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Review

Microbial production of polyhydroxyalkanoates (PHAs) and its copolymers: A review of recent advancements

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ABSTRACT

Traditional mineral oil based plastics are important commodity to enhance the comfort and quality of life but the accumulation of these plastics in the environment has become a major universal problem due to their low biodegradation. Solution to the plastic waste management includes incineration, recycling and landfill disposal methods. These processes are very time consuming and expensive. Biopolymers are important alternatives to the petroleum-based plastics due to environment friendly manufacturing processes, biodegradability and biocompatibility. Therefore use of novel biopolymers, such as polylactide, polysaccharides, aliphatic polyesters and polyhydroxyalkanoates is of interest. PHAs are biodegradable polyesters of hydroxyalkanoates (HA) produced from renewable resources by using microorganisms as intracellular carbon and energy storage compounds. Even though PHAs are promising candidate for biodegradable polymers, however, the production cost limit their application on an industrial scale. This article provides an overview of various substrates, microorganisms for the economical production of PHAs and its copolymers. Recent advances in PHAs to reduce the cost and to improve the performance of PHAs have also been discussed.

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1. Introduction

Petroleum-derived plastics (Traditional mineral oil based plastics) have highly versatile qualities of lightness, robustness, durability and resistance to degradation. They have become valuable materials for modern life successfully replacing many other substances over the years with a wide range of applications in domestic, medical and industrial fields in the form of disposable gears, packaging, furniture, machinery frames and accessories to enhance the quality and comfort in life [1]. Yearly about 150 million tonnes, plastic materials have been consumed worldwide [2]. In last few decades, progression in plastic consumption is expected to continue until 2020 [3].

Large-scale dependence on petroleum-derived plastics has resulted in rapid reduction of crude oil and serious pollution problems. Methods used for the disposal of plastic materials are challenging. In landfills, the degradation rates are tremendously slow. Incineration generates toxic by-products and expensive. Recycling can be done but is very time-consuming process and also cause alteration in the properties of plastic materials. The sorting of a wide range of discarded plastic material is very difficult. Furthermore, the presence of additives such as coatings, fillers and pigments limit the use of the recycled material. Thus, to decrease the environmental impacts of plastics is to replace conventional petroleum-derived polymers with biopolymers such as polylactide, polysaccharides, aliphatic polyesters and polyhydroxyalkanoates that possess similar physicochemical properties as conventional plastics [4].

Biopolymers are important alternatives to petroleum-derived polymers due to their biodegradability, eco-friendly manufacturing processes and enormous ranges of applications in various sectors from consumable goods to medical field. They can be produced through bio-refineries as part of integrated bioprocesses. Naturally biopolymers are produced by variety of microorganisms and plants. Most of the biopolymers are biocompatible; they have no adverse effects on biological systems. It is believed that biopolymers of bacterial origin are produced either as a result of their defence mechanism or as storage material. In both cases, bacteria decompose biopolymers [5,6].

Biodegradable plastics such as polyhydroxyalkanoates (PHAs) are more promising due to their inherent biodegradability, sustainability and environment-friendly properties [7]. PHAs are 100% biodegradable polymers. They are thermoplastic polyesters of several *R*-hydroxyalkanoic acids, which are produced as carbon and energy reserves or reducing power storage materials by numerous microorganisms (Gram-negative and Gram-positive bacteria) in the presence of excess carbon, especially when another essential nutrient such as oxygen, nitrogen or phosphorus is limiting or after pH shifts [8–11]. When a limiting nutrient is provided to the cell, these energy storage compounds are degraded and are used for bacterial growth as carbon source.

Polyhydroxybutyrate (PHB) was the first PHA to be identified in 1926 by Maurice Lemoige in the bacterium *Bacillus megaterium*, which showed intra-cellular granules [12]. PHB is the most widely studied and best-characterized member of PHAs that accumulated upto 80% of the cell dry weight [13,14]. The presence of 3HB as a PHA monomer was reported in activated sludge [15]. The presence of 3HV as a major constituent and 3-hydroxyhexanoate (3HHx) as a minor constituent were explained [16]. Today, approximately 150 different monomer constituents of polyhydroxyalkanoates, which posses straight, branched, saturated, unsaturated and aromatic structures and over 90 genera of microbial species have been reported to accumulate these polyesters [17,18].

2. Structures and classification of PHA

PHAs have general formula [19] shown in Fig. 1. Structurally PHAs family can be classified into two groups; short chain length or medium chain length polyhydroxyalkanoates. This classification is based on the number of carbon atoms in the chain of the branching polymers that range from 3 to 14 carbon atoms and type homopolymers or heteropolymers producing monomeric units [20]. Short-chain lengths PHAs (PHASCL) consist of 3–5 carbon atoms. Examples of this class are poly (3-hydroxybutyrate) P(3HB), poly(4-hydroxybutyrate) P(4HB) and poly(3-hydroxyvalerate) P(3HV) or the copolymer P(3HB-*co*-3HV). Medium-chain lengths PHAs (PHAMCL) consist of 6–14 or more than 14 carbon atoms [21]. Examples include homopolymers poly (3-hydroxyhexanoate) P(3HHx), poly(3-hydroxyoctanoate) P(3HO) and copolymers such as P(3HHx-*co*-3HO) [22].

Difference between two classes is mainly due to the substrate specificity of the PHA synthases that can accept 3HAs of a certain range of carbon length. For example, PHA synthase of *A. eutrophus* can polymerise 3HAs consisting of 3–5 carbon atoms whereas PHA synthase of *Pseudomonas oleovorans* can only accept 3HAs of 6–14 carbon atoms [23]. Hybrid polymers comprising both short-chain and medium-chain monomeric units also exist, such as poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) [4]. Because of the stereo specificity of the biosynthetic enzymes, all monomers are in *R*-configuration. Monomers with various functional groups, such as halogen, hydroxy, epoxy, cyano, carboxyl and esterified carboxyl groups on the chain have also been discovered in mcl-PHAs [24].

Production of PHB homopolymer has been resulted by the utilization of even numbered *n*-alkanoates whereas odd numbered *n*-alkanoates produce copolymers of 3HB and 3-hydroxyvalerate (3HV). Terpolymers of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-3-hydroxyheptanoate) have been produced from alkanoic acids of odd carbon numbers by recombinant strain of *A. eutrophus* harboring the PHA synthase gene of *Aeromonas caviae* [25,26].

PHAs are accumulated as granular inclusions in cell cytoplasm that are typically 0.2 ± 0.5 mm in diameter and can be visualized with a phase contrast light microscope due to their high refractivity or using staining dyes such as Sudan Black B, the oxazine dye Nile Blue A or Nile red. The molecular weight of these compounds ranges from 2×10^5 to 3×10^5 Da, depending on the type of microbial species and the growth conditions such as pH, fermentation

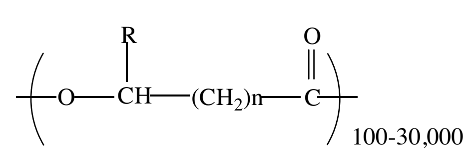

Fig. 1. General Formula of PHA [19].