Chapter 4
Production, Characterization, and Applications of Biodegradable Polymer: Polyhydroxyalkanoates

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Abstract The usage of petroleum-based polymers by the human beings has enhanced the quality and comfort of life in the recent decades. These polymers are extremely persistent in the environment, and none of the conventional techniques can effectively degrade such polymers. A remedy to this issue is the application of biodegradable polymers from different organic sources. Biodegradable polymers are comprised of monomers that are linked with one another through various functional groups with unstable links in the backbone. During degradation, these polymers are broken down into molecules that are degradable by conventional biological techniques. Biodegradable polymers have been synthesized from four different sources: agro-resources, microorganisms, biotechnological renewable sources, and classical chemical synthesis. Polyhydroxyalkanoates (PHAs) are one of the prime substitutes for conventional plastics because they are derived from renewable feedstock by fermentation and are completely biodegradable upon disposal. The fermentation route for the synthesis of PHAs is one of the best substitutes for petroleum-derived polymers. PHAs have excellent physical characteristics, such as low toxicity and high molecular weight, and they can be naturally produced from several carbon sources using numerous microorganisms. Moreover, they possess mechanical and physical properties similar to synthetic plastics such as polyethylene and polypropylene like tensile strength and melting point, etc. More than 300 different types of bacteria, including both gram-positive and gram-negative strains, produce PHAs. In order to reduce the production cost of PHAs, several inexpensive substrates, such as whey, malt, soy and starch waste, palm oil, beet, and cane molasses, are being used. In the

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recent past, several invasive weed biomasses have been used in the microbial fermentation process for the production of PHAs. This invasive alien species (IAS) is non-native to an ecosystem and when introduced outside its natural habitats, affects the native biodiversity in almost every ecosystem. Hence, the production of Poly(3-hydroxybutyrate) (PHB) using these invasive weeds is a brand new technology for the production of biodegradable polymers. Various blends like copolymers have been developed to improve the cost, performance, and physical properties of PHAs. Several PHA nanocomposites have been developed to enhance mechanical properties. PHAs degrade into carbon dioxide and water under aerobic conditions and to methane under anaerobic conditions without any harmful products. These biopolymers can also be degraded either by thermal mode or by enzymatic hydrolysis. The last two decades have seen a shift from bio-stable materials to biodegradable (i.e., hydrolytically and enzymatically) materials for medical and related applications. Initially, PHAs were used in the packaging industry, but their importance was later shifted to the medical industry, pharmacological, and agricultural sectors. This chapter addresses the synthesis and benefits of PHAs over petroleum-derived polymers, their biodegradable characteristics, and applications in several sectors.

Keywords Biodegradable polymer · Polyhydroxyalkanoates · Fermentation · Microorganism · Copolymers · Carbon substrate

1 Introduction

The world has witnessed the consumption and side effects of fossil fuel derivatives for the last two centuries. This extensive usage of petrochemical derived polymers is accountable for worldwide ecological imbalance in several sectors: the plant kingdom, aquatic and terrestrial life, food chains, and stability of the earth's atmosphere [1]. These polymers are extremely persistent in nature, and an alarming accumulation of such plastics have caused problems in safe disposal, recycling, landfilling of waste, bio- or photo-degradation, and incineration. Therefore, the demand for biodegradable polymers has become an urgent need of the hour. Biodegradable polymers consist of monomers that are linked with each other through various functional groups having unstable links in the backbone. Several biologically accepted molecules form in the course of the degradation process of these polymers. Biopolymers are usually developed from four different sources: biomass/agricultural products, microorganisms, biotechnologically renewal sources, and chemically synthesis route. A schematic of various types of biodegradable polymers has been illustrated with examples in Fig. 1 [2].

Among several categories of biodegradable polymers, polyhydroxyalkanoates (PHAs) are one of the most promising alternatives to replace the petroleum-derived polymers because of their excellent physical properties. They resolve the problem of the accumulation of conventional non-biodegradable polymers. These polymers are commonly produced by a wide variety of microbial routes. They can be degraded

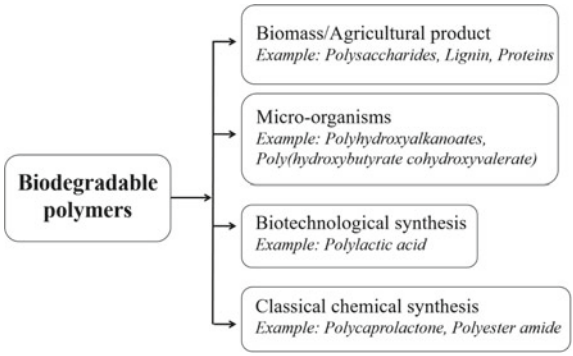


Fig. 1 Classification of biodegradable polymers

under both aerobic conditions (with water) and anaerobic conditions (methane and water). PHAs are the polyesters, which consists of various repeated chains of hydroxyalkanoates (HAs) with different side chains. It is reported that more than 150 types of (R)-3-hydroxy fatty acids have been identified as part of the PHA family [3]. Because of biodegradability and biocompatibility characteristics, PHAs are of interest for potential research and its commercialization. Saturated, unsaturated, branched, and substituted alkyl groups [4] can replace the monomer unit *R* group in PHAs. Polyhydroxybutyrate (PHB) is the most common and simplest polymer of PHAs family. These polymers are produced intracellularly with an excess of carbon source and essential elements required for growth like carbon, oxygen, nitrogen, phosphorus, sulfur, hydrogen, etc. in a limiting condition. Despite the several advantages of PHA over petroleum-derived polymers, the high cost of production of PHA limits its extensive use and makes it inefficient in replacing the conventional plastics. Procurement of pure carbon sources, sterilization of equipment to prevent contamination and pathogenic activity, lower production capacity of fermentation reactors, and the requirement of pure solvents during the extraction process enhance the cost of production [5]. Therefore, in order to reduce production, the cost use of waste organics like industrial sludge, agricultural and food waste, lignocellulosic biomass will play a vital role. Moreover, recently researchers have been focusing on the preparation of mixed cultures (or recombinant microbial strains) compared to refined cultures, which is commercially viable [6]. A typical flowsheet has been illustrated in Fig. 2 to analyze several pathways for the production of PHAs, which includes both conventional and economical routes. This chapter provides a systematic study about PHA-producing microbes and potential carbon substrates, metabolic pathways for their production, extraction and characterization of PHAs, its biodegradability nature, and applications in several sectors.

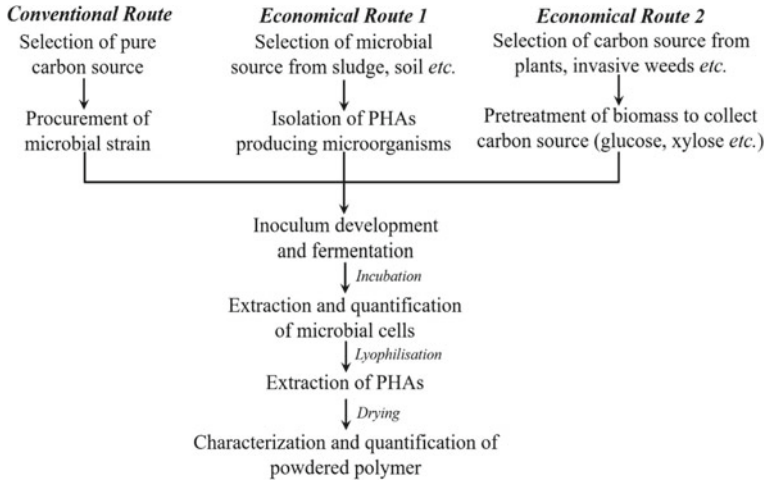


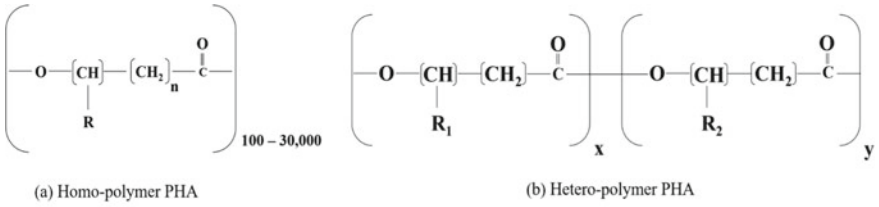
Fig. 2 Pathways for the production of PHAs

1.1 Historical Overview

Maurice Lemoigne first synthesized polyhydroxyalkanoates (PHAs) in 1923, using a gram-positive bacteria *Bacillus megaterium* as a carbon storage compound [7, 8]. He described the biodegradable polymer as an ether-insoluble lipid in a French journal. During the period of 1923–1951, he published 27 articles and postulated that PHB can be produced by a variety of microorganisms [9]. In 1958, Wilkinson Macre observed that by increasing the concentration of carbon sources like glucose and nutrient elements like nitrogen, accumulation of *B. megaterium* granules increased [10]. Later on, researchers synthesized P(3HB) using several microorganisms of bacteria genera like *Pseudomonas*, *Azotobacter*, *Hydrogenomonas*, *Chromatium*, a cyanobacterium, and many others during 1959–1973 [11]. In 1974, Wallen et al. first reported that PHA can be synthesized by extracting the carbon source from activated sludge [12]. Later, numerous compounds of PHAs were developed by several microorganisms using different carbon sources. In 1990s, *Alcaligenes eutrophus* was used for production of PHB, which accumulated up to 75% of dry cell weight [13]. The bacterium was later renamed as *Ralstonia eutropha*, which was again retitled to *Wautersia eutropha*. At present, *R. eutropha* is one of the most promising microorganisms that have been discovered for efficient production of PHAs from several carbon sources.

1.2 General Structure and Classification of Polyhydroxyalkanoates (PHAs)

PHAs are polyesters of HAs and the general structure formula is given in Fig. 3a



(a) Homo-polymer PHA (b) Hetero-polymer PHA

n	*R* group	IUPAC Nomenclature	Abbreviation
1	hydrogen	poly(3-hydroxypropionate)	(3HP)
1	methyl	poly(3-hydroxybutyrate)	(3HB)
1	ethyl	poly(3-horoxyvalerate)	(3HV)
2	hydrogen	poly(4-hydroxybutyrate)	(4HB)
2	methyl	poly(4-hydroxyvalerate)	(4HV)
2	ethyl	poly(4-hydroxycarpoate)	(4HC)
3	hydrogen	poly(5-hydroxyvalerate)	(5HV)
3	methyl	poly(5-hydroxycarpoate)	(5HC)
3	ethyl	poly(4-hydroxyheptanoate)	(5HH)

Fig. 3 Structure of polyhydroxyalkanoates (PHAs)

with its IUPAC nomenclature. In Fig. 3a, the commonly used polymers are shown by varying the pendant group *R*. Generally, based on the monomer units, the PHAs are classified into three different categories. Short-chain-length (*scl*) PHAs are composed of monomers with 3–5 (C_3–C_5) carbon atoms. Carbon numbers ranged from 6 to 14 (C_6–C_{14}) are categorized as medium-chain-length (*mcl*) PHAs. Similarly, the long-chain-length (*lcl*) PHAs have carbon atoms greater than 14 (>C_{14}). The most commonly produced PHAs are *scl*-PHAs that possess a tensile strength as high as polypropylene. Most *mcl*-PHAs are elastomeric and semi-crystalline in nature. *lcl*-PHAs are very infrequent and are the least studied materials among all types of PHAs [14]. Depending upon the type of monomer present in the PHAs, it can be divided into two types: homo-polymer PHA and hetero-polymer PHAs. If the monomer unit consists of one type of 3-hydroxy fatty acid, then it is called as homo-polymer PHA. Hetero-polymer PHA contains more than one type of fatty acid of varying chain length [15]. A schematic of hetero-polymer or copolymer PHA has been shown in Fig. 3b. The polymers can be biosynthesized by a wide range of microbes (i.e., gram-positive and gram-negative bacteria) as an intracellular carbon source and energy storage compounds.

1.3 Physical Properties of PHA

The physical properties of *scl*-PHAs and *mcl*-PHAs are given in Table 1 [3, 5, 16]. It can be observed that different *scl*-PHAs and *mcl*-PHAs show a wide distribution ranging from wooden fibers to petroleum-based polymers (i.e., polyethylene

Table 1 Comparative physical properties analysis of *scl*-PHA and *mcl*-PHA

	scl-PHA	*mcl*-PHA
Melting point T_m (°C)	~180	~60
Glass transition temperature T_g (°C)	~ 0	~ −40
Tensile strength (MPa)	Lower	Higher
Average molecular weight	Higher	Lower
Crystallinity (%)	70	40
Tensile strength	Higher	Lower
Extension to breakage	Lower	Higher
Elasticity	Lower (brittle)	Higher

and polypropylene). The *mcl*-PHAs have lower glass transition temperatures, crystallinity, molar mass, and tensile strength compared to *scl*-PHA. They also have a wide range of melting temperatures varying from 170 to 180 °C and high elongation at break compared to *scl*-PHA. *mcl*-PHAs are flexible and elastomeric, but *scl*-PHAs are stiff and brittle in nature [14, 17].

Similarly, Table 2 shows major physical properties of PHAs, polypropylene, HDPE, and LDPE [18–20]. The PHAs possess similar material properties as polypropylene, whereas polypropylene has better mechanical properties compared to PHAs. In addition, it can be observed that the physical properties can be altered by introducing a co-monomer into the polymer backbone.

Table 2 Physical properties of PHB, P(3HB-*co*-3HV), polypropylene, HDPE, LDPE

	P(3HB)	P(3HB-*co*-3HV)	Polypropylene	HDPE	LDPE
Melting point T_m (°C)	162–181	64–171	160–169	130–137	105–125
Glass transition temperature T_g (°C)	−4 to 18	−13 to 10	−14 to −6	−125 to −90	−125 to −90
Tensile strength (MPa)	19–44	1.8–51	28–40	20–40	7–17
Tensile Young's modulus	1.2–4	0.14–8.7	1.1–2	0.7–1.4	0.14–0.3
Elongation at break (%)	0.8–4.5	1–970	20–75	100–1000	200–900
Density	1.18–1.26	1.18–1.26	0.90–0.91	0.95–0.97	0.92–0.93
Crystallinity (%)	50–80	53–56	50	79.8–81	43

2 Production of PHAs

Biologically derived polymers have created significant impacts on petroleum-derived polymers in terms of the green synthesis method and environmental friendly applications. Biotechnological production of PHA is carried out via two routes: (1) microbial fermentation method and (2) plant-based production system. Significant research has been carried out toward bacterial production of PHAs in recent years, and substantial efforts are in progress to improve the production process [21, 22]. The worldwide production of bio-based polymers is expected to reach 17 million tons (~3 times higher) by the end of 2020 in comparison to 5.1 million tons in 2013 [23].

2.1 PHA-Producing Microorganisms

The biopolymers, PHAs, are synthesized by microorganisms as an intracellular product for storage of carbon and energy under nutrient-limited conditions [24]. In the presence of excess carbon source and limitation of nitrogen, phosphorus or oxygen in the growth media facilitates microorganisms to synthesize PHAs as an energy reserve material [25]. In 1926, French scientist Lemoigne first discovered poly (3-hydroxybutyrate) (PHB) in *B. megaterium* cells [26]. Since the discovery of PHB, more than 90 various bacterial genera and up to 300 species (both gram-positive and gram-negative) have been recognized as PHAs producers in both aerobic and anaerobic conditions [27, 28]. However, *Cupriavidus necator*, formerly known as *A. eutrophus* or *R. eutropha*, is one the most widely used strain for the production of PHAs. This was the first bacterial species used in the industrial production of P(3HB-*co*-3HV) copolymer by Imperial Chemical Industries (ICI) [23]. Types of PHAs depend on the bacterial strain used for the production process, and the production can reach up to a level of 90% dry cell weight depending on the microorganism used. PHAs production process categorizes bacteria community into two groups. In the first group, the PHAs are not accumulated as growth associated product, and they are often produced as energy reserve materials upon depletion of phosphorus, magnesium, nitrogen, or oxygen in the medium. For example, the bacteria such as *R. eutropha*, *P. oleovorans*, and *P. putida* belong to this group, whereas the second group does not require any nutrient limitation to accumulate PHAs, and these are accumulated during growth phase [29]. Recombinant *Escherichia coli* belongs to the second group [30]. The list of PHA-producing genera reported in the literature is provided in Table 3 [31]. From the past several years, PHAs have been known to be an intracellular product, as it accumulates in the cytoplasm of bacterial cells. However, Sabirova et al. reported extracellular production of PHAs using a genetically modified *Alcanivorax borkumensis* SK2 strain [32].

In addition to several wild-type PHA producer strains, certain efforts have also been made to design genetically recombinant strains with improved features for PHAs production. Introduction of appropriate genes into non-PHA-producing strains such

Table 3 List of genera known to be the producer of PHAs

Acidovorax	*Clostridium*	*Leptothrix*	*Rhodobacter*
Acinetobacter	*Comamonas*	*Methanomonas*	*Rhodococcus*
Actinobacillus	*Corynebacterium*	*Methylobacterium*	*Rhodopseudomonas*
Actinomycetes	*Cupriavidus*	*Methylocystis*	*Rhodospirillum*
Aeromonas	*Cyanobacterium*	*Methylomonas*	*Rubrivivax*
Alcaligenes	*Defluviicoccus*	*Methylosinus*	*Saccharophagus*
Allochromatium	*Delftia*	*Methylovibrio*	*Shinorhizobium*
Anabaena	*Derxia*	*Micrococcus*	*Sphaerotilus*
Aphanothece	*Ectothiorhodospira*	*Microcoleus*	*Spirillum*
Aquaspirillum	*Erwinia*	*Microcystis*	*Spirulina*
Asticcaulus	*Escherichia*	*Microlunatus*	*Staphylococcus*
Azomonas	*Ferrobacillus*	*Moraxella*	*Streptomyces*
Azospirillum	*Gamphospheria*	*Mycoplanaa*	*Synechococcus*
Azotobacter	*Gloeocapsa*	*Nitrobacter*	*Syntrophomonas*
Bacillus	*Gloeothece*	*Nitrococcus*	*Thiobacillus*
Beggiatoa	*Haemophilus*	*Nocardia*	*Thiococcus*
Beijerinckia	*Haloarcula*	*Nostoc*	*Thiocystis*
Beneckea	*Halobacterium*	*Oceanospirillum*	*Thiodictyon*
Brachymonas	*Haloferax*	*Oscillatoria*	*Thiopedia*
Bradyrhizobium	*Halomonas*	*Paracoccus*	*Thiosphaera*
Burkholderia	*Haloquadratum*	*Paucispirillum*	*Vibrio*
Caryophanon	*Haloterrigena*	*Pedomicrobium*	*Wautersia (Cupriavidus)*
Caulobacter	*Hydrogenophaga*	*Photobacterium*	*Xanthobacter*
Chloroflexus	*Hyphomicrobium*	*Protomonas*	*Zoogloea*
Chlorogloea	*Klebsiella*	*Pseudomonas*	
Chromatium	*Lamprocystis*	*Ralstonia*	
Chromobacterium	*Lampropedia*	*Rhizobium*	

as *E. coli* can enable the microorganism to synthesize biopolymers. Wang et al. reported an accumulation of 90% P(3HB) by using genetically modified *E. coli* strain [33]. Several studies also reported a successful integration of PHA-producing gene from various microorganisms such as *C. necator*, *Pseudomonas aeruginosa*, *Alcaligenes latus*, *Thiocapsa pfennigii*, and *Streptomyces aureofaciens*, into a *E. coli* strain [23]. These recombinant strains hold several advantages over the wild type of PHA-producing strains in terms of higher production capacity and diversity in the substrate selection.

Apart from using pure cultures of wild or recombinant strains, several studies also reported the use of the mixed microbial culture as an effective technique for PHA production. Different microorganisms belonging to several groups carry out

PHA synthesis in the mixed culture. The major advantages of using mixed cultures in comparison with pure cultures are reduction in process costs, exclusion of sterilization process, and minimum control requirements. The other advantage of mixed culture is its ability to utilize a wide range of substrates, including agro-industrial wastes. Ashby et al. observed an increase in PHA content and maximum substrate (glycerol) utilization through mixed cultures of *Pseudomonas corrugate* and *Pseudomonas oleovorans* [34]. Mixed cultures also proved favorable for PHA production using effluent from the starch and wood mill industry [35, 36]. However, low PHA content and less volumetric productivity (cell mass) are the major drawbacks of using mixed cultures in the PHA production process. These limitations can be surmounted by strain development and improving fermentation methods.

2.1.1 Metabolic Pathways

Three major pathways such as fatty acid β-oxidation, de novo fatty acid synthesis, and carbohydrates biosynthesis associated with the metabolism of PHA are depicted in Fig. 4 [37]. Microorganisms that follow the de novo biosynthesis pathway utilize glucose, gluconate, or acetate as carbon source, while fatty acids are utilized as carbon source in a β-oxidation pathway [38]. The enzymes responsible for PHA synthesis and their respective coding genes are the same in different bacteria. PhaA, PhaB, and

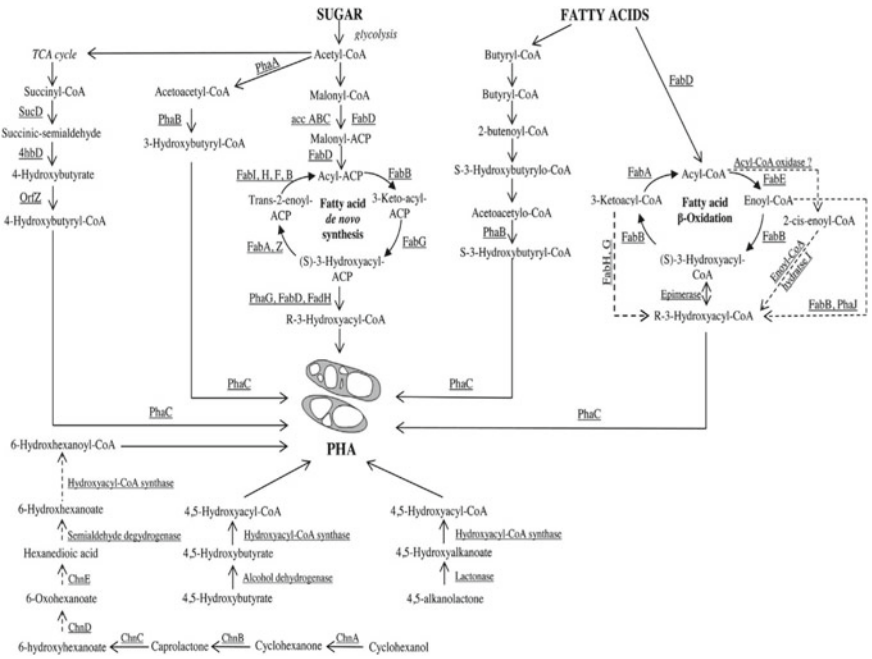


Fig. 4 Metabolic pathways of PHA synthesis

PhaC are some of the essential coding genes synthesizing β-ketothiolase, acetoacetyl-CoA reductase, and PHA synthase enzymes, respectively. At the same time, few other secondary genes required for PHA formation are PhaE, PhaF, PhaZ, and PhaP. Most of the bacteria such as *C. necator*, *Aeromonas hydrophila,* or *Pseudomonas stutzeri* follow the common three-step pathway for PHA biosynthesis using acetyl CoA as the precursor. Acetyl CoA, the end product of carbohydrate metabolism, is further converted into acetoacetyl CoA followed by 3-hydroxybutyryl CoA, the substrate for PHA synthesis. Fatty acid β-oxidation and de novo fatty acid synthesis are the two other pathways for PHA synthesis apart from the above-mentioned pathway. Maximum amount of PHA is reported to be produced from a fatty acid synthesis pathway [37]. The produced polymer composition is directly related to the substrate composition used for the growth of the microorganisms. Short-chain volatile fatty acids are reported to be the most suitable substrates for the PHA production by bacteria [39].

2.2 Substrates for PHAs Production

Appropriate substrate selection improves biomass yield, reduces fermentation cost, and aids in an easy separation of product from fermentation mixture. Selection of carbon source for PHA production primarily depends on the microorganisms used in the fermentation process. The substrate for PHAs production varies from simple commercial sugars (glucose, sucrose, glycerol, etc.) to innumerable waste products including industrial, agricultural, and food waste. All together, the substrates for PHAs production can be divided into three different categories: (i) simple sugars (monosaccharides), (ii) triacylglycerol, and (iii) hydrocarbons [40]. Most of the microorganisms favor the usage of simple sugars as their carbon source, while others use triacylglycerol and hydrocarbons as their carbon sources for PHA biosynthesis. The structural composition of PHAs varies with the use of different carbon sources and microorganisms, which subsequently affects its further applications. For example, several PHA-producing *Pseudomonas* species have the capability of incorporating functional groups such as phenyl, phenoxy, halogens, branched alkyls, olefin, and esters into the PHAs chain when they are grown over substrates containing the respective functional groups [41]. Kim et al. investigated the effect of using 36 various carboxylic acids containing carbon substrates on the PHA production by *Pseudomonas putida* KCTC 2407. Physical properties of PHAs can be significantly improved by incorporating suitable functional group into PHA chain. These groups can be further modified by chemical reactions to extend the potential applications of PHA as biodegradable polymers and possible applications in the medical sector. Table 4 represents production of PHAs using various carbon sources as a substrate. More details about the substrates can be found in the following sections [13, 35, 36, 42–93].

Table 4 Production of PHAs from various feedstocks

Carbon source/waste feedstock	Microorganisms	Fermentation mode	PHA content (%)	Reference
Commercial sugars				
Glucose	*Bacillus cereus*	Batch	47.9	[57]
	Pseudomonas mendocina NK-01	Shake flask (batch)	51.2	[89]
	Pseudomonas stutzeri 1317	Batch	52	[63]
	Pseudomonas putida Δgcd (recombinant)	Fed-batch	67.1	[77]
	Escherichia coli (mutant)	Batch	11.9	[88]
	Pseudomonas aeruginosa ATCC 9027	Batch	10.8	[81]
	Ralstonia. Eutropha	Fed-batch	73.8	[13]
	Escherichia coli K24KP (recombinant)	Aerobic batch	37.2	[42]
Sucrose	*Cupriavidus necator* (recombinant)	Fed-batch	74.3	[43]
	Alcaligenes latus	Fed-batch	50.0	[90]
Glycerol	*Escherichia coli* K24KL (recombinant)	Fed-batch	63	[73]
	Cupriavidus necator DSM 545	Fed-batch	62	[50]
	Shimwellia blattae (recombinant)	Two-step fed-batch	30.7	[83]
Starch				
Starch	*Bacillus cereus* CFR06	Batch	48	[62]
Starch	*Azotobacter chroococcum*	Batch	46	[67]
Cornstarch (extruded)	*Haloferax mediterranei*	Repeated fed-batch	24.2	[64]
Corn starch (extruded)	*Haloferax mediterranei*	Fed-batch	50.8	[55]

(continued)

Table 4 (continued)

Carbon source/waste feedstock	Microorganisms	Fermentation mode	PHA content (%)	Reference
Potato starch	*Ralstonia eutropha*	Fed-batch	55	[60]
Industrial waste				
Crude glycerol	*Pandoraea* sp. MA03	Batch	63.6	[56]
Crude glycerol	Mixed microbial consortia	Batch	47	[72]
Crude glycerol	Mixed microbial culture	Batch	59	[59]
Wood mill effluent	Two different cultures	Aerobic batch	29	[35]
Starch industry wastewater	Mixed cultures	Sequencing batch	60–65	[36]
Palm oil mill effluent	*Comamonas* sp. EB172	Fed-batch	59	[93]
Olive oil mill effluent	*Lampropedia arbour* and *Candidatus Meganema perideroedes*	Anaerobic fermentation and aerobic batch sequencing	–	[44]
Kraft cellulose mill effluent	*Sphingopyxis chilensis* S37 and *Wautersia sp.*	Batch	–	[86]
Paper mill waste	*Plasticicumulans acidivorans*	Sequencing batch	77	[65]
Paper mill waste	*Defluviicoccus vanus*/*Candidatus Competibacter phosphatis*	Aerobic/anaerobic process	42	[46]
Food wastes				
Extruded rice bran: extruded corn starch (1:8)	*Haloferax mediterranei*	Repeated fed-batch	55.6	[64]
Wheat bran	*Halomonas boliviensis* LC1	Batch	34	[87]
Molasses	*Escherichia coli* (recombinant)	Batch	75.5	[82]
Crude palm kernel oil	*Burkholderia* sp. USM (JCM 15050) (recombinant)	Fed-batch	66	[54]

(continued)

Table 4 (continued)

Carbon source/waste feedstock	Microorganisms	Fermentation mode	PHA content (%)	Reference
Crude palm kernel oil	*Cupriavidus necator* (recombinant)	Fed-batch	66	[69]
Palm kernel oil	*Cupriavidus necator* (recombinant)	Fed-batch	79	[48]
Whey	*Haloferax mediterranei*	Fed-batch	66	[68]
Whey	*Haloferax mediterranei*	Batch	53	[75]
Whey	*Thermus thermophiles* HB8	Batch	35.6	[76]
Whey permeate	*Cupriavidus necator* mRePT	Batch	25	[79]
Soy bean and rapeseed oil	*Cupriavidus necator* H16	Two-stage batch	57	[85]
Waste frying rapeseed oil	*Cupriavidus necator* H16	Batch	67.9	[74]
Waste frying sunflower oil	*Cupriavidus necator* H16	Batch	52.4	[74]
Corn oil	*Psuedomonas* species	Batch	35.63	[53]
Sugarcane vinasse	*Haloferax mediterrranei*	Batch	70	[47]
Malt waste	*Alcaligenus eutrophus* DSM1124	Batch	70	[92]
Soy waste	*Alcaligenus eutrophus* DSM1124	Batch	32.57	[92]
Restaurant waste	Recombinant *E. coli* pnDTM2	Batch	45	[58]
Restaurant waste	*Cupriavidus necator* H16	Continuous feeding	87	[61]
Lignocellulosic biomass				
Wheat straw	*Burkholderia sacchari* DSM 17165	Fed-batch	72	[51]
Rice straw	*Bacillus firmus* NII 0830	Batch	89	[84]

(continued)

Table 4 (continued)

Carbon source/waste feedstock	Microorganisms	Fermentation mode	PHA content (%)	Reference
Sugarcane bagasse	*Burkholderia* sp	Fed-batch	48	[71]
Sugarcane bagasse	*Cupriavidus necator*	Batch	57	[91]
Parthenium hysterophorus and *Eichhornia crassipes*	*Ralstonia eutropha*	Batch	8.1–21.6	[80]
Wastewater				
Cassava starch wastewater	*Bacillus tequilensis* MSU 112	Sequencing batch	79.2	[52]
Cassava starch wastewater	Cupriavidus sp. KKU38	Batch	61.6	[78]
Brewery wastewater	Activated sludge consortium	Stirred batch reactor	39	[45]
Food processing wastewater effluent	Activated sludge consortium	Batch	60.7	[66]
Olive oil mill wastewater	Wastewater microbes	Stirred batch reactor	11.3	[49]
Tomato wastewater	Activated sludge consortium	Batch	20	[70]

2.2.1 Commercial Substrates

Carbohydrates are mainly classified into three groups including monosaccharides, disaccharides, and polysaccharides. Monosaccharide carbohydrates are a simple sugar that cannot be hydrolyzed further, whereas disaccharides and polysaccharides can be hydrolyzed to form monosaccharides. PHA-producing microorganisms can directly utilize monosaccharides and disaccharides for PHA production, while polysaccharides need to be hydrolyzed to simple sugars prior to the fermentation. Starch, hemicellulose, and cellulose belong to the polysaccharides category.

The simplest sugar, glucose, is the most widely used carbon source for PHA production. Several wild types of strains such as *R. eutropha*, *Bacillus cereus*, *P. aeruginosa,* and genetically modified strains of *E. coli*, *P. putida* have been used for PHA production using glucose as substrate. Kim et al. reported 73.8% PHA accumulation by using *R. eutropha* strain in a fed-batch process applying glucose as the carbon source [13]. In another study, Poblete-Castro et al. adopted various fed-batch strategies to improve *mcl*-PHAs production from glucose using metabolically engineered *P. putida* strains [77].

The other substrate for PHA production is sucrose, a disaccharide composed of two monosaccharides, glucose, and fructose linked via a glycoside linkage. *R. eutropha* (renamed as *C. necator*), the most wildly used microorganism in PHA production cannot utilize sucrose as a carbon source directly [94]. However, other wild strains such as *Azotobacter vinelandii*, *A. latus,* and *Hydrogenophaga pseudoflava* have been identified to produce PHA by utilizing sucrose directly [95, 96]. These microorganisms hydrolyze sucrose into glucose the wild type of strains, several recombinant strains are also reported for the direct utilization of sucrose as a carbon source. Zhang et al. reported PHA production in a recombinant *Klebsiella aerogenes* by inserting a PHA synthesis gene from *R. eutropha* [97]. In another study, sucrose-utilizing gene from *Mannheimia succiniciproducens* was introduced into *R. eutropha*, which enabled the cells to utilize sucrose for PHA production [94]. The recombinant *R. eutropha* produced approximately 0.0046 g L^{-1} h^{-1} of PHA in a 5-L batch fermenter with sucrose as a substrate.

Glycerol, one of the important commercial chemicals, has been used as a substrate for the production of various fine chemicals, which includes lactic acid, 1,3-propanediol, dihydroxyacetone, ethanol, biohydrogen, etc. Some of the recent studies also focused on the production of PHA from pure glycerol. Using pure glycerol as substrate, 63 and 30.7% PHA content are accumulated with the help of recombinant *E. coli* and *Shimwellia blattae* strains, respectively [73, 83].

Even though utmost efforts have been taken to compete with the petrochemical plastics, biopolymers are still unattractive due to their higher production cost and inferior material properties. Production cost is one of the main constraints in commercialization of biopolymer as compared to conventional polymers. The major fraction of production cost is associated with the carbon substrates, and the cost of the substrate has been reported to contribute about 28–50% of the total production cost [98, 99]. To make the PHA production process economical, worldwide research has been focused on the utilization of several waste materials as carbon source [100]. Wastes or by-products obtained from the biodiesel industry, palm oil industry, paper mill, food waste, agricultural sector, animal wastes, and many more wastes have been explored as a potential carbon substrate for PHA production. More details about the substrates and PHA production processes are described in the subsequent sections.

2.2.2 Starch

Starch is a polymeric carbohydrate consisting of glucose monomers joined by glycosidic linkage. Several plants such as rice, wheat, potatoes, cassava, and maize are known to be the producers of starch. However, many bacterial strains lack the ability to produce α-amylase, the key enzyme responsible for starch hydrolysis. Therefore, α-amylase synthesis gene from external source is essential for starch hydrolysis and to use it as carbon source for PHA production. Along with saccharified waste potato starch, the *R. eutropha* strain resulted a biomass yield of 179 g/L with PHAs content 55% (w/w) of CDW in a fed-batch process [60]. In another study, Chen et al. reported 50.8% (w/w) PHA accumulation in *H. mediterranei* using an enzymatic

extruded cornstarch as a substrate in a fed-batch operation [55]. In these studies, saccharification of starch was carried out by the enzymes from an external source (i.e., commercial enzymes) prior to the main PHA production process. Halami et al. isolated α-amylase producing native *B. cereus* CFR06 strains from soil sample, which could accumulate about 48% of PHA after 72 h of fermentation in a starch-containing medium [62]. These studies clearly indicate a higher production of PHA using starchy food wastes as the substrates with minimal or no pre-saccharification process.

2.2.3 Industrial Wastes

Effective utilization of industrial wastes for value-added products are considered as an economical solution to reduce production cost, while simultaneously addressing the environmental issues. Waste from biodiesel industry and effluents from palm oil, paper, olive, and wood industry have been used as a substrate for the PHA production using various microbial strains. More details about the industrial wastes, microorganisms used and PHA content are listed in Table 4.

Nowadays, biodiesel is considered as one of the major alternative green fuels for petroleum diesel. The global biodiesel production is expected to reach 32 billion liters (8.2 billion gallons) by the end of the year 2020. This would concurrently produce 2.6 million tons (5.9 billion gallons) of crude glycerol, a major by-product from biodiesel industry [101]. On the weight basis, approximately 1 kg of crude glycerol is produced as a by-product for every 9 kg of biodiesel. The effective utilization of enormous quantity of crude glycerol for PHA production is a viable solution to trim down the production cost of both biodiesel and biopolymers. Freches and Lemos reported a final PHA content of 59% from acclimatized mixed microbial cultures using crude glycerol as substrate in a sequencing batch reactor (SBR) [59]. de Paula et al. reported PHA production from biodiesel-derived crude glycerol by newly isolated *Pandoraea* sp. from Atlantic rainforest in Brazil [56]. PHA accumulation by this strain ranged from 49.0 to 63.6% CDW using crude glycerol as substrate, which reported to be higher than the pure glycerol. Presence of NaCl contaminants in crude glycerol enhanced biopolymer accumulation in *Pandoraea* species.

Effluents from wood mill, palm mill, olive oil mill, paper mill, etc. are rich in organic sources, which are further converted to value-added chemicals including PHA. Palm oil mill effluent (POME), rich in carbohydrate, protein, lipids, nitrogenous compounds, and minerals, provides nutritious support for bacterial growth concurrently degrading the waste to reduce its environmental hazard [102]. Zakaria et al. isolated eleven potential PHA-producing strains from the treated palm oil wastewater [93]. Among the isolated strains, *Comamonas* sp. accumulated highest PHA content (44%) and they exhibited a wide range of substrate utilization. Hassan et al. recommended a two-stage process for the PHA production from POME [103]. In the first stage, production of acids, particularly acetic and propionic acids was carried out in the anaerobic process, which was subsequently converted to PHA using *Rhodobacter sphaeroides* at the second stage. This study also reported inhibition effect of formic acid in PHA production process and a decrease in PHA content

from 67 to 18% was noticed with an increase in formic acid concentration. A three-stage treatment (anaerobic and aerobic) of olive oil mill effluent for PHA production was investigated using activated sludge, dominated with *Lampropedia arbour* and *Candidatus Meganema perideroedes* species [44]. Volatile fatty acid (VFA) content, the most direct precursor for PHA production, was increased from 18 to 32% at the first stage (anaerobic) of the process. Similarly, Jiang et al. reported that the PHA production from the organic-rich paper mill effluent in a three-stage process [65]. The conversion of paper mill effluent to VFA is carried out in the first stage, followed by the enrichment of PHA-producing bacteria, and later maximizing their storage capacity at the second and the third stages of the process, respectively. This process accumulated maximum PHA content up to 77% of cell dry weight within 5 h of enrichment process. Similarly, Bengtsson et al. reported a three-stage process for PHA accumulation (42%) using glycogen accumulating organisms, and observed a total PHA yield 0.10 kg per kg of influent soluble chemical oxygen demand [46]. Further, the optimization of process and purification of the final product is required to make the process more economical and feasible for an industrial-scale production.

2.2.4 Food Wastes

Food wastes are one of the major form of wastes generated worldwide, starting from harvesting of the crop to the end of life [104]. Food and Agriculture Organization (FAO) estimated that one-third of the world food production is lost or wasted during this process. It was reported that approximately 89 million tons of food waste are produced by the EU-28 Member States in 2012 [105, 106]. Many developing countries are observing significant increase in the quantity of food waste in recent years due to the increasing population growth, increase in food consumption, and lack of proper treatment process. A sustainable, economic, and efficient alternative for the conversion of these surplus food wastes to several value-added products can simultaneously reduce environmental pollution and health risk hazard caused by the perishable food waste. These food wastes are rich in proteins, carbohydrates (e.g., cellulose, hemicellulose, starch, and sugars), minerals, oils and fats, which can be used as a substrate for microbial or enzymatic processes. The exploitation of food waste for the production of several value-added products (lactic acid, biohydrogen, ethanol, and biogas) has been reported in past several years. PHA, another value-added product produced from various food wastes using microbes, is summarized in Table 4. These wastes include rice or wheat bran, molasses, whey, palm kernel oil, soy waste, malt waste, restaurant waste, waste frying oils, etc.

Whey, the major by-product from cheese-making process, has been extensively studied for the production of PHA. Whey is a rich source of lactose, proteins, fats, vitamins, and other essential nutrients that does not require any extensive pre-treatment step for the use in the fermentation process conducted by microorganism [107]. Most of the studies reported the use of recombinant *E. coli* for the production of PHA, as the wild microbial strains have limited ability to utilize lactose directly. Studies reported the use of *Haloferax mediterranei*, *Thermus thermophiles* HB8, and

C. necator for whey fermentation, and the final PHA content varied from 25 to 66% [68, 75, 76, 79].

Recently, various plant oils from household and industry have also used as an economic substrate for PHA production. Oils produced from industries such as palm oil [54], soybean oil [85], sunflower oil [108], olive oil [53], and from household such as waste frying rapeseed and sunflower oil [74, 85] are successfully tested for the production of PHA. These oils are devoid of costly pre-treatment processes, and they can be directly added to the fermentation media as carbon source for the production of PHA. A study by Taniguchi et al. reported the conversion of various waste edible oils and fats for the production of PHA using *R. eutropha* H16 strain [85]. A highest PHA yield of 83%, and final cell dry weight 6.8 g/L were observed from this study with the use of palm oil with lard as carbon sources. Production of copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) occurred instead of pure PHB when tallow was used as the carbon source. In another study, Obruca et al. reported the utilization of waste frying oils (rapeseed, palm, sunflower) as a carbon source in fermentation using *C. necator* H16 microbial strain [74]. The cell dry weight and PHB content using these substrates varied in the range from 10.8 to 11.9 g/L and from 52.4 to 67.9%, with a higher PHB production level from waste frying rapeseed oil. Other than whey and oils, studies also reported the use of several other food wastes such as molasses, wheat bran, rice bran, malt waste, soy waste, sugarcane vinasse, and restaurant waste as an economical carbon source for the production of PHA. Nielsen et al. have given a detailed review of the utilization of various food wastes for the production of PHA [109].

2.2.5 Agricultural Waste and Lignocellulosic Biomass

In recent years, agricultural wastes and its coproducts have become the major contributor of waste into the environment, and its annual global production has reached 16 million metric tons [110]. Stubble burning of these lignocellulosic wastes such as sugarcane bagasse, rice straw, rice husk, corn stover, wheat straw, and wood chips is the major source of environmental pollution in many agrarian countries. Cellulose (40–50%), hemicellulose (25–30%), and lignin (15–20%) are the three major constituents of lignocellulosic biomass, with some amount of extractives such as ash, proteins, pectins [111, 112]. Cellulose is a homo-polymer, consisting of a linear chain of D-glucose linked with a β-1,4 glycosidic bond, whereas pentose sugars (xylose, arabinose), hexose sugars (glucose, galactose, mannose), glucuronic acid, and uronic acids are the major components of hemicellulose. Lignin is a complex polymer of aromatic ring compounds (sinapyl, coniferyl, and p-coumaryl alcohols) that provide mechanical strength to the plant cell wall. Pre-treatment of biomass partially removes the hemicellulose and lignin fractions, which also decreases the cellulose crystallinity and improves the porosity of biomass for accessibility of enzymes or acids to hydrolysis [113]. Hydrolysis of biomass releases hexose and pentose sugars, which are subsequently used in fermentation for production of various value-added products.

Sindhu et al. reported the utilization of hemicellulosic rich, acid hydrolyzed rice straw hydrolysate for the production of PHB using *Bacillus firmus* NII 0830 strain [84]. Acid hydrolysis was carried out in the presence of 2% (w/w) H_2SO_4 for the release of pentose sugar. The bacterium accumulated 1.697 g/L of PHB with a final cell mass 1.9 g/L, after 90 h of fermentation using pentose hydrolysate without any prior detoxification. Another study by Cesario et al., used wheat straw hydrolysate in batch and fed-batch fermentation, which resulted in a PHB production of 0.7 and 0.72 g of PHB/g of CDW, respectively [51]. The microorganism *Burkholderia sacchari* DSM 17165 has the ability to utilize both C_6 and C_5 carbon sugars (i.e., glucose, xylose, and arabinose) in hydrolysate for the PHA production. Sugarcane bagasse is one of the other abundant agricultural wastes used in the PHB production using *Burkholderia* sp and *C. necator* microbial strain [71, 91]. Apart from the agricultural waste, limited studies also explored the possible utilization of invasive weeds as potential feedstock for the production of PHA. In one of our studies, we selected two different invasive weeds such as *Eichhornia crassipes* and *Parthenium hysterophorus* as substrates for PHB production using *R. eutropha* strain [80]. Biomasses were hydrolyzed using dilute acid pre-treatment (1% v/v H_2SO_4) and enzymatic hydrolysis (cellulase and cellobiase) process for obtaining pentose and hexose-rich hydrolyzates. These hydrolyzates were further fermented separately using *R. eutropha* strain, and the PHB content in dry cell mass varied in the range of 8.1–21.6% w/w with yield 6.85×10^{-3}–$36.41 \times 10^{-3\%}$ w/w of raw biomass. Significant variation in thermal properties of the produced polymers derived from two different hydrolyzates was noticed. Higher maximum thermal degradation observed for PHB derived from hexose-rich hydrolyzate, whereas the pentose-rich hydrolyzate derived PHB showed higher glass transition temperature.

Further research on effective utilization of other invasive weeds and mixed lignocellulosic feedstock for biopolymer production need to be carried out. Combination of suitable pre-treatment techniques can increase the fermentable sugars production, which can be further used for higher PHA yield.

2.2.6 Wastewater

The production of PHA using cassava starch, brewery, and wastewater obtained from food processing industry has gained much attention in recent years due to the use of mixed microbial culture and open reactors despite a costly sterilization process. The treatment of wastewater for removing impurities and organic content with a simultaneous production of biopolymers is an efficient solution to address the problems of environmental pollution. Chaleomrum et al. investigated the potential of cassava starch wastewater for PHA production using *Bacillus tequilensis* in a sequencing batch reactor (SBR) [52]. The effect of different inlet COD concentrations in cassava starch wastewater on the treatment efficiency and the PHA production was determined. Highest PHA production (79.2%) was reported with a COD concentration of 4000 mg/L, while the maximum COD removal efficiency (94.8%) was at

5000 mg/L COD. The PHA accumulation of 58 and 43% (w/w) was reported using swine wastewater and paper and pulp wastewater, respectively [114, 115].

3 PHAs Extraction from Microorganism

PHAs are intracellular polymers, i.e., they are stored inside the extracted cells post-fermentation cycle. Therefore, extraction of these polymers is often complex and expensive. Many researchers have developed various economical methodologies for extraction of the intracellular PHAs. Before performing any extraction technique, the cells are harvested after fermentation by centrifugation at low temperature (~10 °C). The centrifugation should be performed at lower temperature to prevent damage/disruption to the cells. The biomass should be freeze dried or lyophilized prior to performing the extraction of PHAs.

3.1 Solvent Extraction

The solvent extraction method is commonly used for recovery of PHAs from microbial cells; it is divided into two phases. First, the breakage of cellular membrane is done by solubilization of PHA molecules. Several researchers observed that solvent containing at least one hydrogen and one chlorine atoms provide best recovery of PHA with high purity. Using solvents like 1,2-propandiol, or glycerol formal, or diethyl succinate, or butyrolactone, 79–90% of PHB recovery can be achieved with purity ranging from of 99.1 to 100% [116]. The cellular debris can be removed by suitable filter papers to get dissolved PHA solution. Then, the soluble PHA is subjected to precipitation in the form of granules using non-solvent such as alcohols and hexane. Repeating the precipitation step up to three times yields maximum amount of PHAs precipitate [117]. The precipitated PHB can further be dried at room temperature or in vacuum oven to evaporate all the residual solvents to get PHB in powder form.

3.2 Digestion Method

For extraction of PHAs, the digestion method is a potential alternative to solvent extraction, which can be achieved by either enzymatic or chemical reactions. Applications of surfactants like sodium dodecyl sulfate (SDS) not only disrupt the cellular membrane but also break the membrane to make micelles and phospholipids [116]. The surfactant (betaine) and chelate aqueous system help in solubilization of protein and non-polymeric cellular materials [118]. After solubilization of all non-PHA

components, the PHA molecules are released to the solution surrounded by the surfactants. The chemical digestion method is achieved by addition of chemicals such as sodium hypochlorite followed by addition of chloroform. After addition of sodium hypochlorite, the entire solution is stabilized into three phases. The first or upper phase is the hypochlorite solution, the second or middle phase consists of cellular debris, and the third or bottom phase is the PHA dissolved in the chloroform. The chloroform soluble phase is separated by filtration which can be further precipitated by addition of non-solvent solution [119]. Similarly, the enzymatic digestion for extraction of PHA is achieved by proteolytic enzyme digestion, which minimized the degradation of HB polymer [120]. Similarly, Kathiraser et al. reported the performance of the enzymatic degradation of PHAs cells using *Alcalase* enzyme, SDS, and EDTA and obtained a purity of 92.6% [121].

3.3 Mechanical Disruption

Cellular disruption of bacterial cells using mechanical agitation method is a very widely used technique for extraction of PHAs as it is economically viable possessing lesser chance of degradation to PHA polymers. It is an environmentally friendly method because no chemicals are required and contamination of PHA polymer can be minimized. This method can be categorized into two categories such as solid shear and liquid shear methods. The extraction method using bead mill includes solid shear method, and high-pressure homogenization includes liquid shear method [116, 122]. The bead mill is comprised of a vertical cylinder-grinding chamber where the microbial cells are allowed to enter at the base and flow through the annular space between the rotor and the stator exiting at the top. The heat generated during the homogenization process is minimized by cooling water supply in the jacket around the chamber [116]. The efficiency of cells disruption is a strong function of bead loading, agitation speed, cell concentration, residence time distribution, bead diameter, and geometry of loading chamber, etc. For large-scale PHA cell disruption, high-pressure homogenization is one of the most effective mechanical methods for extraction. This extraction process involves homogenization under high-pressure via an adjustable and restricted orifice discharge valve. This extraction process depends on operating pressure, number of passes, valve design, and operating temperature [122]. However, this process is not efficient for low biomass concentrations inside the homogenizer [122].

3.4 Supercritical Fluid Extraction

Usage of supercritical fluids is one of the latest technologies for extraction of PHAs because of its physical properties such as high density, low viscosity, and less toxicity. Because of lower viscosity with almost negligible surface tension, the speed

of percolation is very high resulting in better diffusion of fluid for extraction process compared to liquid solvents [123]. A wide range of supercritical fluids such as carbon dioxide (CO_2), ammonia (NH_3), and methanol (CH_3OH) can be used for recovery of PHAs. Hejazi et al. reported that the application of supercritical CO_2 in addition to methanol results in 89% recovery of PHB polymer from microbial cells obtained from *R. eutropha*. As reported by Williams et al., extraction of PHAs using supercritical fluid results in recovery of 100% pure PHA, which is 150 times less contaminated compared to PHAs produced from other methods [124].

3.5 Aqueous Two-Phase Extraction

Aqueous two-phase extraction method is one of the potential alternatives for extraction of PHAs using microorganism such as *B. flexus*. Aqueous two-phase systems are observed when two immiscible phases coexists. Such scenario is observed when the two polymers show chemical incompatibilities in nature and are present at low concentrations. Similar aqueous phase can also be observed when one of the polymers and inorganic phase are present at low concentration at the same time. During the extraction process, enzymatic hydrolysis of *B. flexus* cells is done followed by introducing polymer-salt two-phase system to recover PHAs. During this process, high molecular PHAs are obtained with purity of around 97% [122]. This method of PHA extraction is relatively less time consuming along with lesser cost and lesser energy consumption. In some cases, the two phases are observed as water and non-volatile phase. The isolation, purification, and recovery of PHA are done by water phase. The cellular debris can be recovered from the bottom layer as it settles down during extraction process. This method is an environmentally viable and effective non-solvent method for isolation of PHA from bacterial cells [123].

3.6 Ultrasound-Assisted Extraction

Ultrasound-assisted cell disruption is a new potential alternative for the extraction of PHA. The ultrasound waves are very effective for the rupture of cell walls/membranes, emulsification, and homogenizing, as it focuses on a localized volume [80]. It employs the instantaneous sinusoidal movement of a soundwave in a liquid medium. During sonication process, formation of microbubbles takes place, which leads to the generation of cavitation phenomena. Cavitation process or phenomena is controlled by two forces, viz. pressure force and inertial force, under the influence of pressure variation induced by ultrasound acting simultaneously on the radial motion of the bubble. The dominant force between these two forces governs the expansion of the bubble in the rarefaction half cycle, which is governed by the amplitude of the ultrasound wave. During the compression half cycle, the

inertial force dominates over pressure force resulting in the compression of the bubble. This phenomenon continues until the bubble is compressed to extremely small size (minimum radius or maximum compression). Further variation in any force will result in transient collapse of the bubble. Temperature and pressure can reach up to ~5000 K and ~50 MPa, respectively, during the transient collapse [125]. As the bubble collapses during cavitation, it generates high-energy (temperature and pressure) concentration in a very small (nano) spatial and temporary scale [126]. The transient collapse of bubbles leads to chemical (generation of free radicals) and physical (shockwaves and micro-turbulence) effects [127]. During the cavitation process, the sonic/vibrational energy is converted into mechanical energy due to conservation of momentum, resulting in generation of high-pressure shockwave causing the cell disruption.

4 Characterization of PHA

A number of PHA homo-polymer and copolymers have been developed through different microbial process in order to fulfill the research gap for biodegradable polymers for the last five decades. To understand the chemical compositions, crystallinity, glass transition temperature, thermal properties, mechanical properties, molecular weight, etc. of PHAs, physical, and chemical characterizations are very important for their quantification and identification and are discussed in the following subsections.

4.1 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy helps to identify the functional groups and quantifies of PHA content present in the polymer chain by observing magnetic field around the nucleus of the atom. The chemical shifts for methyl, methylene, and methine groups are observed in the range of 1.25–1.28, 2.17–2.65, and 5.25–5.26 ppm, respectively, for ^{1}H NMR of *scl*-PHA (e.g., polyhydroxybutyrate). Similarly, for ^{13}C NMR the corresponding chemical shifts are observed in the range of 19.95–21.4, 31.09–41.3, and 67.8–68.5 ppm, respectively. The –C=O group is observed in ^{13}C NMR, which is in the range of 169.31–170 ppm [128–130]. For *mcl*-PHAs (e.g., P(3HB-*co*-3HV)) the methyl, methylene, and methine groups are detected at 1.26–1.6, 2.3–2.7, and 5.2–5.25 ppm, respectively. For detection of –C=O group, the chemical shift observed in ^{13}C NMR is in the range of 169.1–169.5 ppm [131–133]. A summary of the NMR characterization results for both *scl*-PHA and *mcl*-PHA is presented in Table 5.

Table 5 Summary of functional groups observed from NMR and FTIR analysis for *scl*-PHA and *mcl*-PHAs

Group or moiety	Chemical Shift (ppm)		Group or moiety	Wave number (cm^{-1})
	^{1}H NMR	^{13}C NMR		
– CH	5.2–5.26	67.8–68.5	– CH	2962–2853
– CH_2	2.17–2.7	31.09–41.3	– C = O	1742–1709
– CH_3	1.25–1.6	19.95–21.4	– C–O or –C–C	1300–1000
– C=O		169.1–169.5	– OH	3460–3407

4.2 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy is used to identify the organic functional groups present in PHAs by measuring absorption of infrared radiation as a function of wavenumber. For both *scl*-PHA and *mcl*-PHAs (including copolymer), the range of wavenumber shifts for different moieties is in the similar range. The absorption of hydroxyl groups (–OH) is observed in the range of 3460 to 3407 cm^{-1} for wide range of PHAs [134, 135]. The major strong bands are observed for carbonyl (–C=O) and unsaturated ester (–COO) groups and are within the range of 1742–1709 cm^{-1} [128, 136]. The coupling of –C–O and –C–C intense stretches are observed in the range of 1300–1000 cm^{-1}. For methine (–CH) group, mild stretch vibrations have been observed in the range of 2962–2853 cm^{-1} [121]. The details of wavenumber shifts ranges for all types of functional groups present in different types of PHAs are summarized in Table 5.

4.3 X-Ray Powder Diffraction (XRD) Analysis

X-ray powder diffraction measurement helps to understand the crystalline nature and morphology of PHAs. In the XRD profile of PHAs, two strong intense peaks are observed around $2\theta = 13°$ and 17° having miller indices of (020) and (110), respectively, suggesting the α-PHB crystal and orthorhombic unit cells appeared on the plane. Relatively weaker reflections are observed at around $2\theta = 21°$ and 22°, which corresponds to miller indices of (101) and (111), respectively for α-PHB crystal. Other similar weaker reflections are observed at $2\theta = 26°$ and 27° showing (130) and (040) reflections, respectively [128, 137, 138]. Senhorini et al. reported that the crystallinity (χ_c) of PHAs can be calculated by measuring the area of crystalline and amorphous peaks. The empirical formula is stated as follows:

$$\chi_c = \frac{A_t - A_a}{A_t} \times 100 \tag{1}$$

where A_t and A_a are the areas under crystalline and amorphous peaks [139]. Bhaskaran et al. reported a comparative study for crystallinity (χ_c) of PHAs synthesized from palm oil in which the χ_c of PHAs are observed in the range of 34–52% [135]. Therefore, from the observation of peaks and calculation of χ_c of different PHA samples, it can be concluded that the PHAs are partially crystalline in nature.

Similarly, the apparent crystal size (D_{hkl}) of PHA samples can be determined by Scherrer's equation, which is stated as follows:

$$D_{hkl} = \frac{K\lambda}{\beta_0 \cos\theta} \tag{2}$$

where K is the geometrical shape factor, β_0 is the half width (radian unit) of the reflections corrected for instrumental broadening, θ is the peak position, and λ is the wavelength (nm) [140]. Mottin et al. reported that the average sizes for PHB film, nanofibers, and crystals are observed in the range of 19–79 nm. [137].

4.4 Differential Scanning Calorimetry (DSC)

The thermal properties of PHAs such as melting point, glass transition temperature, and crystalline temperature on heating and cooling can be obtained from differential scanning calorimetry (DSC) analysis. Apart from these properties, the crystallinity (χ_c) of PHAs can be calculated from DSC curve by measuring the melting enthalpy or heat of melting (H_f). The degree of crystallinity can be estimated by the following equation:

$$\chi_c(\text{Percentage}) = \frac{H_f}{H_{100\%}} \times 100 \tag{3}$$

where $H_{100\%}$ is the fusion enthalpy of 100% crystal PHB, which is 146 J/g [141]. The crystallinity of PHB obtained from soy waste and commercial PHB is in the range of 46–53% [142], whereas the corresponding value for PHB copolymer, e.g., P(3HB-*co*-3HV) lies within 39–47% [141]. The crystallinity range is similar to the values obtained from XRD analysis confirming the partial crystalline nature of PHAs. The melting point range of PHB obtained from molasses, corn steep liquor, and soy waste using different microorganisms (i.e., *C. necator and B. megaterium*) are around 169–177 °C. The crystalline temperature on heating (T_{hc}) and cooling (T_{cc}) and glass transition temperature (T_g) for corresponding PHBs is in the range of 40–48, 86–114, and −1–1 °C, respectively [142, 143]. It has also been reported that the melting point and glass transition temperatures of PHA copolymers decrease with increase in 4HB (hydroxybutyrate) and HH (hydroxyhexanoate) content over 3HB content. Cheng et al. reported that for co-polyester P(3HB)(4HB), as the 4HB content increases from 0 to 40%; the melting point was decreases from 173 to 51 °C, the glass transition temperature decreases from −6 to −20 °C, and the crystallinity decreases form 59

to 0.5%. Such variations in thermal properties are due to destruction of crystalline structure of PHB, which not only affect the surface free energy, but also change the biocompatibility nature of the polymers. In addition to this, the differences in crystallinity and molecular weight of different polymer matrices result change in the aforementioned thermal properties [144, 145].

4.5 Thermogravimetric and Differential Thermogravimetric Analysis (TGA and DTG)

Thermogravimetric analysis or thermal gravimetric analysis (TGA) is an analytical technique that helps to analyze the thermal properties such as thermal stability, resistance, and rate of degradation that are measured as a function of temperature or as a function of time. The decomposition of PHAs can be categorized into three different phases. The first phase or initial phase of mass loss results due to evaporation of physically adsorbed impurities and solvents during the fermentation process. The second stage of mass loss is the major degradation step, which occurs after the melting point of a particular PHA polymer. In this stage, the β-chain scission process results in cleavage of –C–O and –C=O bonds in ester functional group with the formation of crotonic acid. During this heating process, the crystalline regions are destroyed and depolymerization of hemicellulose results in rapid mass loss of PHAs [80]. The third and final degradation stage occurs after residual mass left in the PHA sample, in which the rate of mass loss is negligible. Several researchers have assessed the thermal stability by analyzing the quantitative details of weight loss (e.g., 5% mass loss) with respect to temperature. The temperature at 5% mass loss of PHB obtained from recombinant *A. hydrophila* was in the range of 226–235 °C. The corresponding mass loss for copolymers such as P(3HB-*co*-4HB-*co*-3HH$_x$), P(3-HB-*co*-3-HV), P(3HB-*co*-3HH$_x$), P(3HB-3HV-3HH$_x$), and P(3HB-*co*-3H4MV) lies within 247–306 °C, respectively [146–150]. The temperature details of PHAs at 5% mass loss with their compositions are given in Table 6 [146–150]. Therefore, it can be concluded that the incorporation of various monomers such as 4HB, 3HH, 3HV, and 3H4MV provides more thermal stability to the polymer as it has shown higher thermal degradation temperature. The rate of mass loss of PHAs with respect to temperature is measured by differential thermogravimetric (DTG) analysis which is obtained from the first derivative of the weight loss curve, and it indicates the thermal stability with respect to temperature at which maximum degradation occurs in the polymer matrix. He et al. studied the DTG analysis of three different types of PHAs such as PHB, P(HB 70 mol%: HV 30 mol%), P(HB 85 mol%: HH$_x$ 15 mol%) in which the maximum degradation temperatures are observed at 349, 352, and 359 °C, respectively. They observed that copolymers (e.g., P(HB-HV) and P(HB-HH$_x$)) have higher thermal degradation temperatures relative to homo-polymer PHAs such as PHB, and hence, the thermal stability of such polyesters improves by increasing the number of structural hydrocarbon units [151].

Table 6 TGA analysis of 5% mass loss temperature (T_5%) of different types of PHA

Type of copolymer	Composition of PHAs (mol%)	T_5% mass loss (°C)	Name of the strain
P(3HB)	3HB: 100	226–235	Recombinant *A. hydrophila*
P(3HB-*co*-4HB-*co*-3HH$_x$)	3HB: 73.8, 4HB: 5.1, HH: 21.2	257	Recombinant *A. hydrophila*
P(3HB-*co*-3HV)	3HB: 80, 3HV: 20	247–253	*Alcaligenes eutropha*
P(3HB-*co*-3HH$_x$)	3HB: 57%, 3HH: 43%	285	Recombinant *Cupriavidus necator*
P(3HB-3HV-3HH$_x$)	HB: 5.4, HV: 9.9, HH: 86.7	273	Recombinant *A. hydrophila*
P(3HB-*co*-3H4MV)	3HB: 81, 3H4MV: 19	306	*Burkholderia* sp.

4.6 Gel Permeation Chromatography (GPC)

Gel permeation chromatography (GPC) is primarily used for analysis of biological, polymeric and macromolecule samples to estimate weight-average molecular weight (M_w) and number average molecular weight (M_n). The polydispersity index (M_w/M_n) which is a measure of molecular mass distribution is defined as the ratio of average molecular average to the number average molecular weight. The two types of molecular weights and polydispersity index are shown in Table 7, for a wide range of PHA samples. From Table 7, it can be observed that the weight-average molecular weight (M_w) of several homo- and hetero-polymers lies in between 9 and 16 $\times$ 10^5 Da [146, 148–150, 152]. The PHA copolymer containing HH$_x$ monomer unit poses lesser molecular weight compared to P(3HB). The lower molecular weight of such copolymers is due to the higher accumulation of PHA synthase (bulkier monomer like HH$_x$) containing both soluble bound and granule bound PHA synthase [149]. Mizuno et al. have studied the time-dependent change of molecular weight of PHAs

Table 7 Molecular weight analysis of several PHAs

Type of PHA sample	M_w (10^5 Da)	M_n (10^5 Da)	M_w/M_n
P(3HB)	9–16	1.66–8.4	1.7–2.9
P(3HB-*co*-32 mol% 3HH$_x$)	3.47	2.24	1.55
P(4HB)	8.54	4.87	1.75
P(HV)	10.56	8.15	1.3
P(3HB-co-77 mol% 3HV)	9.24	1.59	5.8
P(3HB- 6.6 mol% 4HB- 19.5 mol% 3HH$_x$)	7.61	5.54	1.37
P(3HB-5.4 mol% 3HV- 9.9 mol% 3HH$_x$)	3.73	1.71	2.17

by extracting the cultured cells at different time intervals. It is observed that weight-average molecular weight drastically decreases from 19.3×10^5 Da to 1.46×10^5 Da between 14 and 72 h of cell cultivation. Such rapid reduction of molecular weight is because of depolymerization of intracellular PHA at late stationary growth phase for a particular microorganism [153]. Since higher molecular weight PHAs are useful for several domestic and industrial applications; therefore, the reduction in molecular weight of such polymers should be avoided by optimizing the culture time period.

4.7 Mechanical Properties (Tensile Strength, Young's Modulus, and Elongation at Break)

The principal mechanical properties of polymers that govern response of the polymer to mechanical forces are: tensile strength, Young's modulus, and percentage elongation. In this section, mechanical properties of several types of PHAs have been discussed. A comparative study between different types of PHAs (e.g., *scl*-PHA, *mcl*-PHA, and copolymer) and petroleum-derived polymer has also been summarized in Table 8, to understand the mechanical viability of PHA [117, 148, 149, 154]. From Table 8, it can be observed that for P(3HB-*co*-HHx) polymers, there is a sharp decrease in both tensile strength and Young' modulus. Therefore, it suggests that as the monomer fraction of HHx increases from 32 to 70 mol%, the polymer become more brittle and stiff. All the P(3HB-*co*-HHx) polymers have elongation at break up to 107% indicating a better elasticity compared to polyethylene and polypropylene. Based on the mechanical properties observed, it can be inferred that the P(3HB-*co*-HHx) polymers are gluey and sticky [149]. Similarly, the P(3HB-*co*-3HV) polymer possesses much better mechanical properties (in all the three cases) compared to the simple polymer like P(3HB), whereas it possesses better stiffness (i.e., Young's modulus) compared to polyethylene and polypropylene. The copolymer P(3HB-*co*-3HV-*co*-4HB) with 34% 3HV and 55% 4HB have similar stiffness compared to polypropylene and HDPE, whereas the copolymer with 3% 3HV and 93% 4HB have comparable elasticity like polypropylene, HDPE, and LDPE [154]. Zhao et al. study the mechanical properties of terpolyester such as P(3HB-*co*-3HV-*co*-3HHx), by varying the mol% of HV and HHx. They observed that with varying HHx concentration from 10 to 13%, the elongation at break varies from 277 to 481%, with a reduction in Young's modulus from 319 MPa to 110 MPa, respectively. Therefore, the introduction of HHx decreases the mechanical strength and improves the stiffness/flexibility of PHAs [148].

Table 8 Comparative study for mechanical properties of PHAs and petroleum-derived polymers

Polymer sample	Young's modulus (MPa)	Elongation at break (%)	Tensile strength (MPa)
P(3HB)	3.5	0.4–5	40–43
P(3HB-*co*-32% #HH_x)	101	856	8
P(3HB-*co*-43% #HH_x)	75	481	5
P(3HB-*co*-56% #HH_x)	12	368	1
P(3HB-*co*-60% #HH_x)	3	424	1
P(3HB-*co*-70% #HH_x)	1	1075	1
P(3HB-*co*-3% 3HV)	2900	–	38
P(3HB-*co*-9% 3HV)	1620	37	190
P(3HB-*co*-14% 3HV)	1500	35	150
P(3HB-*co*-20% 3HV)	1450	32	120
P(3HB-*co*-25% 3HV)	1370	30	70
P(3HB-*co*-40% 3HV-*co*-50% 4HB)	503	4	9
P(3HB-*co*-34% 3HV-*co*-55% 4HB)	618	3	10
P(3HB-*co*-23% 3HV-*co*-66% 4HB)	392	5	9
P(3HB-*co*-12% 3HV-*co*-76% 4HB)	142	9	4
P(3HB-*co*-6% 3HV-*co*-84% 4HB)	118	300	9
P(3HB-*co*-3% 3HV-*co*-93% 4HB)	127	430	14
P(3HB-*co*-5% 3HV-*co*-3HHx)	63	123	2
P(3HB-*co*-3HV-*co*-12% 3HHx)	135	108	5
P(3HB-*co*-1% 3HV-*co*-10.7% 3HHx)	319	277	10
P(3HB-*co*-2.4% 3HV-*co*-13.4% 3HHx)	110	481	8
P(3HB-*co*-5.4% 3HV-*co*-11.7% 3HHx)	291	340	16
Polypropylene	590	435	27
High-density polyethylene	640	576	19
Low-density polyethylene	50–156	126–700	13–79

5 Biodegradability of PHAs

The ultimate advantage of PHAs that distinguishes it from other biopolymers is due to its biodegradability and biocompatibility characteristics. These polymers can be degraded under both aerobic and anaerobic environments [155]. The final products after the degradation of PHA are carbon dioxide and water in aerobic environments, whereas the end products during anaerobic degradation are carbon dioxide, methane, and water [156]. Moreover, they can also be degraded via thermal degradation, enzymatic hydrolysis, using microbial depolymerases, and by enzymatic and non-enzymatic hydrolysis in animal tissues [155]. The biopolymer is made up of 100% organic and bio-based resources having multiple degradable options. The carbon cycle of biopolymers is shown in Fig. 5 to have a better understanding on life cycle of biopolymers [157]. The degradation period ranges from few months in anaerobic condition and up to few years in brackish water. However, the rate of degradation can be accelerated under the application of UV light. As reported by Lee et al., it takes 6, 75, and 350 weeks for PHB to degrade in anaerobic condition, soil, and saline water, respectively [158]. During the degradation process, the microorganisms develop extracellular PHA depolymerases resulting in the conversion of PHAs into water-soluble oligomers and monomers as a carbon source. Similarly, the PHA-producing microorganisms hold the capability to degrade the PHA intracellularly. The PHA depolymerase break the polymer to hydroxyalkanoic acid [159]. It is biocompatible showing no lethal effect on animals for biomedical applications [124]. There are some generalized concepts, i.e., physical and chemical properties on which the biodegradability nature of PHA depends. The rate of biodegradation decreases with increase in melting point, stereo-regularity, and crystallinity of the polymer, whereas low molecular weight PHAs are prone to biodegradation. Biodegradation

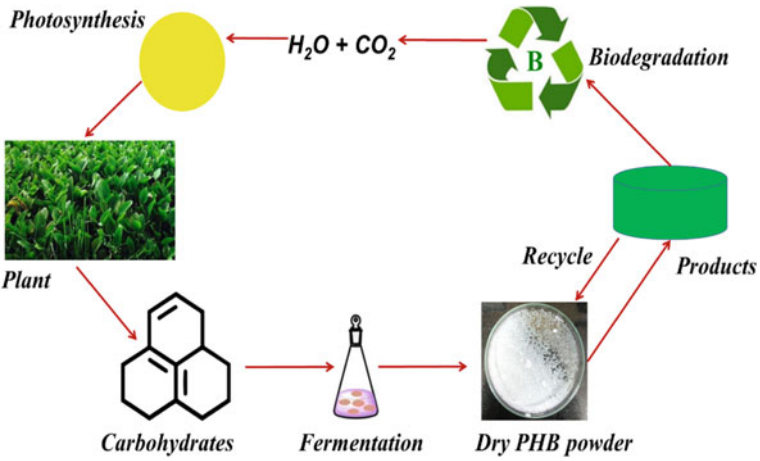


Fig. 5 Carbon cycle of biopolymers

of PHA depends on several factors such as temperature, pressure, moisture, surface area, pH, and microbial effect in disposal environment [156]. Mergaert et al. studied the effect of temperature on biodegradation of simple PHA and its copolymer [160]. They noticed that for PHB, the weight loss was observed at 40 °C, which is higher compared to 28 °C for P(3HB-*co*-20% 3HV), because of the increase in microbial activity. Similarly, at higher temperature the rate of degradation for P(3HB-*co*-3HV) was very fast compared to PHB. It has also been observed that, with an increase in HV content in the copolymer, the rate of degradation increases [160].

6 Application of PHAs

The applications of PHAs have attracted not only the researchers but also the industries because of its competitive physical properties such as mechanical properties, crystallinity, and melting point with polyethylene and polypropylene. In this section, applications of PHAs in medical, agricultural, and industrial sector are discussed.

6.1 Medical Sector

The PHAs having higher mechanical strength are extensively used for preparation of medical scaffold in the form of screws, pins, etc. These PHAs are durable, harmless and help in the stem cell growth, and cartilage repair [161]. The PHAs can be used for enhancing the mechanical strength of bone tissues by copolymerizing with hydroxyapatite. Scaffolds made from PHBV coated with collagen are used to repair injured spinal cord. The fabrication of fibers and tubes into P(3HB)-*co*-HHx is used for curing Achilles tendon injury observed in rats. Because of biodegradability characteristics, PHAs can be easily placed inside the body as a carrier for controlled drug release [162]. PHAs such as P(3HB) and P(HB-*co*-HV) are used for healing the wounds of domestic animals. Due to promising mechanical properties, P(4HB) can be used to prepare suture, clinical meshes, etc. In spite of several medical applications, preparation of pure PHAs through industrially viable method must be developed to increase the demand for PHAs in medical sector [163]. The poly(ester urethane) based on poly(3-hydroxyhexanoate-co-3-hydroxyoctanoate) (PHHxHO) in the synthesis is more hydrophobic and used for synthesis of wound remedial and hemostatic materials. The graft copolymers contains poly(methyl methacrylate) as backbone and PHB as side chain. These graft polymers are commercially used as orthopedic application in the form of acrylic bone cement [164]. Similarly, PHAs such as PHB and PHBV help in activation of blood enzymes. These phenomena are achieved by reducing the concentration of LPS endotoxins from 100 to 120 U/g to 20 U/g using bacteria such as *A. eutrophus* and a recombinant strain of *E. coli* [165]. The nonwoven PHB sheets are used in preparation of effective superficial conduits for treatment of peripheral nerve and spinal cord injuries. For diseases like hernias

and gastrointestinal tract, PHB is proposed for repairing the soft tissues. However, all the applications are verified on animals like rats. Similarly, P(3HB-*co*-3HV) membranes are used for treatment of jaw bone defects and in growing the height of the rat mandible [166]. PHAs have also shown potential applications in diagnostic and therapeutic disease treatments. 3-HB has been commercially used for the preparation of low-cost point-of-care device for treatment of diabetic ketoacidosis (DKA) to help hyperglycemic patients. Moreover, 3-HB monomer is useful to maintain blood levels for the epilepsy and neurodegenerative disorders [167]. Some of the PHAs are used for anti-microbial treatment, e.g., 3-hydroxy-n-phenylalkanoic acid is used to counter-attack the universal strains like *Listeria monocytogenes*, which has the ability to grow at both extremely high and low temperatures and low pH [168].

6.2 Agricultural Sector

The PHA nanocomposites are used as plastic mulch in the cultivated land to prevent the growth of weeds and suppress the evaporation from the soil. They act as a protective layer to preserve the vital ingredients in the soil. It not only reduces the labor cost but also helps in ecofriendly recycling process [169]. The PHAs act as bacteria inoculants to improve nitrogen fixation in plant kingdom using strains such as *Azospirillum*. The plant growth is observed to be very consistent (irrespective of carriers) having intracellular PHA with *Azospirillum brasilense* strain [155]. The strain shows a greater permanence to withstand UV radiation, heat, osmotic pressure, etc. PHAs help in applying control release fertilizers by embedding pesticides in PHA polymer (e.g., P(3HB-3HV)) matrix to control the pest activity in farmer's crop fields. The copolymer gradually degrades by bacteria, microscopic algae, and fungi. However, it is essential to optimize the polymer to pesticide ratio and regulate the application of pesticide to obtain best mechanism to prevent pest activity [14, 155]. PHAs can also be used to act as a career to prevent herbicidal action to destroy invasive weeds from soil. Loading the chemicals like Zellek Super on poly(3HB-*co*-3HV) as carrier is one of the most effective methods during tillering phase on plants. Release of such pesticides in a controlled way helps in diminishing its adverse effect on human health, environment, and ecology [155].

6.3 Industrial Sector

The P(3HB-*co*-3HV) is thermoplastic copolymer which is being used by BIOPOL® for preparation of coat papers and paper boards, electrical appliances packaging, fishing nets and ropes, and several types of containers for storage of shampoo, razors, and motor oil. Another biopolymer produced by Nodax™ company synthesizes copolymer using P(3HB) and small amount of *mcl*-PHA. These polymers are available in the forms of foams, fibers, latex, and films [155]. PHAs have been applied

for renewable biofuel production by hydrolysis of PHAs followed by esterification that produces 3-hydroxyalkanoates methyl esters. These biofuels have similar energy content compared to bioethanol. Because of high glass transition temperature (T_m), PHB has limited applications in packaging industries. However, the glass transition temperature can be increased by incorporating hydroxyvalerate, which extends its application in packaging industry [170]. PHAs are also being used as toners for printing purposes and adhesives for coating applications [171].

7 Challenges in PHAs Production

Because of similar properties like petroleum-derived polymers and biodegradability characteristics, PHAs have acquired much consideration toward several academia research groups and industries. However, many factors limit its production for several manufacturing industries. It is estimated that PHAs are approximately 15 times more expensive than the conventional polymers [23]. The cost of PHAs production increases due to the carbon source substrates used for microbial growth. The usage of pure carbon sources such as glucose, fructose, and xylose constitutes significant amount of production cost to PHAs as their price is increasing very fast. Therefore, extraction of carbon sources from activated sludge, industrial waste, and food waste (rice, whey, malt, etc.) would be helpful in reducing the substrate cost. Similarly, with the advancement of technologies, the production of petroleum resources such as crude oil and shale gas increases significantly, and hence, the production cost of petroleum-derived plastics is not going to increase drastically in near future [172]. Hence, lowering the PHA cost is still a crucial challenge for industries. As it can be observed from the above-mentioned sections, the structure and physical properties of different types of PHAs (i.e., *scl*-PHA, *mcl*-PHA, and copolymers) are not similar and lie in a wide range of values. Therefore, it affects the economic situation of PHA-producing industries.

Apart from the basic challenges mentioned above, there exist a few technical challenges associated with the production process. The major limitation is the determination of optimized growth conditions of bacteria and production of microbial cells. Lower fermentation time results in reduction of PHAs yield, whereas higher fermentation time results in degradation of physical properties (as mentioned in Sect. 4.7). The entire synthesis process is extremely tedious and time consuming (takes several weeks to complete), which includes inoculum preparation, fermentation growth, lyophilization, extraction of PHA, and drying. One apparent limitation of the bioprocessing industries includes operation through batch process or non-continuous process, and fed-batch systems during synthesis process. This results in consumption of fresh water and additional energy requirements for sterilization process [172]. The conversion of PHA from its carbon source is another vital challenge for synthesis of PHA. Conversion of the substrates to PHA varies from 10 to 89% depending on the type of strains and carbon sources used [4]; however, the conversion of polyethylene and polypropylene can be achieved close to 100% from its

monomer. It can be noted that the 89% conversion of substrate to PHA is extremely difficult to attain and can be achieved under certain rigorous conditions and only for few particular types of PHA. Another critical factor that affects the economics is the oxygen limitation during high cell density culture. Supplying enough oxygen to obtain aerobic condition can increase the running cost of PHA production [158, 173]. Since PHA is an intracellular polymer, the extraction method results in additional costs of production. The solvent extraction of PHA leads to the accumulation of harmful wastes in the environment causing disposal and recycling issues. Moreover, the extraction methods such as digestion method are very expensive [23], whereas mechanical methods have abundant energy requirements for breakage of cell wall for releasing the PHA polymer. Thus, combination of latest mechanical method like ultrasonication with the usage of reduced amount of solvent like chloroform could be an effective alternative in terms of energy requirements for extraction of intracellular polymers [128].

8 Conclusion

This chapter has attempted to present a comprehensive discussion on various facets of microbial production (both upstream and downstream) and characterization of the biodegradable polymer of PHA. In addition, different aspects of biodegradation of PHAs, applications of PHA in several sectors, and challenges in commercial scale PHA production have also been discussed. PHAs were first synthesized in 1923 and since then, particularly after 1959, the developments in the preparation of *scl*-PHA, *mcl*-PHA, and copolymers have been quite phenomenal. From the properties of PHAs, it can be inferred that PHAs are one of the potential alternatives to substitute the conventional petroleum-derived polymers (e.g., HDPE, LDPE, and polypropylene). Approximately 90 various bacterial genera and up to 300 microorganism species were found to be PHA producers in both aerobic and anaerobic conditions. Carbon substrates such as commercial sugars, starch, industrial and food waste, biomass, and wastewater help in production of PHAs, along with different microorganisms, having PHA content in a wide range of 10–89%. These biopolymers are intracellular in nature, and therefore, several extraction methods have been proposed in this chapter to obtain pure form of PHA. Characterization techniques such as NMR and FTIR help in detection of functional groups present in PHA after extraction process. ^{1}H NMR helps in detecting –CH, –CH_2, and –CH_3 functional groups, whereas ^{13}C NMR helps in detection of additional –C=O moiety in the form of chemical shifts. However, all the moieties can be observed in FTIR analysis by measuring absorbance as a function of wavenumbers. The crystalline nature of PHAs can be studied using various characterization techniques like XRD and DSC analytical tools. The crystallinity of PHA can be determined by measuring the area under the crystalline and amorphous region or using Scherrer's equation from XRD diffractogram. From DSC curve, the crystallinity can be measured from melting enthalpy of PHA samples. It has been observed that the PHAs are partially crystalline in nature

having crystallinity in the range of 34–53%. The properties such as melting point, crystallinity, tensile strength, and Young's modulus can be varied to a wide range by copolymerization of PHA depending upon the requirements and applications. The TGA and DTG analysis have shown that better thermal properties can be achieved by synthesizing copolymers of PHAs compared to simple PHB polymers. These polymers have excellent biodegradability and biocompatibility characteristics depending on several factors such as temperature, pressure, moisture, surface area, and pH of the disposal environment. They can be degraded in a few months to a few years in both aerobic and anaerobic conditions to carbon dioxide, methane, and water. However, cost of production, duration of fermentation process, and selection of ecofriendly extraction mechanisms are a few major challenges in commercial synthesis of PHA. It is anticipated that with advancement in basic research aimed at cheaper carbon sources and high yielding strains, commercialization of PHAs can be realized in near future.

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