Exploring the Polyhydroxyalkanoates: Bioplastic for a Green Tomorrow

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Abstract

Conventional plastics, though a necessity in modern society, come with significant disadvantages due to the non-biodegradability, health risks, resource depletion, limited recycling and limited thermal resistance, ultimately effecting a wide range of life forms on earth. Polyhydroxyalkanoates (PHAs) are a family of polyesters that serve as an efficient alternative for conventional plastics. PHAs are hydroxy fatty acids polyesters, water insoluble, storage granules produced intra-cellular by a variety of bacteria under stress. They offer several advantages including biodegradability and biocompatibility, non-toxic nature and versatile properties. Polyhydroxyalkanoates (PHAs) exhibit diverse properties that make them highly valuable across various industries, including material packaging, biofuel production, fine chemical synthesis, agriculture, and animal feed. In the medical field, PHAs hold significant potential in applications such as neuronal regeneration, bioimplant patches, drug delivery systems, and cardiovascular therapies. Currently, PHAs are produced industrially via fermentation, but scalability remains constrained by high production costs. But recent advances in synthetic biology and metabolic engineering have provided a new potential to increase production efficiency. Mixed microbial culture (MMC) also reduces the cost by utilizing wide range of waste without the need of strict sterilization condition. Overall, PHAs are a big step towards sustainable materials that can reduce the detrimental effects of conventional plastic waste.

Keywords: Biocompatible, Biodegradable, Biopolymer, Drug Delivery, Food Packaging, Mixed Microbial Culture, Polyhydroxyalkanoates.

Introduction

Synthetic polymers, known for their lightweight, durability, strength, and flexibility, are widely used in the production of various commodities however, being petro-chemically derived and non-

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biodegradable, they pose a significant threat to environmental chemistry. According to Chow et al, globally 360-400 million metric tons of plastic is produced annually (Chow et al., 2023). However these figures are around 155 million tons in 2002 (Marciniak & Możejko-Ciesielska, 2021). The value will surpass by 25 billion metric tons by 2050 (Geyer et al., 2017). After utilization, only a small amount of this plastic is being recycled and the left over is castaway on landfills. Figure 1 illustrate the processing of the plastic after the utilization of the products (Lee et al., 2021).

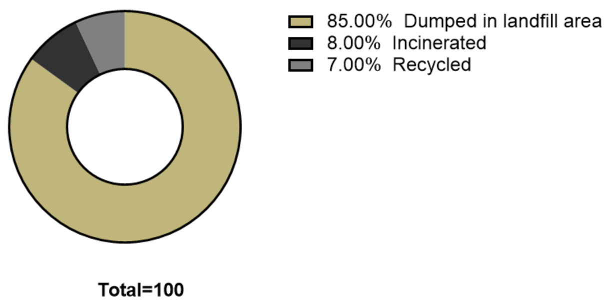


Figure 1: Dumping of conventional plastic material into various forms causing environmental problems.

Plastic debris has been documented to affect 267 species of marine life globally through ingestion, entanglement, or chemical contamination, and this figure is expected to rise due to escalating plastic pollution (Moore, 2008). Chemical additives like phthalates and bisphenol A (BPA) are present in plastic that act as endocrine disruptors. Phthalates and BPA cause growth issues, cognitive impairment and reproductive problems. Moreover, Endocrine-disrupting compounds (EDCs) are responsible for changes in gene expression that ultimately cause long-term health problems by turning genes “on” or “off”. Phthalates in plastics can harm the cardiovascular system by modifying heart rate fluctuation and raising blood pressure, leading to coronary heart disease. Living in plastic-polluted environments might cause mental health disorders including anxiety and sadness. Plastic exposure is especially damaging to children and developing fetus. It can cause neurological issues, poor lung growth, and a higher chance of birth abnormalities. These consequences are frequently caused by the presence of toxic substances that can penetrate the placenta and disrupt fetal development. They have been linked to oxidative stress, DNA damage, organ malfunction, and metabolic problems in experimental settings (American Lung Association, 2022; Li et al., 2023). Koller claimed that a small amount of this plastic will become part of all food chains in the future, which is detrimental to humans and animals (Koller, 2020).

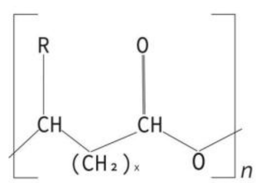
Considering the harmful effects posed by petrochemically derived plastic, there is a need to substitute them by other environment friendly substance. Substituting these synthetic polymers with the biopolymers, synthesized through microbial processes can solve the associated problems with the synthetic polymers (Sheldon & Norton, 2020).

Polyhydroxyalkanoates (PHAs), polylactic acid, polytrimethylene and starch blends are some biobased polymers. Polyhydroxyalkanoates (PHAs) are linear polyesters of hydroxy fatty acids that accumulate as intracellular microbial storage granules under nutrient-limiting conditions (Choi et al., 2020). PHAs are among the biopolymers capable of replacing conventional petrochemically derived plastics (Sabapathy et al., 2020). The first PHA production is reported in 1926 in Bacillus megaterium (Lemoigne, 1926). PHAs are naturally occurring biopolymers accumulated by microorganisms as intracellular granules under conditions of excess carbon and limited nutrients (Dalton et al., 2022). Bacteria produce PHAs for their survival in adverse environmental conditions including high temperature, acidic pH, UV radiation, oxidative, osmotic pressure, and freezing. Azotobacter vinelandii mutant strain, Alcaligenes lactus and recombinant E. coli are those in which PHA is produced during the growth phase of the organism. Most bacterial species exhibit enhanced production of PHAs under conditions of carbon excess and deficiency in phosphorus, nitrogen, oxygen, or magnesium. However, certain bacterial genera can synthesize this biopolymer without requiring such stringent conditions (Kumar et al., 2020). Archaea and several bacterial strains, including photosynthetic bacteria, gram positive bacteria, and a variety of other microbes, accumulate PHAs both aerobically and anaerobically. According to (Ansari et al., 2021), 92 bacterial genera are able to synthesize it both aerobically and anaerobically. Among 92 genera, Ralstonia eutropha H16 is the model organism along with several Pseudomonas spp. bacteria that have capability for the production of biopolymers by the utilization of the versatile carbon sources (Vicente et al., 2023). However, the production of PHAs by halophilic bacteria that can tolerate the high salinity environment is advantageous because the high salinity can reduce the risk of microbial contamination and increase the intracellular osmotic pressure, which makes microbial cell lysis for PHA extraction easier. There is a dominating interest of scientific community and industry in PHAs as they are capable of replacing the conventional petrochemically derived plastic in order to reach the circular economy (Haddadi et al., 2019).

This review aims to address the existing gaps in the comprehensive understanding of PHAs, particularly in terms of their metabolic pathways, genetic regulation, and their diverse applications. While PHAs have been studied extensively, their full potential across various sectors, such as agriculture, medicine, food, and biofuels, remains underexplored. By synthesizing current knowledge and highlighting emerging trends, this review provides a timely and critical examination of PHAs' significance as a sustainable biopolymer, offering insights that could drive future research and innovation in these fields.

Structure and Classification

PHAs are composed of monomeric subunits of (R)-hydroxyalkanoic acid (HA), which give rise to a variety of PHAs. This diversity is due to the versatility of their side chains, which vary with changes in the carbon chain length of the precursor molecules. Molecular mass of PHAs ranges from 50 KDa to 100 KDa based on their monomeric subunits (Haddadi et al., 2019). There general formula is shown in Figure 2 that indicates PHA are commonly composed of (R)-b-hydroxy fatty acids, where the R group varies from methyl (C1) to tridecyl (C13) (Reddy et al., 2003). Short-chain length (SCL), medium-chain length (MCL), and long-chain length (LCL), are the three main categories of PHAs on the basis of side chain length. Research reveals that SCL-PHAs are composed of 3-5 carbon atoms and extensively studied biopolymer, PHB also belongs to this category. *Bacillus megaterium, Burkholderia cepacia, Ralstonia eutropha, Cupriavidus necator* and several other bacterial species are found to be involved in the production of SCL-PHAs.



R = Alkyl groups
x = 1,2,3,4
n = 100-3000

Figure 2: General structure of PHAs.

In contrast to SCL, MCL-PHAs (medium-chain-length polyhydroxyalkanoates) contain 6-14 number of carbon atom and mainly Pseudomonas species are involved in their production. When carbon number exceeds above 14 then PHAs are categorized as LCL-PHAs that are occasionally produced by microbes (Vicente et al., 2023). The classification of polyhydroxyalkanoates (PHAs) is important because it aids in understanding their structural features and adapting them for specific applications, such as biomedical use or packaging (Muneer et al., 2020).

Metabolic Aspects

PHAs biosynthesis and degradation is revealed by several metabolic and genomic studies. The production of PHAs generally involves two main steps: the production of hydroxyacyl-CoA and its subsequent polymerization into PHA polymers. Hydroxy acyl-CoA production is carried out by acetoacetyl-CoA, fatty acid synthesis and β-oxidation of fatty acids (Haddadi et al., 2019). These three pathways synthesize and regulate acetyl-CoA and acyl-CoA that leads to the production of PHAs that are clearly mentioned in the Figure 3.

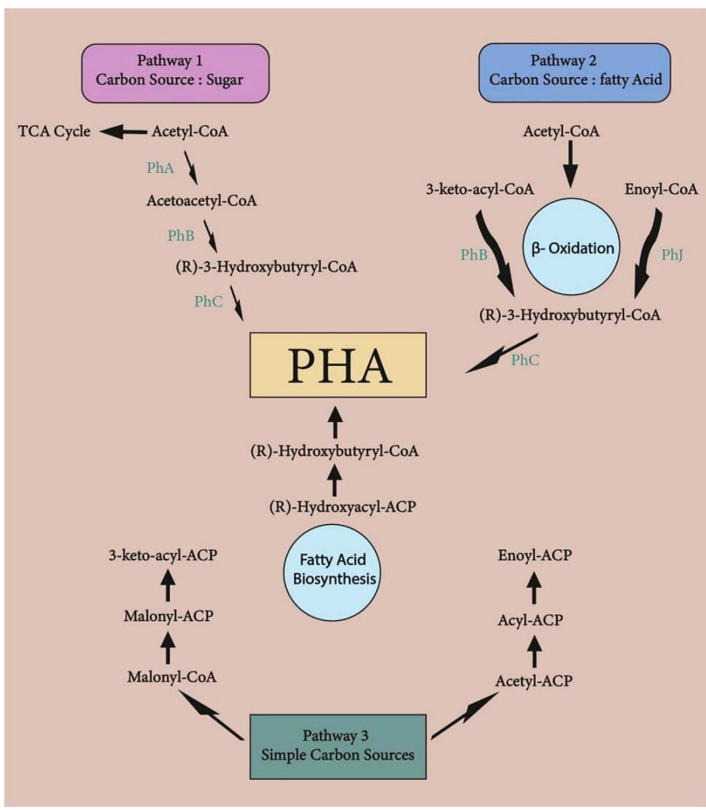


Figure 3: Different metabolic pathways and enzymes involved in PHAs production.

The activation of specific pathways for PHA production depends on the availability of the carbon source. When sugars serve as the carbon source for microbes, pathways involving acetoacetyl-CoA and fatty acid synthesis are activated to facilitate PHA biosynthesis. When fatty acid synthesis pathway is activated, such precursors are generated that leads towards the production of MCL-PHAs. In this pathway, (R)-3-hydroxyacyl-CoA is produced from (R)-3-hydroxyacyl-ACP by the action of CoA transacylase. β-oxidation is the main route for biopolymer synthesis when fatty acids act as carbon source and several precursors are formed that ultimately generate (R)-3-hydroxyacyl-CoA under the action of variety of enzymes (reductases, hydratases, epimerases). This pathway give rise to a variety of MCL-PHAs. SCL-PHAs (short-chain-length polyhydroxyalkanoates) can also be synthesized when enoyl-CoA hydratase (PhaJ) is involved. PhaJ acts on enoyl-CoA intermediates, converting them into (R)-3-hydroxyacyl-CoA, which is then incorporated into the polymer during biosynthesis. Through any of three primary metabolic pathways, (R)-hydroxyacyl-CoA monomers are synthesized. These monomers are then polymerized by PHA synthase into PHA chains via ester bond formation. These pathways allow the production of PHAs from varied carbon sources and the production of tailored biopolymers appropriate for various uses (Gao et al., 2022).

Since PHAs are typically produced during the stationary phase of microbial growth, this ability can potentially be enhanced through the use of genome editing tools (Zhang et al., 2020). Genes that are involved in PHA production are present in gene clusters. For instance, the most studied gene cluster PhaCBA present in R. eutropha H16 while PhaA (β-ketoacyl-CoA thiolase), PhaB (NADP-dependent acetoacetyl-CoA reductase), and PhaC (PHA synthase) are the genes that are present in this cluster. Three important proteins are encoded by the gene cluster present in PHA producing bacteria. When this gene cluster is utilized, PHA is produced in three steps. Initially, acetyl-CoA is converted into acetoacetyl-CoA by the action of PhaA-β-ketothiolase which is encoded by the PhaA gene. Then acetoacetyl-CoA is reduced by the action of PhaB-acetoacetyle-CoA reductase into (R)-3-hydroxybutyryle-CoA and this enzyme is encoded by PhaB gene. In the final step, the PhaC gene encodes PhaC-PHA synthase that is essential for the polymerization of the subunits for the production of PHAs as shown in figure 3. By modifying key genes involved in PHA biosynthesis, it is possible to optimize microbial strains to improve yield.

Role of Different Enzymes in PHA Production

Under stressful conditions, PHAs are synthesized by microorganisms to mitigate the harmful effects of freezing and thawing cycles, enabling them to survive extreme temperature fluctuations. A wide range of enzymes are present on the surface of these granules that are involved in the production and degradation of biopolymer as well as its stabilization and mobilization as shown in Figure 4. The attached molecules are regulatory proteins (PhaR and PhaQ), phasins, PHA synthases, PHA degrading enzymes. Phasins i.e., PhaP, PhaI and PhaF, are not involved in the production of biopolymer instead they stabilize its structure. Different enzymes that are involved in the synthesis of PHAs but the key role is mainly performed by PhaC, having four types i.e., type I, type II, type III, type IV and present in all PHA producing bacterial species. Through functional analysis it is revealed that SCL-PHAs are synthesized by type I and type III, while type II is mainly associated with the production of MCL-PHAs. *Ralstonia eutropha* has type I and while type II is present in *P. seudomonas*. Both of these types I and II comprise of single subunit having molecular weight of 60-70 kDa while type III found in *Allochromatium vinosum* (formerly called as C*hromatium vinosum*), is formed by PhaC and PhaE subunits. Likewise, type IV found in Bacillus spp., also formed by PhaC and PhaR subunits. The degradation of this biopolymer, which can occur both aerobically and anaerobically, is primarily facilitated by enzymes such as PhaY and PhaZ. The rate and extent of degradation are influenced by factors including the length of the side chains, crystallinity, chemical composition, and overall complexity of the PHAs.

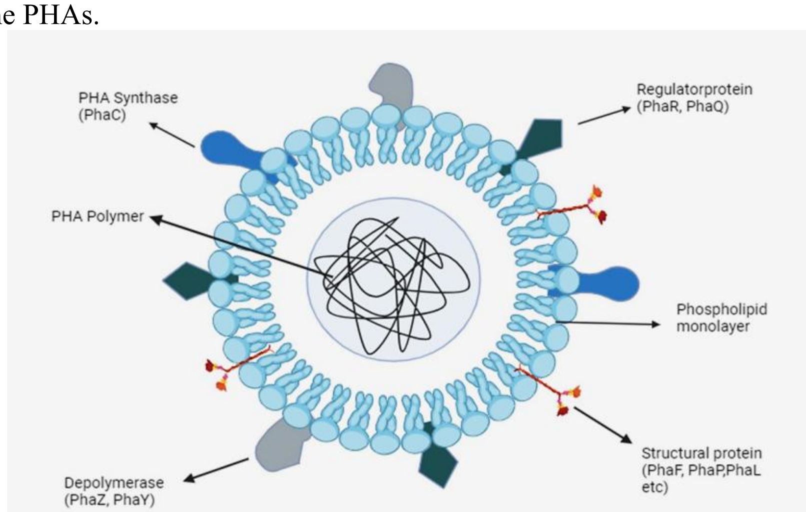


Figure 4: Structure of PHAs granule.

Properties of PHAs

PHAs are non-toxic, biologically degradable, and biocompatible, making them an attractive alternative to conventional plastics. They exhibit good processability, are insoluble in water, and can be produced from renewable sources, offering a sustainable solution for various applications. They are optically active and studies have revealed that stereochemical regularity is present in their repeating units. These are piezoelectric, non-electric and have greater ability of polymerization. Mechanical and thermal properties vary with the change in concentration of the subunits and in this way, we can get the desired product easily (Leong et al., 2014).

Biodegradability and Biocompatibility

The term "biodegradation" refers to the dissolution or decomposition of materials by bacteria, fungi, or other biological processes that can be aerobic and anaerobic. In general, materials that break down in biological contexts via enzymatic and non-enzymatic hydrolysis rather than heat oxidation, photolysis, or radiolysis are known as biodegradable polymers. PHAs are called biodegradable because these are broken down by nature into CO2, water, and organic fertilizer, also known as humus. A variety of microbes under aerobic and anaerobic conditions degrade it easily. Their insolubility in water, stability in atmosphere, non-reactive nature and disintegration into carbon dioxide and water make them appreciable. On the other hand, anaerobic environment is responsible for the production of the methane along with carbon dioxide that usually occurs in soil, sewage and other water bodies (Anjum et al., 2016). Degradation is done by depolymerase enzyme that are encoded by genes which can be present on chromosome or plasmid or in many cases on both of them together as in case of *Rhizobium meliloti*. It is usually considered that *E. coli* strains don't contain PHA depolymerase but a study disclosed that different gene products of *E. coli* caused degradation of the PHB.

Several bacteria are responsible for biodegradation of the PHA where the dominating genera includes Variovorax, Stenotrophomonas, Acinetobacter, Pseudomonas, Bacillus, Burkholderia, Cupriavidus, Mycobacterium and Streptomyces under both aerobic and anaerobic conditions. Fungi are also effective in the degradation of the PHA that are usually members of Ascomycota, Basidiomycetes, Deuteromycetes, and Zygomycota. Many gram-negative bacteria i.e., *Pseudomonas fluorescensce* GK13 (PhaZPflGK13) secretes extracellular depolymerase. Extracellular depolymerase is the first to be thoroughly studied that is involved in the degradation of the mcl-PHA granules (Melchor-Martínez et al., 2022). Several species of thermophilic bacteria, particularly *Thermus thermophilus* HB8 and *Streptomyces venezuelae* SO1 have different genes that are involved in the synthesis of mcl-PHA depolymerase. *Acidovorax* sp. DP5 have extracellular depolymerase with higher activity to decompose PHB polymer under alkaline conditions. *Streptoverticillium kashmirense* AF1 is also a source of the depolymerase, responsible for the breakdown of PHBV polymer. Composition of monomers and crystallinity play key role in its degradation. Research revealed that the longer the side chains, better the degradation of PHAs. Similarly, homopolymers are difficult to degrade while hetero-polymer and copolymers lead to low crystallinity and porosity which is then responsible for better degradation (Yean et al., 2017).

Polyhydroxyalkanoates (PHAs) are biopolymers that are extremely biocompatible and thus suitable for a wide range of biomedical applications. Their biocompatibility is linked to their inherent existence in living beings, which is demonstrated by the presence of PHA building blocks such as 3-hydroxybutyrate in human and animal bloodstreams. This intrinsic compatibility enables PHAs to be employed in medical applications such as scaffolds, implants, and drug delivery systems without producing adverse reactions. Furthermore, PHAs' biocompatibility is further proved by their capacity to promote cell proliferation and incorporation into tissues, making them useful materials for tissue engineering and regenerative medicine (Koller, 2017).

Thermal Properties

To express the thermal properties of PHAs, Tg and Tm are used. Tm is the melting temperature of the crystalline phase, on the other hand Tg is glass transition temperature of amorphous phase. Experiments reveal that their values change with the change in the length of side chain. When side chain increases, Tg decreases but this is different for Tm because it increases from 45 to 69° C by an increase in C4 to C7. An experiment is conducted on *Pseudomonas putida* to investigate its thermal properties, specifically the glass transition temperature (Tg), and to examine how variations in the chain length of PHAs affect these thermal characteristics. When *Pseudomonas putida* is grown on coconut fatty acids, a glass transition temperature (Tg) of -43.7°C is recorded. In contrast, when the substrate is linseed or flaxseed oil, the Tg drops to -61.7°C, indicating that the type of substrate influences the thermal properties of the resulting PHA (Rai et al., 2011). In conclusion, the thermal properties of the MCL-PHAs varies greatly depending upon the type of the substrate.

Mechanical Properties

The mechanical properties of PHAs vary significantly, ranging from hard and crystalline to flexible and elastic, depending on factors such as the polymer's composition, molecular weight, and the length of its side chains. SCL-PHA are highly crystalline as well as stiff and brittle. On the other hand, MCL-PHAs (medium-chain-length polyhydroxyalkanoates) are less crystalline compared to SCL-PHAs (short-chain-length polyhydroxyalkanoates) and exhibit greater flexibility and elasticity, making them suitable for applications requiring these specific mechanical properties (Anjum et al., 2016). P(3HB) possess good thermoplastic properties but its mechanical properties such as young's modulus and tensile strength are very poor just like petroleum-based polymer. It is brittle in nature and can be improved by the addition of the copolymers and other additives. Flexibility can be improved by the use of other polymers and copolymers for different applications in industry and medical field. In a study, it is shown that mechanical properties such as flexibility, hardness and rigidity of P(3HB-co-HHx) are mainly affected by 3HHx units. It is evident that when 5.95 3HHx mol fraction is present, the break value is recorded as 163%. On the other hand, this is 25.7 MPa with 2.5% 3HHx. When it is estimated in term of Young's modulus, 631.3 MPa break value is noted with 2.5% 3HHx. Thermal stability of PHAs can be improved through modifications that increase their melting temperatures and mechanical qualities, making them suitable for a variety of applications.

Production of PHAs

The fermentation process is commonly used for the production of various valuable compounds at the industrial level like enzymes, chemicals, biofuels, and pharmaceutical products. By using different organic waste from various sources, fermentation is an effective method for the production of PHAs. Product yield is greatly dependent on the type of strain that is used in a process as well as on other parameters including temperature, pH, concentration of carbon and nitrogen, micronutrients, and incubation period. Batch, continuous, and fed-batch fermentation strategies are employed for the production while the process is itself divided into continuous and discontinuous mean (Koller, 2017). A lot of the work is done on its production at laboratory scale, as shown in Table 1, by the utilization of the numerous wastes and a variety of strains. The main problem associated with PHAs production is the cost of the substrate along with recovery and purification while conventional plastic gives such kind of advantages. Although the cost of producing polypropylene (PP) and polyethylene (PE) is approximately US$ 0.60–0.87/lb, the cost of producing PHA is estimated to be US$ 2.25–2.75/lb, which is three to four times more expensive than that of traditional plastic. Technological developments in microbe-based PHA manufacturing have considerably enhanced efficiency and scalability. Recent advances include the use of mixed microbial cultures (MMC) and current industrial biotechnology (CIB) to improve PHA manufacturing. MMCs enable the use of varied microbial communities capable of efficiently converting complicated substrates usually waste from different industries into PHAs, lowering manufacturing costs while boosting yield. CIB methods concentrate on optimizing fermentation processes and genetic engineering to improve microbial strains' ability to collect PHAs. For instance, genetically modified bacteria such as *Halomonas* and *Cupriavidus necator* have been developed to increase PHA production and effectively utilize renewable feedstocks. These initiatives try to make PHA manufacture more economically viable than traditional polymers (Sabapathy et al., 2020).

Table 1: Production of PHAs using different bacterial strains and carbon sources at laboratory scale.

S.No.	Bacterial strain	Carbon source	Type of PHA	Yield
1	*Cupriavidus necator*	Cassava peel waste	PHBV	28.6% (g PHA/100 g DCW)
2	*C. necator* DSM 545	Date wastes	-	79.20 % (w/w on dry mass)
3	*Cupriavidus necator*	Waste cooking oil	P(3HB-co-3HHx)	75.5 wt%
4	*Cupriavidus necator*	Cassava peel waste	PHBV	31% (gPHA/gDCW)
5	*Thauera mechernichensis* TL1	Wastewater	-	56.3 % gPHA/gMLSS
6	*Bacillus pacificus* NAA2 (NAA2)	Glycerol	PHB	2.92 g/L (79 %)
7	*Klebsiella quasipneumonia* NAA4 (NAA4)	Xylose	PHB	2.89 g/L (73 %)
8	*Pseudomonas aeruginosa* MCC 5300	Oleic acid	mcl-PHA (copolymer of 3-hydroxydecanoate and 3- hydroxydodecanoate)	75.6 % DCW
9	*Halomonas* sp	Crude glycerol	PHB	52.67 wt%
10	*Methylosinus trichosporium* OB3b (KCTC 12568),	Methane	PHB	25 % DCW
11	*Methylocystis* sp. MJC1	Methane	PHB	46.23 % DCW
12	*Methylocystis* sp. OK1	Methane	PHB	27.60 %DCW
13	*Enterobacter cloacae*	Molasses	-	48 - 56%
14	*M. zhoushanense*	Sucrose	PHB	64.05 wt%
15	*C. necator*	Sugar beet molasses	P(3HB-co-3HV)	80%
16	*Pseudomonas citronellolis*	Acetic acid	mcl-PHA	3.4g/L
17	*Purple phototrophic bacteria*	Urban organic waste	-	42% (wt%)
18	*Paracoccus alcalophilus and Azoarcus sp.*	Wastewater from Yeast Industry	PHB	1.2 g /L
19	*Priestia flexa*	Brewers spent grain	-	3.01g/L
20	*Burkholderia cepacia*	Volatile fatty acids (VFAs)	-	1.088 g/L
21	*Burkholderia cepacia*	Waste glycerol	PHB	1.6 g/L
22	*Burkholderia cepacia*	Palm oil	PHB	3.17 g/L
23	*Paraburkholderia* sp. PFN29	Glucose	PHB	82.58%

24	*Bacillus* sp. YHY22	Lactate	PHB	64.7%
25	*Bacillus circulans*	Potato peel	-	0.232 ± 0.04 g/L
26	*Photobacterium* sp. TLY01	Soybean oil	PHBV	16.28 g/L
27	*Agromyces indicus*	Glucose	PHB	3.86 g/L
28	*Cupriavidus necator* H16	Waste cooking oil	PHB	0.8 g/g
29	*Cupriavidus necator* H16	Waste fish oil	PHB	0.92 g/g
30	*Streptomyces toxytricini* D2	Tapioca molasses	-	86.56%
31	*Thauera mechernichensis*	Food waste anaerobic digestate	PHB	23.98 ± 0.52 wt %
32	*Priestia sp*	Molasses	PHB	61.7 %
33	*Bacillus* sp.	Cashew industry wastewater (CIW)	-	34%
34	*Halomonas organivorans*	Galactose	PHB	5.61 ± 0.01
35	*Halomonas cerina*	Galactose	PHB	78.1%
36	*Zoogloae* sp	Discarded fruit juices (DFJ)	P(3HB-co-3HV)	43.27 ± 2.13 %
37	*Azotobacter vinelandii*	Sucrose	PHB	85%
38	*C. necator* DSM 13,513	Dairy wastewater effluents	PHB	1.34% DCW
39	*Chelatococcus daeguensis* TAD1	Glycerol	PHA	0.81 g/g
40	*Bacillus thuringiensis* DF7	Glucose-rich hydrolysate	PHB	14.28 g/L (68.3%)
41	*Paracoccus* sp. KKU01	Sugarcane juice (SJ)-based medium	PHB	32.1 gL-1
42	*Pseudomonas chlororaphis* DSM 19,603	Crude glycerol	mcl-PHA	19.0 gL-1

Technological Advancements in PHAs Production by Microbes

Current improvement in the fields of synthetic biology in addition to genetic engineering, genomics and proteomic instrument and devices have paved the route for various purposes including the prediction and identification of the proteins and manipulation of the genes to modify a particular pathway in a cell. By the application of the genetic engineering, different cellular process can be modified and metabolism can be regulated in microbes, *P. putida* KT2440 is studied at proteomic level and modifications can enable it an efficient producer of PHAs as production cost can be reduced. *Pandoraea* sp. ISTKB is analyzed at both proteomic and genomic level for making the strain an efficient producer of PHAs along with greater ability to valorize the lignin. The application of the genetic engineering for the modification and optimization of the metabolic pathways involved in PHA production can help in cost reduction and improved polymerization and molecular weight control.

Role of Mixed Microbial Culture (MMC) in PHA Synthesis

There are some drawbacks in scaling up its production at the industrial level. One of the major challenges is the high cost of carbon sources including glucose, fructose, and sucrose, which is about 28-50% of the total production cost. This problem can be solved by using different waste as carbon source including municipal waste, syngas production, agriculture waste and food waste. The feast and famine (FF) carbon availability approach is frequently used to choose a PHA accumulating culture in the PHA synthesis process with mixed microbial culture (MMCs). MMCs are considered cost effective as there is no need for strict sterilization techniques that is a major problem for PHAs production at industrial level. MMCs have wide range of metabolic activities that allow them to use a variety of substrates. MMCs are highly tolerant as well as adaptive to waste substrates which makes their combination essential for efficient waste treatment and bioremediation processes. The actual PHAs accumulation usually takes place in a second reactor, where waste from the chosen culture is fed constantly or intermittently until the cells have created the maximal PHA. This selection is often carried out in reactor (Sabapathy et al., 2020). Given that the PHA polymer synthesized by MMCs is of sufficient quality for a variety of applications, it is necessary to better understand the relationship between the microbial structure and function of these enrichments.

CIB (Current Industrial Biotechnology) for PHAs Production

For the production of the PHAs from laboratory to industrial scale, following steps are being followed as the strain development that can be wild type or engineered bacteria, optimization of the shake flask, then laboratory and pilot fermenter studies before industrial up-scale that is the last step. Both wild and engineered bacteria can be used for example, *Alcaligenes latus* and *Ralstonia eutropha* are used by researchers for the production of SCL-PHAs, *R. eutropha* and *Aeromonas hydrophila* for PHBHHx and *Pseudomonas* sp. for MCL-PHA. Among genetically modified bacteria, *E. coli* is used for the production of several different types of PHAs and researchers have achieved success. These bacteria are major producer of PHAs which are based on CIB that has initiated the new wave in the production of PHAs at industrial level. *R. eutropha* and recombinant *E. coli* are frequently used in industry. 232 g/l cell dry weight is obtained with highest cell density with *R. eutropha* while in case of *E. coli*, highest volumetric productivity is obtained with the value of 4.63 g/l/h (Marciniak & Możejko-Ciesielska, 2021).

Next-Generation Industrial Bioprocessing (NGIB), which is based on extremophiles, is created to address the major obstacles to the industrialization of PHAs that are related to CIB. *Extremophilic bacteria* i.e., *Halomonas* spp. have gained popularity for producing PHA in large quantities at a lower cost. Additionally, NGIB has been developed which is based on contamination-resistant extremophilic bacteria that use low-cost carbon sources under artificial intelligence (AI) instead of labor-intensive handling and monitoring processes to address the major obstacles related to CIB's PHA accumulation (Chen & Jiang, 2018). Despite of all challenges production of PHA at industrial scale is mainly launched by the following as shown in table 2.

Table 2 Major producer of biodegradable plastic at industrial scale.

Manufacturer	Production	Type of PHA
MedPHA (China)	1000 ton/year	PHBand/or P34HB
Pha Builder (China)	10000 ton/year	PHB, PHB-co-4HB and/or PHB-co-3HV
Tianan (China)	3000 ton/year	PHBV
Danimer Scientific (USA)	6000 ton/year	PHBHHx
Keneka (Japan)	5000 ton/year	PHBHHX

Applications of PHAs

PHAs have properties that make it the center of attraction for different industrial branches especial medical field. Transportation of goods and for the safe packaging of material leading to the increasing global demand of the PHAs. It can be used in paper industry for its coating ability, agriculture field, for the packaging of materials, formation of films and molding of products. They can be used in medical field because of their tissue friendly nature i.e., these can be incorporated into bone, blood, cartilage and different cell lines. A wide range of applications are reported in the treatment of cardiovascular diseases. Not ending there, being non-cytotoxic compounds, these are used for the development of orthopedic pins, adhesion barriers, stents, guided tissue repair/regeneration devices, nerve guides, wound dressings, tendon repair devices and 3D custom-made bone marrow scaffolds. Tissue growth can also be promoted by them (Ansari et al., 2021). PHAs are becoming the great resource in market for being able to protect the environment and having compatibility with living tissues.

Packaging and Commodity Items

PHA's uses in the packaging sector have expanded since the 1990s, encompassing packaging films, lids, tubs, and containers like shopping bags. Paper cups can also be lined with PHA, making cups that currently have PE linings recyclable. PHAs are considered to be used for the packaging of those food materials where there is a need to prevent the oxidative spoilage of the food and this due to the property of PHAs to act as a barrier for oxygen permeation. PHBV is commonly used for this purpose. Those food products which are declared as organic, containing high oil concentration as well as frozen can be packaged via PHAs based packaging material. Therefore, packaging plays a crucial role in maintaining food quality during storage, transit, and consumptio. Danimer Scientific (USA) has collaborated with Bacardi© to substitute the poly (ethylene terephthalate) (PET) bottles with poly (3- hydroxypropionate) (P3HP). It is an efficient step that will rule out 80 million PET bottles per year (Mukherjee & Koller, 2023). FDA has approved many of them for food like PHBV and poly((R)-3-hydroxybutyrate-co- (R)-3-hydroxyhexanoate) (PHBHHx). In 2020, packaging items accounted for 40% of the total 1.6 million tons of bioplastic market (Sehgal & Gupta, 2020).

Agriculture

PHAs are used in agriculture sector i.e., for the formation of protection nets, greenhouse film as well as grow bags. Thus, these are replacing the conventional plastic-based mulch that is lethal for our environment. Mulch is being used in agriculture sector for the improvement of soil fertility, weed control, pollution control and water conservatio. Traditionally, high-density polyethylene (HDPE) and low-density polyethylene (LDPE) are used that are hazardous for environment. It can be replaced with PHAs derived mulch that is designed by the utilization of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). Films and coatings can also be formed by PHA due to the low permeability of the oxygen through them. Growth bags is another encouraging application exhibiting numerous advantages i.e., reduced toxicity, small change in root deformity, enhanced growth of plants, improved immunity against pathogens. Scl-PHA can also be used as a fertilizer where they enhance the crop production as well as improve carbon in the soil (Kontárová et al., 2022; Souza & Gupta, 2024).

Biofuels and Fine Chemicals

Production of biofuels is possible by the utilization of PHAs has become possible and for this purpose methyl esterification of used bioplastic is done and the products act as sustainable fuel. In 2009, PHAs as source of biofuel is reported. SCL and MCL-PHAs are utilized for this purpose. When they are treated with methanol, esters of hydroxyalkanoates are produces that are used as biofuel. It is reasonable to produce biofuel from PHA as we can synthesize PHAs by the use of sludge and other nutrient rich waste. PHAs production by the utilization of activated sludge and further its applications for the production of biofuels costs about US$ 1200 per ton. PHB methyl ester (HBME) along with hydroxyalkanoate methyl ester (HAME) are evaluated after the esterification of PHB. Besides production of biofuels, PHAs are also used for the production of industrially important compounds like pheromones, antibiotics and aromatics (Muneer et al., 2020).

Animal Feeds

After pretreatment, Scl-PHA has its application as a feed for fingerlings and piglets that improved their health due to positive impact on digestive system. Single-cell proteins are rich source of nutrition that can be produced from waste stream which are also used for nutrient recovery. PHA has its applications for improving animal food. according to research, single-cell proteins in association with PHB can replaced traditional aquaculture feed. It is evaluated for a number of wastewater and agricultural waste for the sake growth and resistance towards infection of brine shrimp Artemia which is taken as model organism. Mcl-PHA showed greater efficiency as a biocontrol agent even in a smaller amount (Raza et al., 2018).

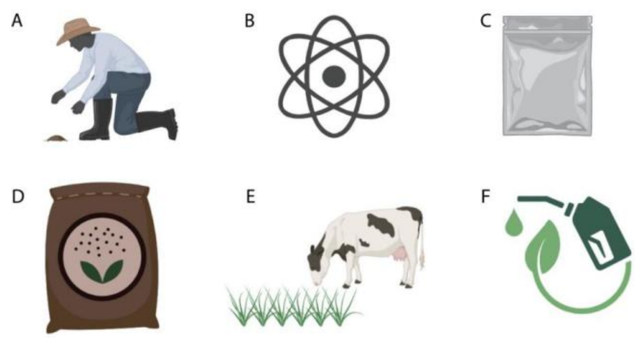


Figure 5: Applications of PHAs in different industries indicating its important. PHAs used as (A) Mulch (B) Fine chemicals, (C) Packaging material, (D) Fertilizer, (E) Source of animal feed, (F) Fuel.

Medical Applications

Due to low cytotoxicity, biodegradability and biocompatibility of PHAs, these are used in medical field. These are used as drug delivery system for various therapies, neuronal regeneration and heart valves (Figure 6). These are also used to stimulate the growth of the bones as being the osteosynthesis material. Not only this, they have an important role as replacement of blood vessels, surgical sutures due to their piezoelectric properties (Grigore et al., 2019).

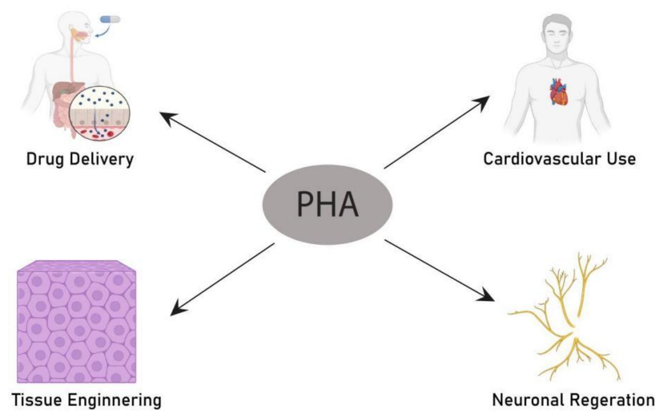


Figure 6: Biomedical use of PHAs.

Neuronal Regeneration

Scientists and researchers are trying their best to find the solution for various medical problems such as neuronal regeneration. They are trying to find an ideal material that should allow nutrient penetration, biocompatible, show minimal cytotoxicity, biodegradable and inflammatory responses should be very low and all such qualities are being reported in PHAs. 3 Hydroxybutyrate (3HB) is the major degradation product of PHA that plays an important role in the proliferation and viability of cells I.e., glial cells, osteoblasts and fibroblasts. Not only this, 3HB enhances the differentiation of neuronal progenitor as well as neuronal stem cells. Such properties make it as a center of attraction for medical field. To check the efficiency of 3HB and its derivatives, experiments are being made and positive responses are obtained. PHBV is used in research for the formation of 3D tubes that mimics the sciatic nerve conduit and then impregnated with Schwann cells. It is implanted and regeneration of the neuron occurred (Biazar & Heidari Keshel, 2013). PHBV/collagen nanofibers are greatly important in tissue engineering of neurons. It is also discovered in a different study that PHB/PHBV scaffolds promote myelin sheath regeneration. The correct alignment of PHB, PHBV, and collagen fibers promoted the proliferation of Schwann cells. Notably, seven days, there is an increase in glia derived neurotrophic factor (GDNF) gene expression and nerve growth factor (NGF) secretion (Masaeli et al., 2013).

To treat the peripheral nerve lesions of wrist or forearm, P(3HB) can be used to replace the epineural sutures as these are safe having no product complications and adverse reactions. An investigation is made on hBMSC-seeded P(3HB-co-3HHx) scaffolds to repair the nerve damage. This framework enhanced the proliferation and differentiation of the nerve cells. PHAs are preferred on other material to be used in CNS (central nervous system) for the regeneration of the neurons because of their mechanical support. Combined with Schwann cells, these are used for the repairing of the spinal cord. It is observed that after 4 weeks, this scaffold is well-integrated and enhanced the survival and proliferation of the Schwann cells.it can also be used for the recovery of the damaged ureter, as scaffolds, and wound dressing (Raza et al., 2018).

Cardiovascular

There are many materials that are used for the production of the heart valves i.e., PCL, PLA, PGA and PHAs. MCL-PHAs being more flexible among the family of the PHAs are being implicated for the production of these valves both as a scaffolding and covering. For example, near-ideal flow conditions are being showed by decellularize heart valves that are made up of P(4HB) or P(3HB) in case of the polymeric heart valves (Luo et al., 2019).

Drug Delivery Systems

In medicine, development of the effective drug delivery systems is necessary. Chemotherapy and radiations are being employed for the treatment of the cancer but these results in toxicity and nanomedicines are considered to be the better alternative. Drug delivery system including the nanoparticles, micelles and liposomes helps the medicines to circulate and reach the target tissue. This system is established in 1960s in US. To increase the efficiency of the drug delivery system, PHAs can be used as ideal material due to their biodegradable and biocompatible properties (Souza & Gupta, 2024). Surface erosion can lead to the degradation of the SCL-PHA that make it effective for this purpose. However, pores are formed in these polymers because of crystallinity and hydrophobicity due to this fact quick medication is recommended. Since MCL-PHAs show low crystallinity, these are preferred over others as better drug delivery system in the treatment of the cancer (Pouton, 2001).

In a recent study for the treatment of the cancer, photodynamic therapy is developed in which PHAs act as carrier for phthalocyanines and porphyrins that shows an excellent accumulation in the membranes of the cell organelles. When experiments are made on it, anti-plastic effects are being induced in myelocytic cell line K562 by these porphyrin sensitizers. Under such conditions harmful effects of the porphyrins can be overcome by the use of PHAs. They can also act as drug delivery system for the treatment of gingivitis. Micro and nanosphere that act as shell can be made by using PHAs and drugs are inserted in these shells that are later on degraded easily. For example, to treat the chronic osteomyelitis, Sulbactam-cefoperazone is loaded in the rods of PHBV (Raza et al., 2018).

Bio-implant Patches

PHAs produce low inflammatory response that enable them to be used for the implantation of patches in humans. Transmural abnormalities in gut are commonly treated with local incision and closure by sutures. These can be done by the use of biodegradable polymer such as PHB (Raza et al., 2018). For such applications, endotoxin units (EU) should be less than 20 US Pharmacopeia that FDA usually approves. It is shown by research that no vascular reaction is observed by PHB/PHBV and PHBHHx sutures for a year. TephaFLEX is a commercially available P4HB used for this purpose that has been approved by FDA. It has many other uses as heart stents and valves and tablets (Martin & Williams, 2003; Pandey et al., 2022).

Challenges and Future Prospects

In the last several years, the interest towards PHA has been increased because of its important characteristics such as biodegradability, bio-compatibility, non-toxic, and exhibit high structural diversity hence, providing sustainable and eco-friendly plastic worldwide. Despite of its unique characteristics, there are still a few challenges that limit the production as well as applications of PHA. One of the primary challenges is the high production cost of the PHA as compared to conventional plastics because of use of expensive carbon sources like glucose, fructose, sucrose, etc. at commercial level thus, hindering the commercialization of PHA. Another major hurdle is the low polymer productivity. Furthermore, as compared to conventional plastics, bioplastics have limited range of properties for instance, their mechanical properties often fall short. But such challenges must be solved to overcome the non-biodegradable plastic pollution globally and provide PHAs with significant market share. The one possible solution is to continue research into microbial strain and genetically modify them for producing different variety of PHAs at high productivity and utilizing inexpensive carbon sources. Optimizing different fermentation conditions can also contribute to cost reduction and scalability of PHAs. Moreover, increasing awareness regarding major disadvantages of using conventional plastics and alternatives to them can drive market demand for PHAs and for this the collaboration between government, industry, and academia is essential.

Conclusion

As indicated by applications, PHAs are sustainable solution of conventional plastic. It is used in a different sector and a fabulous bio-polymer for medical field due to its non-toxic, non-immunogenic and bio-compatible nature. It is estimated that in near future its production rate will surpass the present-day scenario as several projects are being made world widely to reduce the production cost that act as a barrier in its proper implementation. This rise in interest is because of its bio-compatibility and biodegradability that plays a key role in circular economy that is the need of the hour for the protection of our environment. A large number of bacterial species are used for the production of the PHAs and waste from different industries is given as a carbon source to reduce the production cost. Moreover, genetic engineering and synthetic biology can also play an essential role to enhance the production of dry cell mass at industrial level.

Authors' Contributions

Fareeha Rauf: Conceptualization, writing original manuscript, Diagrams, review the original articles
Asad Ur Rehman: Supervision, conceptualization, and Writing manuscript – editing and correction
Syed Sib Tul Hassan Shah: Conceptualization, review the original article, diagrams, writing manuscript – editing and correction
Areej Atta: Conceptualization, review the original articles
Sikander Ali: Conceptualization, review the original articles
All authors have seen and approved the manuscript for submission to this journal.

Competing Interests

The authors declare that they have no competing or conflict of interest.

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