industrial production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)], which was sold under the trade name of Biopol™ [30]. However, there are some limitations associated with the use of natural PHA-producers. Natural PHA-producing bacteria usually harbor native machinery for polymer degradation and are often hard to lyse, making the recovery of PHA difficult [31]. For industrial production of PHA, it is desirable to develop strains that can reach high final cell density in a relatively short period of time and produce high PHA content from simple, inexpensive substrates [32]. Thus, genetic engineering serves as a powerful tool in the development of strains that can produce PHA from inexpensive renewable resources efficiently. Genetically engineered bacteria such as recombinant strains of Escherichia coli, which produce PHA containing 3HB, 3HHx and 3HO monomers from soybean oil and a recombinant strain of C. necator harboring the Aeromonas caviae PHA synthase gene, which produces P(3HB-co-3HHx) from palm oil have been developed [33, 34].

2.2 Fermentation processes

Depending on the culture conditions that favor PHA accumulation, bacteria that are used for the production of PHA can be classified into two groups. The first group of bacteria requires limitation of essential nutrients such as nitrogen, oxygen and presence of excess carbon source for the efficient synthesis of PHA. The representative bacteria belonging to this group include C. necator, Protomonas extorquens and Protomonas oleovorans. On the other hand, the second group of bacteria does not require nutrient limitation for PHA synthesis and can accumulate PHA during exponential growth phase. Some of the bacteria included in this group are Alcaligenes latus, a mutant strain of Azotobacter vinelandii and recombinant E. coli harboring the PHA biosynthetic operon of C. necator [32, 35]. The culture conditions required for PHA biosynthesis are important criteria to be taken into consideration for the development of cultivation techniques used in the large scale production of PHA. Batch and fed-batch fermentations are widely used in the industrial fermentation processes. Fed-batch cultivation is more efficient than batch cultivation in terms of achieving high product and cell concentration because the medium composition can be controlled by substrate inhibition. Therefore, high initial concentration of substrates fed can be avoided. The limitations of fed-batch are the long downtime between two batches, which results in high operation costs [36]. Fed-batch culture is suitable for bacteria belonging to the first group. A two-stage cultivation method is most often employed, which was initially adopted by ICI for the industrial production of P(3HB-co-3HV) and the technology has not been changed very much since then [37]. In the first stage, the bacterial cells are grown until a pre-determined cell mass concentration is reached without nutrient limitation. The cells are then transferred to the second stage medium with limiting nutrients and the carbon substrates fed, are utilized by the cells to make PHA. During this nutrient limitation stage, the cells are unable to multiply and...
remain almost constant. However, the cells begin to increase in size and weight due to the intracellular accumulation of PHA as a storage product [35].

2.3 Development of renewable resources

Despite the numerous advantages of using biodegradable plastics, the commercialization of PHA has been ongoing since 1980s with limited success. The high production cost of PHA has been a major drawback to their replacement of petrochemical plastics [37, 38]. Considering the price of most commodity plastics derived from petroleum, such as polyethylene and polypropylene are below US$1 kg⁻¹ [35], PHA cannot currently compete with the bulk production of petrochemical plastics. Substantial efforts have been devoted to reduce the production cost through the development of efficient bacterial strains, fermentation and recovery processes [39, 40]. The major cost in the PHA production is the cost of the substrate [41]. Thus, the selection of suitable carbon substrate is a critical factor that determines the overall performance of the bacterial fermentation and the cost of the final product. Therefore, the simplest approach is to choose renewable, inexpensive and most readily available carbon substrates that could support both the microbial growth and PHA production efficiently.

Microorganisms are capable of producing PHA from various carbon sources ranging from inexpensive, complex waste effluents to plant oils [42], fatty acids [43], alkanes [44] and as well as simple carbohydrates. Each year, a large amount of waste materials are discharged from agricultural and food processing industries and these wastes represent a potential renewable feedstock for PHA production. Utilizing these waste materials as carbon source for PHA production not only reduces the substrate cost, but also saves the cost of waste disposal [36].

Table 1. Bacteria used for production of PHA from plant oils and wastes.

<table>
<thead>
<tr>
<th>Strains</th>
<th>PHA type</th>
<th>Substrates</th>
<th>PHA content (wt %)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcaligenes latus DSM 1124</td>
<td>P(3HB)</td>
<td>Soya waste, malt waste</td>
<td>33, 71</td>
<td>[45]</td>
</tr>
<tr>
<td>Bacillus megaterium</td>
<td>P(3HB)</td>
<td>Beet molasses, date syrup</td>
<td>~50</td>
<td>[46]</td>
</tr>
<tr>
<td>Burkholderia sp. USM (JCM 15050)</td>
<td>P(3HB)</td>
<td>Palm oil derivatives, fatty acids, glycerol</td>
<td>22-70</td>
<td>[47]</td>
</tr>
<tr>
<td>Comamonas testosteroni</td>
<td>MCL-PHA</td>
<td>Castor oil, coconut oil, mustard oil, cottonseed oil, groundnut oil, olive oil, sesame oil</td>
<td>79-88</td>
<td>[48]</td>
</tr>
<tr>
<td>Cupriavidus necator</td>
<td>P(3HB)</td>
<td>Bagasse hydrolysates</td>
<td>54</td>
<td>[49]</td>
</tr>
<tr>
<td>Cupriavidus necator H16</td>
<td>P(3HB-co-3HV)</td>
<td>Crude palm kernel oil, olive oil, sunflower oil, palm kernel oil, cooking oil, palm olein, crude palm oil, coconut oil + sodium propionate</td>
<td>65-90</td>
<td>[50]</td>
</tr>
<tr>
<td>Cupriavidus necator DSM 545</td>
<td>P(3HB)</td>
<td>Waste glycerol</td>
<td>50</td>
<td>[51]</td>
</tr>
<tr>
<td>Recombinant Cupriavidus necator</td>
<td>P(3HB-co-3HHx)</td>
<td>Palm kernel oil, palm olein, crude palm oil, palm acid oil</td>
<td>40-90</td>
<td>[34]</td>
</tr>
<tr>
<td>Recombinant Escherichia coli</td>
<td>P(3HB-co-3HHx-co-3HO)</td>
<td>Soybean oil</td>
<td>6</td>
<td>[33]</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa IFO3924</td>
<td>mcl PHA</td>
<td>Palm oil</td>
<td>39</td>
<td>[52]</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa NCIB 40045</td>
<td>mcl PHA</td>
<td>Waste frying oil</td>
<td>29</td>
<td>[53]</td>
</tr>
<tr>
<td>Pseudomonas guezennei biovar. tikehau</td>
<td>mcl PHA</td>
<td>Coprah oil</td>
<td>63</td>
<td>[54]</td>
</tr>
<tr>
<td>Thermus thermophilus HB8</td>
<td>P(3HV-co-3HHp-co-3HN-co-3HU)</td>
<td>Whey</td>
<td>36</td>
<td>[55]</td>
</tr>
</tbody>
</table>

MCL-PHA: medium-chain-length polyhydroxyalkanoate; P(3HB): poly(3-hydroxybutyrate); P(3HB-co-3HV): poly(3-hydroxybutyrate-co-3-hydroxyvalerate); P(3HB-co-3HHx): poly(3-hydroxybutyrate-co-3-hydroxyhexanoate); P(3HV-co-3HHp-co-3HN-co-3HU): poly(3-hydroxyvalerate-co-3-hydroxyheptanoate-co-3-hydroxynonanoate-co-3-hydroxyundecanoate)

©FORMATEX 2010
2.3.1 Plant oils

Plant oils such as soybean oil, palm oil and corn oil are desirable carbon sources for PHA production as they are relatively cheaper than most sugars. The production of PHA using sugars has been optimized to achieve high productivity. However, the cost of PHA production using sugars is higher than the ‘acceptable’ level as it results in low PHA yield [56]. Approximately 0.3 to 0.4 g of P(3HB) per g of glucose has been reported to be the highest yield of PHA production. On the contrary, plant oils are predicted to provide higher yield for both cell biomass and PHA production (0.6 to 0.8 g of PHA per g of oil) as they contain higher carbon content per weight compared to sugars [57].

Kahar and coworkers [58] used C. necator H16 and its recombinant strain (harboring the PHA synthase gene from A. cavae) with soybean oil as the sole carbon source for synthesizing P(3HB) homopolymer and P(3HB-co-5 mol% 3HHx) copolymer. The production of PHA from soybean oil was effective, whereby up to 80 wt% PHA of the dry cell weight was obtained [42, 58]. In particular, the incorporation of a small molar fraction of 3HHx monomer into 3HB improved the unfavorable physical and thermal properties of P(3HB). The same phenomenon also occurred when palm oil was used as the sole carbon source to feed the recombinant C. necator H16. The molar fraction of 3HHx monomer in the copolymer remained unchanged regardless of the type and concentration of palm oil products used [34].

Besides C. necator H16, there are also several other bacteria that are known to produce PHA from plant oils, namely Burkholderia cepacia [59] and Comamonas testosteroni [48]. Owing to the absence of lipase activity in P. putida, plant oils in the form of triglycerides could not support both the cell growth and PHA production in P. putida. Therefore, an additional saponification step was needed to break down the triglycerides into free fatty acids, which can be assimilated by P. putida for growth and PHA production [60]. Kim and coworkers [61] performed a two-stage fed-batch cultivation using P. putida by supplying octanoic acid in the first step, which resulted in good growth and could stimulate the biosynthesis of MCL-PHA efficiently.

C. testosteroni has been studied for its ability to synthesize MCL-PHA from vegetable oils such as castor seed oil, coconut oil, mustard oil, cotton seed oil, groundnut oil, olive oil and sesame oil [48]. This bacterium was shown to accumulate PHA up to 80 wt% of dry cell weight with major monomer compositions consisting of 3HO and 3HD [48]. B. cepacia was used by Ramsay’s group to produce 47 wt% of P(3HB) from fructose in batch fermentation [62]. Chee and co-workers have isolated Burkholderia sp. USM (JCM15050) from oil polluted wastewater and reported that this bacterium could produce P(3HB) up to 70 wt% of dry cell weight from palm oil [47]. However, this bacterium was only capable of producing P(3HB) homopolymer from plant oil as the sole carbon source. In order to obtain a copolymer of P(3HB-co-3HV) from this bacterium, structurally related carbon sources such as valeric acid and propionic acid were needed as precursors for synthesizing this copolymer. In a separate study, it was reported that B. cepacia had the ability to produce poly(3-hydroxybutyrate-co-3-hydroxy-4-pentenoate), (3HB-co-3 mol% 3HVx) amounting to 70 wt% of the dry cell weight from unrelated carbon sources such as sucrose or gluconate [63, 64].

2.3.2 Glycerol

Glycerol is a by-product of the palm oil refining process. It is used as a humectant as well as a plasticizer in certain applications such as oral-care products, tobacco, cosmetics, food and beverages. Glycerol is generated in bulk particularly from the co-product stream of biodiesel, and thus possesses great potential to be an attractive carbon source for PHA production by certain microorganisms [47, 65, 66].

Recent studies have shown that P(3HB) could be synthesized from glycerol by Burkholderia sp. under appropriate growth conditions [47, 67]. Previous works by Ashby and coworkers [65] had revealed the possibilities of producing PHA from glycerol. A mixed culture fermentation consisting of wild type P. oleovorans NRRL B-14682 and Pseudomonas corrugata 388 was used. Both bacteria with similar growth requirements produced different PHA from the same substrate. P. oleovorans NRRL B-14682 and P. corrugata 388 were able to convert glycerol to 20 wt% of P(3HB) and 40 wt% of MCL-PHA blends, respectively.

2.3.3 Carbon dioxide

Carbon dioxide (CO₂) is the most abundant carbon source in the ecosystem. As naturally occurring CO₂ and sunlight serve as carbon and energy sources, obtaining PHA from genetically modified crop plants represents an interesting approach [68]. The first investigation reported on the use of the transgenic plant, Arabidopsis thaliana harboring the PHA genes of C. necator. Poirier and coworkers [69] reported the successful expression of the C. necator genes encoding acetoacetyl-CoA reductase and PHA synthase in the cytoplasm of A. thaliana.

Similar to plants, cyanobacteria also pose as attractive PHA producers that utilize CO₂ and sunlight as carbon and energy sources. Cyanobacteria are oxygen-evolving photosynthetic bacteria that naturally possess the key enzyme for the production of PHA, which is the PHA synthase [70]. To date, only P(3HB) homopolymer has been identified in most cyanobacteria. Among the various cyanobacteria that are capable of synthesizing PHA, Spirulina platensis UMACC 161 [17] and Synechocystis sp. PCC6803 [71] are the topic of interest. P(3HB) content in both S. platensis UMACC 161 and Synechocystis sp. PCC6803 could reach a maximum of about 10 wt% of the dry cell weight under nitrogen-limited conditions and in the presence of acetate [17, 71]. However, studies on the ability of S. platensis
UMACC161 to incorporate new monomers other than 3HB by the addition of other precursor carbon sources has not been successful [17].

2.3.4 Other attractive renewable resources

Currently, the use of inexpensive carbon sources in the biosynthesis of PHA is predicted to reduce its production cost. Studies on other carbon sources such as whey [55], xylose [32], beet molasses and date syrup [46] have been carried out using different bacteria. *B. cepacia* ATCC 17759 grown on 10 g/L of xylose, could produce 2.6 g/L of cell biomass with 60 wt% of dry cell weight of P(3HB) [32]. A recombinant *E. coli* strain has been reported to produce P(3HB) on molasses as carbon source. The final dry cell weight, P(3HB) content and P(3HB) productivity were 39.5 g/L, 80 wt% and 1 g/L/h, respectively [72].

3. Downstream processing (PHA recovery and purification)

Since PHA is an intracellular product, the method applicable for the effective separation of PHA from other biomass components can be complex and costly. The most common method for the extraction of PHA from biomass is solvent extraction by using chloroform. Chloroform extraction is a very simple and effective method to separate PHA granules from the biomass. By using this method, highly purified PHA can be obtained without the degradation of PHA molecules [73, 74]. Other halogenated hydrocarbon solvents such as dichloromethane, dichlorehane and chloropropane can be also used to extract and purify PHA from the cell biomass [74-76]. However, these methods are not suitable for the mass production of PHA as the solvent is potentially hazardous to health and environment [74].

Besides solvent extraction, another simple and effective method that has been employed to recover PHA is cell lysis by using sodium hypochlorite [77]. In this method, the cell biomass is initially treated with sodium hypochlorite solution before the PHA granules are isolated from the cell debris by centrifugation [77]. The use of sodium hypochlorite to extract PHA from biomass always results in severe degradation of PHA and yields PHA with a lower molecular weight ($M_w$). In contrast, the use of surfactant pretreatment to recover PHA resulted in lower purity but less degradation of $M_w$. Ramsay and co-workers developed a modified method by combining hypochlorite digestion and surfactant pretreatment to obtain PHA with higher degree of purity and $M_w$ [78]. Reasonably high $M_w$ of PHA can be obtained rapidly by using surfactant-hypochlorite method compared to other treatment such as solvent-based extraction. The purity of P(3HB) recovered in this manner was 97–98% with $M_w$ between 730,000–790,000 g/mol, compared to 680,000 g/mol for hypochlorite extraction [73]. Another modified separation process involves the dispersion in sodium hypochlorite solution and chloroform, taking advantage of both differential digestions. Degradation of P(3HB) is significantly reduced by using this separation method [78]. The rationale behind this method is the immediate separation of hydrophobic P(3HB) from the hydrophilic lyophilized cells and dissolved into the chloroform, thus avoiding the polymer from further severe destruction by hypochlorite [16, 79]. Purity of P(3HB) recovered in this manner was approximately 98% with reduction of about 17% in $M_w$ [73]. In general, different extraction and pretreatment methods will affect the recovery of P(3HB). For example, pretreatment with a more polar solvent such as acetone will recover about 70% of P(3HB) with 95–98% purity and $M_w$ of 930,000 and 1,050,000 g/mol, when extracted with chloroform or 1,2-dichlorehane [74].

Enzymatic digestion technique is a gentle, yet selective separation method, which has attracted the interest of many researchers [74, 80]. Enzymes such as protease (trypsin, chymotrypsin, rennin, papain and bromelain), cellulose and lysozyme, are commonly used in this method [80, 81]. The reaction of enzymes is specific and requires only a mild operational condition for high reaction rates with little product damage. Extraction of PHA in this manner is known to result in high recovery and purification of PHA with no polymer degradation [80-82]. However, in this recovery process, a short period of heat shock treatment is applied to the culture broth before the enzymatic treatment in order to rupture the cells as well as denature and solubilize the nucleic acids. Without this preliminary heating step, the release of nucleic acid into the medium will result in a very viscous suspension [82]. In addition, the efficiency of enzymatic treatment can be increased with the aid of several chemicals such as proteinase K, Alcalase®, sodium dodecyl sulfate (SDS) or ethylene diamine tetra acetic acid (EDTA) [81, 83].

Compared to the extraction of PHA from bacterial cells, recovery of PHA from transgenic plants is more challenging. Extraction of PHA from agricultural crop in a large scale process by solvent extraction is an expensive, impractical and trivial task. Due to the drawback of solvent extraction, much attention has been given to centrifugal fractionation, a simple and economical process for separating specific resin components from the recovered PHA. The crop has to be pretreated with hexane in order to eliminate molecular lipids and oil; and 40% water/60% ethanol to eliminate soluble compounds such as sugars. Up to 85% of PHA with purity higher than 95% can be obtained from continuous centrifugal fractionation [82-84]. Besides centrifugal fractionation, recovery of PHA from crops using air classification has also been investigated. This process involves the separation of finely ground solid particles based on weight or size. The finer fractions accomplished at the end of the process will have higher PHA concentration and PHA can be recovered from the particles by any other convenient method such as filtration and centrifugation. Ultimately, 85–90% PHA with purity within the range of 85–95% can be recovered by using this method [85].
4. Applications of PHA

PHA has a wide range of potential applications because of its desired features such as biocompatibility, biodegradability and negligible cytotoxicity to the cells. Hence, the potential application of PHA as replacement for petrochemical based polymers is gaining popularity in various fields involving packaging, medical and coating materials. These desirable properties in compounding and blending have broadened their performances as potential end-use applications.

PHA has been manufactured for non-woven materials, polymer films, sutures, and pharmaceutical products used in surgery, transplantology, tissue engineering, and pharmacology [86]. In tissue engineering, the cells are grown *in vitro* on biodegradable polymers to construct “tissue” for implantation purposes [87]. A high level of biocompatibility is usually needed before foreign materials can be incorporated into human body. Shape, surface porosity, chemistry of the materials and the tissue environment play important roles in biocompatibility [5, 88, 89]. PHA has a distinct advantage

---

**Fig. 1:** Observation of PHA granules under phase-contrast and transmission electron microscope. Micrographs showing P(3HB) granules in wild type *C. necator* H16 (A), *R. meguaterium* MC1 (C, D) and transformant *C. necator* PHB-4 (pBBR1MCS-Csp2) (E, F); P(3HB-co-3HHx) and P(3HB-co-3HV) granules in *C. necator* PHB-4/pBBREE32d13 (G, H) and *D. acidovorans* (B) respectively. Black arrows indicate PHA granules inside the cells.
in the medical field over silicone, a traditionally used polymer, which is believed to have maligned effects and contribute to cancer cell growth [90]. Although PHA can serve as substitute biomaterials for silicone, five key elements need to be fulfilled for successful application of PHA in tissue engineering, i.e. biocompatibility, support cell growth and cell adhesion, guide and organize the cells, allow in growth of cells and allow passage of nutrients as well as waste products, and finally biodegradable without producing any harmful compounds [91].

P(3HB-co-4HB) has been evaluated as scaffold in tissue-engineering. The supporting scaffold polymer was implanted together with tissue-engineered cells [89]. For instance, this approach was carried out in the fabrication of a tri-leaflet heart valve scaffold. The heart valve scaffold was seeded together with vascular cells from ovine artery and consequently tested in a pulsatile flow bioreactor to determine the functionality of the heart leaflet. P(3HB) as scaffolds, has been evaluated to augment the pulmonary artery for the regeneration of arterial tissue. These living vascular grafts engineered from autologous and biodegradable polymers functioned well in the pulmonary circulation as a pulmonary artery replacement [87]. The suitability of P(3HB) as nanoparticles in animals has been proven by toxicity tests according to ISO 10993 [92]. Apart from that, P(4HB) has also been found to be a suitable scaffold for preparing autologous cardiovascular tissue. These fast-absorbing P(4HB) scaffolds were seeded with autologous cells before implantation to augment the pulmonary artery in a juvenile sheep model [93]. Many surgeons agreed that patching of P(4HB) implant materials would be more convenient compared to conventional polytetrafluoroethylene (PTFE) as no bleeding was observed during implantation or after suturing, thus decreasing the risk of blood leakage.

P(3HB) and P(3HB-co-15 mol% 3HV) copolymer sutures were implanted with a purpose to test animals intramuscularly. The tissue reaction was investigated and compared with the reaction to silk and catgut. The sutures featured the strength necessary for the healing of muscular-fascial wounds and they were implanted intramuscularly for an extended period of up to one year. The results showed that these two sutures did not cause any acute vascular reaction or any adverse events at the site of implantation. It is biocompatible against suppurrative inflammation, necrosis, and calcification of the fibrous capsule or malignant tumor formation [94].

Biomaterials such as P(3HB) and P(3HB-co-3HHx) were among the most extensively studied PHA used in the applications of tissue engineering and controlled drug-released. Regulations of PHA molecular weight are now possible to control the degradation rate of this polymer [88, 95, 96]. Following the development in nanotechnology, nano-sized PHA was penetrated deeply into the targeted body tissues and it was shown that PHA has good absorption efficiency by the cells [97]. Recently, PHA has also been studied for its application as cosmetic oil-blotting film. PHA was found to absorb and retain the oil rapidly; and simultaneously also act as an oil-indicator [98]. The biocompatible property of PHA makes it well-suited for skincare products [99]. Another emerging application of PHA is as a potential source of organic acids in animal feed. Short-chain fatty acids (SCFAs) has also been used as a novel biocontrol and antimicrobial agent in animal production [100]. P(3HB) which is comprised of β-hydroxy-SCFA was found to degrade slowly in brine shrimp, and the resulting fatty acids served as a biocontrol protection from pathogen [101].

5. Conclusion and future outlook

Development of PHA as potential substitute material to some conventional plastics has drawn much attention due to the biodegradable and biocompatible properties of PHA. The potential applications of PHA in various industries and in the medical field are encouraging. Nevertheless, the production cost of PHA has been a major drawback. Consequently, scientists have shown immense progress in searching for new bacterial strains, creating new types of recombinant strains and tailoring various kinds of PHA to reduce the cost of production. The ongoing commercialization activities in several countries are expected to make PHA available for applications in various areas soon.

Acknowledgements The research of the authors on polyhydroxyalkanoate has been supported by Techno Fund provided by Ministry of Science, Technology and Innovation, Malaysia (MOSTI). J. Y. Chee acknowledges Malaysia Toray Science Foundation. N. S. Lau and S. C. Ling thank USM Fellowship for financial support.

References


Rodrigues MFA, Da Silva LF, Gomez JGC, Valentin HE, Steinbüchel A. Biosynthesis of poly (3-hydroxybutyric acid-co-3-hydroxy-4-pentenoic acid) from unrelated substrates by *Burkholderia sp.* Applied Microbiology and Biotechnology 1995;47:880-886.


