

# A Review on Production of Polyhydroxyalkanoates in Microorganisms

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## Abstract

Plastic waste is a concern that is growing due to its nondegradable nature. Many species of microorganisms naturally produce polyhydroxyalkanoates, macromolecule-polyesters that may be used to replace conventional plastics. A variety of microorganisms can completely biodegrade PHAs within a year, which is unlike petroleum-derived plastic that takes decades to degrade. Carbon dioxide and water are produced during this biodegradation, which is returned to the environment. Many methods of mass-producing PHAs have been attempted. Genetically modified microorganisms can be used to establish novel production mechanisms. PHA production, as well as the expression of a few genes, must be maximized in the host for this task. Despite significant advances that have been achieved in generating transgenic organisms, acquiring large quantities of PHA at reasonable costs continues to remain a difficulty. The increasing awareness as well as the promising nature of utilizing microorganisms as a source of polyhydroxyalkanoates are highlighted in this review.

**Keywords:** Polyhydroxyalkanoate, Microorganism, Bioplastic, Polyhydroxybutyrate

## Introduction

Our planet has been plagued by a massive accumulation of non-degradable wastes, due to the growth of the human population. As far as the environment is concerned, plastic waste has become a major issue (Padervand *et al.*, 2020). In addition to taking decades for conventional plastics to decompose in nature, they may also generate toxins in the process of degradation. There is therefore a strong interest in producing plastics in a manner that is "environmentally friendly" by using recycled materials (Bilhalva *et al.*, 2018). Also contributing to the popularity of bioplastics is the declining availability of petrochemicals. Petroleum products have been widely employed as a major energy source in industrial processes and as building materials in advanced economies. Non-renewable resources, on the other hand, are scarce, and the latest work predicts that, by 2025, consuming fossil fuels will surpass discovering them, given the rate of discovery and previous fuel usage trends. Because our economy is still heavily dependent on

oil, this is a global issue. Plastic is currently consumed in the world in an amount of 140 million tons per year. The production of these plastics consumes about 150 million tonnes of carbon fuels. The characteristics of all structural materials made of carbon are determined by long sequences of carbon-carbon bonds (e.g., plastics, foams, coating, and adhesives). The world's problem is to see if we can replace the non-sustainable source of these lengthy carbon arrays with a sustainable and renewable supply.

Bioplastics, unlike plastics made from petroleum, are made up of natural biopolymers that are manufactured and catabolized by a variety of species and do not harm the host organism (Reddy *et al.*, 2019). As a result of stress, these polymers accumulate in microbial cells as storage materials (Bátori *et al.*, 2018). Polyhydroxyalkanoates (PHAs) and their derivatives are the most widely used microbial bioplastics (Kawamura *et al.*, 2021). A lucent granule of PHA was first observed in bacterial cells by Beijerinck in 1888 (Choi *et al.*, 2020). PHAs were first reported by Lemoigne as an unknown compound in the form of polyhydroxybutyrate (PHB), a home polyester comprising the three hydroxybutyric acids (Sathya *et al.*, 2018). In the subsequent 30 years, there was little interest in this unknown material. By 1958, Macrae and Wilkinson had published their first report on the PHB's functions (Bhatia *et al.*, 2019). They discovered that PHB generated by *Bacillus cereus* and *Bacillus megaterium* biodegrades quickly. PHB's popularity grew dramatically from this point forward. Researchers began experimenting with PHBs and other PHAs in the years that followed and realized how these biopolymers were useful (Zia *et al.*, 2017). An overview of PHAs' chemical structure and properties is presented in the current review. After that, microorganisms are examined for their synthesis of PHA. Last but not least, several ways for synthesizing PHA in plants using genes encoding PHA synthases are demonstrated. The market for PHA polymers has been discussed in detail.

### *The Physical Properties of Phas and Their Monomer Makeup*

Poly-hydroxyalkanes comprise 3-hydroxy fatty acid-based linear head-to-tail polymers (Figure 1). In general, PHA has  $10^3$  to  $10^4$  monomers gathered into inclusions with a diameter of up to 0.5 microns, with accumulating monomers accumulating as inclusions. Both Gram-negative and Gram-positive bacteria can produce and store these inclusions without harming the hosts (Ravi Teja *et al.*, 2020). A nutrient imbalance can lead to PH accumulation when cells receive too much carbon with insufficient nitrogen, phosphorus, and oxygen (Reddy *et al.*,

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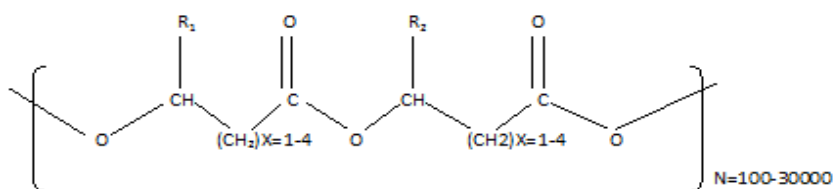
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2019). Insoluble biopolymers are formed from soluble molecules by the bacteria to store excess nutrients. When normal growth

conditions return, biopolymers become mobilized.



**Figure 1.** Structure of Polyhydroxyalkanoates with R<sub>1</sub> and R<sub>2</sub> are alkyl groups (C-C)

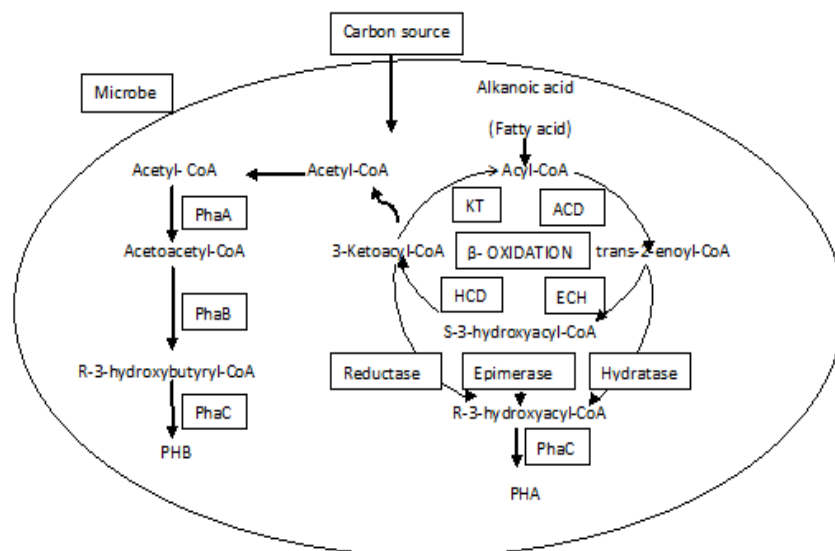
Depending on the organism's physio-chemical characteristics the composition, number, and size of granules may change. (Buzarovska *et al.*, 2018). The number of carbons in alkyl groups, which take up the R configuration at the C-3 position in all characterized PHAs, can range from one (C1) to more than 14 carbons (C14). PHAs can be categorized into three types based on the size of their monomers. PHAs with up to C5 monomers are known as short-chain length PHAs (scl- PHA). mcl-PHAs and lcl-PHAs are PHAs that include C6–C14 and N C14 monomers, respectively. Mcl-PHAs are considered rubbers and elastomers, but scl-PHAs are akin to traditional plastics (Raberg *et al.*, 2018). As an example of functional modification of the monomers, unsaturated and halogenated branched chains have been introduced into the bioplastic to improve its properties. Additionally, heteropolymers can be produced through polymerization between different kinds of monomers. PHB, the most prevalent kind of scl-PHA, is the most thoroughly researched 3-hydroxybutyric acid homopolymer. PHA polymers including 3-hydroxybutyrate (3HB), 3-hydroxyvalerate (3HV), and 3-hydroxyhexanoate (3HH) monomers can be made in a variety of ways. The majority of bacteria produce either scl-PHAs with predominantly 3HB units or mcl-PHAs with 3-hydroxyoctanoate (HO) and 3-hydroxydecanoate (HD) as primary monomers (Ryan & Walsh, 2016). It has been reported that bacteria synthesize more than 150 different types of PHAs (Wu *et al.*, 2016). PHAs derived bacterial cells have characteristics analogous to that of conventional polymers such as polypropylene (Hungund *et al.*, 2018). Many microorganisms can degrade PHAs quickly (3–9 months) by using their own PHA depolymerases to produce carbon dioxide and water (Jendrossek 2020). Biodegradable materials, recyclable and considered natural materials, are available from renewable resources. This makes PHAs an ideal substitute for petrochemical thermoplastics (Iordanskii *et al.*, 2017). The broad variety of monomers found in PHAs can create an array of physical characteristics. Homopolymer PHBs are stiff and brittle bioplastics, which have limited applications. In addition, mcl-PHAs can also be modified to modify rubber properties. Elastomers and sticky materials are often formed of PHAs with longer monomers. PHA copolymers largely composed of HB with a small percentage of longer chain monomers such as HO, HV, or HH are flexible and robust. In addition to food containers, they can also be used in bottles, razors, and packaging materials (Alexandrovich *et al.*, 2018).

Paper, film, and cardboard can be treated with PHA latex to create a water-resistant layer (Ravi Teja *et al.*, 2020). PHB and co-polymer P(HB-HV) were utilized as a water-resistant coating behind the diaper sheets in the United States (Gajjar & King, 2014). ICI/Zeneca and Monsanto marketed this copolymer P(HB–HV) for its fluidity and wear resistance under the brand name Biopol™ until 1995. PHAs are also employed in the production of fiber products, such as nonwoven textiles. Long-chain hydroxyacid PHAs have been utilized in pressure-sensitive adhesive compositions (Requena *et al.*, 2020). Many PHAs are also biocompatible in addition to being biodegradable. They are broken down into 3-hydroxyacids, which are found in mammals naturally. Gauzes, implants, suture, osteosynthetic materials, filaments, as well as matrix materials for a gradual release of medicines and in vitro cell cultures are among the medical applications of these PHAs (Anjum *et al.*, 2016; Chen & Zhang, 2018).

#### PHA synthesis in Microorganisms

##### Genes and enzymes involved in PHA synthesis

The Halobacteriaceae family of the Archea encompasses several species of bacteria that possess the ability to produce PHAs. It was found that there are nearly 300 species of such PHA-producing microorganisms exist that add up to the ever-growing list (Morya *et al.*, 2018). Although there are numerous chemical forms of PHAs present, the most eminent and widely synthesized form is PHB (Das *et al.*, 2018). PHB production is one of the most uncomplicated biosynthetic pathways (Gao *et al.*, 2018). The action of three enzymes and their associated transcribing genes is required for the synthesis of PHB (**Figure 2**) (Zhao *et al.*, 2019). The fusion of two acetyl-CoA molecules to generate acetoacetyl-CoA is the first step in the process, and it is catalyzed by the enzyme -ketothiolase, which is encoded by the *phaA* gene. The acetoacetyl-CoA formed in the earlier step is now reduced into (R)-3-hydroxybutyryl-CoA with the aid of an NADPH-reliant enzyme acetoacetyl-CoA reductase (Sharma & Dhingra, 2021). The *phaB* gene is responsible for the encoding of the acetoacetyl-CoA reductase enzyme. The polymerization of (R)-3-hydroxybutyryl-CoA monomers occurs in the last stage, which is catalyzed by the PHA synthase enzyme expressed by the *phaC* gene (Hiroe *et al.*, 2018).



**Figure 2.** Biosynthetic pathway of Polyhydroxybutyrate in microbes (Ranganadhareddy & Chandrasekhar, 2021)

*Ralstonia eutropha*, earlier known as *Alcaligenes eutrophus*, comprises a PHA synthase that interacts with a limited set of substrates with chain lengths ranging from C3 to C5, with a preference for C4 substrates (Brigham, 2019). Hence, the PHA end product synthesized by this process is monomers with a short chain length. All of the enzymes involved in PHB formation are found in the cytosol of the cell, and PHB accumulation is likewise found in the same region within the cell (Mannina *et al.*, 2020). Bacteria can produce a variety of PHAs, and this synthesis is not just limited to PHB (Skariyachan *et al.*, 2018). Many bacteria, including *Ralstonia eutropha*, have been able to produce various PHAs with C3 to C5 monomers as a result of changes in the amount and source of carbon in the nutrient broth. The addition of propionic or valeric acid in glucose medium, for example, results in the creation of a copolymer with HB and HV [P(HB-HV)]. The condensation of propionyl-CoA with acetyl-CoA is catalysed by ketothiolase in this process (3-ketothiolase, bktB). The reduction of 3-ketovaleryl-CoA to (R)-3-hydroxyvaleryl-CoA and subsequent polymerization to form P(HB-HV) are then subjected to similar enzymes which participate in the manufacture of PHB. Acetoacetyl-CoA reductase and PHA synthase are enzymes that are involved in both stages and PHB synthesis (Ranganadhareddy & Chandrasekhar, 2021). A variety of hydroxyacyl-CoA thio-esters are used as substrates by PHA synthases extracted from numerous bacterial species. The PHA synthase classification is done based on the content in their subunit, and they are segregated into four classes on this basis. The genes that encode the enzymes that are vital for the synthesis of PHA are cloned from the source of a natural origin of the polymer. Anti-thiolase antibodies were used to clone the phaA gene from *Zoogloea ramigera* for the first time (Sharma & Dhingra, 2021). The presence of the phaB gene in *Zoogloea ramigera*, *Paecococcus denitrificans*, and *Rhizobium meliloti* was discovered to be in the same operon, but phaC was in a separate operon (Miao *et al.*, 2021). In *Acinetobacter spp.*, *Pseudomonas acidophila*, *Ralstonia eutropha*, and *Alcaligenes latus*, a phaCAB operon is formed by the pha genes of these

microorganisms even though the genes do not share the same sequence in these species. The genomes might carry numerous copies of operons in some situations. There is a probability of the PHA synthase having two sub-units (PhaC and PhaE) and this situation holds true in the case of *Chromatium vinosum*, *Thiocapsa fennigii*, *Thiocystis violacea*, and *Synechocystis sp.* PCC 6803. The function of this type-3 synthase includes the catalysis of the production of scl-PHAs, and the polymerization of scl- and mcl-monomers (Katayama *et al.*, 2018).

Not only have these fundamental enzymes but there existed few other enzymes that enable PHA synthesis indirectly. The phaJ gene in *Aeromonas caviae* is responsible for encoding the enoyl-CoA flanks PHA synthase gene (phaC). Enoyl-CoA hydratase plays a role as a catalyst in the (R)-specific hydration of 2-enoyl-CoA which in turn supplies (R)-3-hydroxyacyl-CoA monomers for the synthesis of PHA by fatty acid  $\beta$ -oxidation pathway (Zhang *et al.*, 2019). *P. oleovorans*, *P. aeruginosa*, *Burkholderia caryophylli* can synthesize mcl-PHAs while, *R. eutropha* is unable to do so (Pérez-Nava *et al.*, 2021). These organisms accommodate two phaC genes which are split apart by a phaZ gene in the phaC1ZC2D operon. These genes encode for an enzyme called PHA depolymerase, whose significance remains mysterious, even though it somehow contributes to the PHA synthesis (Mozejko-Ciesielska *et al.*, 2019). *P. aeruginosa* and *P. oleovorans* utilize the fatty acid-oxidation pathway's intermediates directly, resulting in large molecules of 3-hydroxyacyl CoA (Raza *et al.*, 2018). The *P. oleovorans* contains PHA synthase which enables the polymerization of monomers leading to the formation of polymers with higher molecular weight and better elastic properties. An extra cluster (phaFI) is present in many microorganisms, and the location of this cluster is downstream from the phaC1ZC2D operon. The role of PhaI is the stabilization and formation of granules. And the function of PhaF is to regulate and stabilize the granules (Lim *et al.*, 2018).

*Production of PHA copolymers*

Stiff crystalline materials that are brittle were formed by scl-PHA homopolymers (C3-C5) such as PHB. The limited flexibility of these scl-PHA homopolymers results in a restricted number of applications. The PHB homopolymer consists of a C4 monomer as its only component tends to degrade at temperatures slightly beyond the melting point, making the homopolymer difficult to handle (Ryan & Walsh, 2016). Unlike the PHB homopolymer made of C4 monomer, the polymers that are built only with mcl-PHA tend to be semi-crystalline thermoplastic elastomers. This makes the mcl-PHA containing polymer possess mechanical properties improved by reinforcement. Based on the mol percent composition of subunits that make up the polymer, the scl-mcl-copolymers preserve better material characteristics than polymers having either mcl- or scl- monomers (Snoch *et al.*, 2019). The majority of the scl-mcl copolymers are made up of c4 monomers with a minimal count of c6 monomers and exhibit the property that is identical to polypropylene. Flexible and tough material can be obtained from the scl-mcl-PHA copolymer of HB and HH [P(HB-HH)]. This HB and HH [P(HB-HH)] copolymer comprise many desirable qualities such as improved flexibility, reduced crystallinity, melting point, simple processing, and increased strength. Many laboratories tried to reap the benefits of these enhanced properties by trying to manufacture a few selective mcl-PHA copolymers in bacteria. The synthesis of mcl-PHA and copolymers is initiated by inserting pha genes from several biological compounds into *E. coli*, such as the phaC gene from *Pseudomonas sp.* The phaC1 gene from *P. oleovorans* in fadA and fadB strains collected mcl-PHAs when cultured on C8 to C18 fatty acids, with an increase in yield attained by using inducible promoters. By inserting the phaC1 and phaC2 genes from *Burkholderia caryophylli* and *P. aeruginosa* into the recombinant fadB mutant of *E. coli*, PHA copolymers including HH, HD, and HO were synthesized. The hbcT gene from *Clostridium kluyveri*, with a function of encoding 4-hydroxybutyric acid-CoA transferase, and phaC from *R. eutropha* were co-transformed with *E. coli* cells (Gutiérrez-Gómez *et al.*, 2019). The bacteria when supplied with 4HB during its growth resulted in the accumulation of P(4HB) homopolymer, constituting up to 20% of the cell dwt of the cell. The presence of glucose plays a major role in the type of polymer that will be produced, while its presence results in the production of P(4HB) homopolymer, whereas its deficiency leads to the synthesis of P(HB-4HB) copolymer despite the absence of phaA and phaB genes (Najah *et al.*, 2019). Varying the concentration levels of fatty acid and glucose in the medium proven to affect the monomer makeup of PHA copolymer. Through the selection of specific bacterial strains, 43% cell dwt P(HB-HV) copolymer synthesis was obtained in modified *E. coli* (Lim *et al.*, 2018). To maximize the copolymer synthesis, various fermentation and feeding procedures were implemented. Because *E. coli* is not a natural PHA producer, boosting its multiplication was challenging.

## Conclusion

A substitute to petroleum-based plastics is a substance stored by microorganisms that is biodegradable which is known as PHA. For the exploration of various PHB accumulating strains, the environment can be excavated. By displacing non-biodegradable inorganic plastics, Polyhydroxybutyrate contribute to the

creation of a sustainable environment. To compete in a market driven by thermoplastics, it is important to produce cost effective biodegradable polymers. By reducing the production cost of biodegradable polymers their application range can be broadened. The imperative properties of biopolymers like biodegradability and biocompatibility have made them compatible for usage in packaging, biomedical field, aquaculture field and antifouling. Therefore, with the help of this report, it can be said that PHB is a resilient class of biopolymers that can be used for better designing of new applications in various fields.

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