Chapter 11
Polyhydroxyalkanoates Production from Renewable and Waste Materials Using Extremophiles/Recombinant Microbes

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Abbreviations

3HV	3-Hydroxyvalerate
3HPA	3-Hydroxypropionaldehyde
CDW	Cell dry weight
mcl	Medium-chain length
MMC	Mixed microbial culture
PHAs	Polyhydroxyalkanoates
PHB	Poly(3-hydroxybutyrate)
PHBV	Poly(3-hydroxyvalerate)
PHP	3-Hydroxypropionate
scl	Short-chain length
T_d	Thermodegradation temperature
T_g	Glass transition temperature
T_m	Melting temperature
VFA	Volatile fatty acids

What Will You Learn from This Chapter?

- Polyhydroxyalkanoates (PHAs) are biodegradable polyesters produced by various species of *Bacteria* and *Archaea* as reserves of energy and carbon in nutrient poor environments.

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- Being biodegradable and biocompatible, PHAs have found many industrial and medical applications as attractive bio-based alternatives to petroleum-based polymers.
- To compete with petroleum-based polymers and garner a bigger market share, cost-effective PHA production processes are needed.
- Therefore, renewable, cheap, sustainable, and readily available carbon sources from industrial wastes and agricultural by-products should be considered in PHA production.

11.1 Introduction

Since their discovery, petroleum-based plastics have turned out to be the most widely utilized materials in almost every area of daily life such as packaging, home appliances, electronic devices, and medicine. In accordance with the environmental concerns caused by discarded petrochemical plastics, increasing carbon dioxide emissions, global warming, and fluctuations of petroleum prices, development of eco-friendly biodegradable bio-based plastics from cheap, renewable resources is becoming increasingly important. In order to produce bioplastics with chemical and mechanical properties matching those of conventional plastics, microorganisms belonging to *Bacteria* and *Archaea* domains are employed.

Various monomeric building blocks such as lactic acid, bioethylene, *cis*-3,5-cyclohexadiene-1,2-diol, and 1,3-propanediol can be produced by microorganisms and used to synthesize polylactic acid, polyethylene, poly(*p*-phenylene), and poly (trimethylene terephthalate), respectively. Polyhydroxyalkanoates (PHAs) are linear aliphatic polyesters composed of hydroxycarboxylate monomers. They differ from these microbial originated biopolymers because their polymerization is conducted in vivo as cytoplasmic inclusions in *Bacteria* and *Archaea* under certain nutrient-deprived growth conditions (Chen 2010).

More than 300 bacterial and archaeal species have been found to synthesize PHAs consisting of approximately 150 different (*R*)-hydroxyalkanoic acid monomers (Arcos-Hernández et al. 2013). According to their chemical and physical properties, PHAs may be classified into two major groups: often stiff and brittle short-chain-length (scl) PHAs consisting of C3–C5 monomers and medium-chain-length (mcl) PHAs consisting of C6–C16 monomers which are elastomeric in nature (Fig. 11.1). Due to the stereospecificity of the enzymes involved in the biosynthesis, all of these monomers are in the *R*-configuration (Steinbüchel and Valentin 1995).

Their structural diversity and unique properties such as biodegradability and biocompatibility allow PHAs to be used in various applications, including packaging materials, biomedical implants, biofuels, carriers in drug delivery, and bioactive compounds (Tan et al. 2014).

Despite their great advantages, PHAs held only a relatively small fraction of the biopolymer market share because of their relatively high production cost and concurrent availability of low-cost petrochemical plastics. Since most of the cost

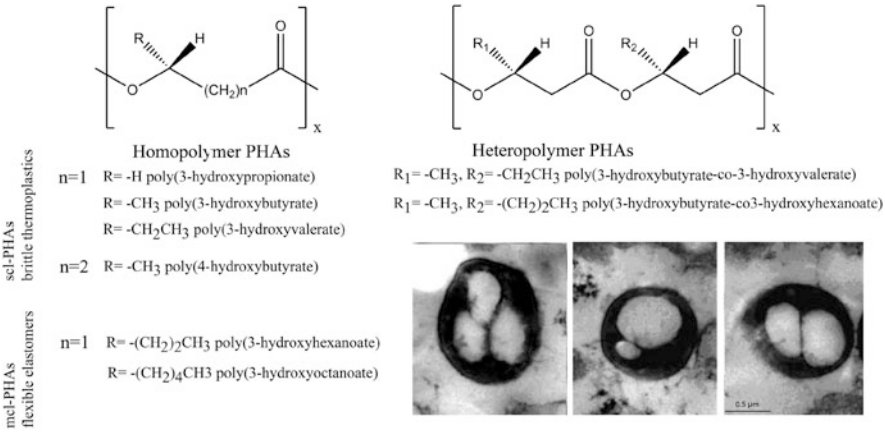

Fig. 11.1 General structure of common polyhydroxyalkanoates and transmission electron microscope pictures of PHBV granules in extremely halophilic archaea *Natrinema* 1KYS1 isolate

arises from the need for expensive carbon sources, industrial, agricultural, or municipal wastes may be used as feedstock for bacterial production of PHAs in order to overcome this obstacle (Anterrieu et al. 2014). Hence, in this chapter we focused on the biosynthesis and applications of PHAs from renewable resources and waste materials.

11.2 Biosynthesis and Applications of PHA

PHA polymers can be synthesized biologically or chemically; while obtaining materials with desired structures is easier with the latter approach, biosynthesis of PHAs via microorganisms has certain advantages. Biosynthetic PHAs generally have high molecular weights. Since they can be produced from sustainable resources, by-products of large industrial processes, and waste materials, they are accepted as eco-friendly (Chen 2010).

PHAs are synthesized by numerous organisms in the domains of *Bacteria*, *Archaea*, and *Eukarya* (fungi and animals including higher vertebrates). In *Bacteria* and *Archaea*, when essential nutrients such as nitrogen, oxygen, and phosphorus are limited and there is an excess carbon source present, PHA biosynthesis and accumulation as carbon and energy sources are favored (Chee et al. 2010). Molecular structure and properties of the synthesized PHAs are dependent on the particular bacterial and archaeal species in use, type of the feedstock, and substrate specificities of the enzymes in the biosynthetic pathways (Lu et al. 2009). Accumulation of PHAs by *Pseudomonas aeruginosa*, *Aeromonas caviae*, *Chelatococcus daeguensis* TAD1, *Lactobacillus reuteri*, *Azotobacter vinelandii* UWD, *Bacillus subtilis*, *Escherichia coli*, *Ralstonia eutropha*, and *Burkholderia cepacia* was investigated by researchers

(Koller et al. 2010; Insomphun et al. 2014; Cui et al. 2015; Linares-Pastén et al. 2015; Oh et al. 2015; Rodríguez-Contreras et al. 2015).

The most extensive studies were done for PHA synthesis in *Cupriavidus necator*, *Pseudomonas aeruginosa*, and *Pseudomonas putida*. More than eight different pathways for the synthesis of PHAs have been discovered to date in various organisms (Tan et al. 2014).

In addition to bacterial strains, extremely halophilic archaea have a remarkable capacity for accumulation of PHAs, which are used for production of biodegradable and biocompatible plastics (Quillaguamán et al. 2010). Poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) are the most familiar and analyzed PHAs (Tan et al. 2014). Researchers demonstrated that species belonging to genera *Haloferax*, *Halorubrum*, *Haloarcula*, *Halococcus*, *Halobacterium*, *Haloterrigena*, *Natronobacterium*, *Natrinema*, *Natronorubrum*, *Halostagnicola*, *Halobiforma*, *Halogeometricum*, and *Halalkalicoccus* produced PHAs (Table 11.1).

In addition to species listed in Table 11.1, *Halorubrum coriense* DSM 10284T (Legat et al. 2010), *Natronococcus occultus* DSM 3396T (Legat et al. 2010), *Halobiforma haloterrestris* (Hezayen et al. 2002), *Halopiger aswanensis* (Hezayen et al. 2010), *Halococcus hamelinensis* JCM 12892T (Legat et al. 2010), *Halococcus saccharolyticus* DSM 5350T (Legat et al. 2010), *Halococcus qingdaonensis* JCM 13587T (Legat et al. 2010), *Halorubrum chaoviator* DSM 19316T (Legat et al. 2010), *Halorhabdus utahensis* (Wainø et al. 2000), *Haloquadratum walsbyi* (Burns et al. 2007), *Halorhabdus tiamatea* (Antunes et al. 2008), and *Natrinema altunense* (Xu et al. 2005) have been recognized as PHA producers.

The first study on PHB accumulation by extremely halophilic archaebacterium (*Haloarcula marismortui* previously known as *Halobacterium* sp. from the Dead Sea) was carried out by Kirk and Ginzburg (1972). PHB accumulation of *Haloferax mediterranei* in the test medium containing glucose, yeast extract, and marine salts was subsequently observed by Fernandez-Castillo et al. (1986). Researchers found that PHB accumulation of this strain increased to 45% cell dry weight (CDW) in a medium with glucose, yeast extract, and low salt concentration. Lillo and Rodriguez-Valera (1990) discovered that high PHB accumulation of *Haloferax mediterranei* (60% CDW) could be obtained in the test media containing 2% (w/v) starch.

Although different species of halophilic archaea produce biopolyesters, most studies on PHAs were done using *Haloferax mediterranei*, which accumulates high quantities of PHBV. *Haloferax mediterranei* is a highly preferable source for inexpensive and easy production of high-quality PHA because of its stable genetic structure, rapid growth rate, minimum sterility requirement, growth in high saline waste materials, aseptic condition, broader substrate spectrum, and capacity for high-yield accumulation of PHA (Lillo and Rodriguez-Valera 1990; Koller et al. 2007; Koller 2015). It was emphasized that the other advantage of this strain was the production of PHA without antibiotic addition (Koller 2015).

Don et al. (2006) reported that PHA accumulated by *H. mediterranei* was PHBV. Koller et al. (2007) explained that *H. mediterranei* DSM 1411 accumulated 50% (CDW) poly-3-(hydroxybutyrate-co-8%-hydroxyvalerate) from hydrolyzed whey

Table 11.1 PHAs produced by extremely halophilic archaea

Extremely halophilic archaea	Carbon sources	Biopolymer	References
Haloferax mediterranei	Yeast extract/glucose	PHB	Fernandez-Castillo et al. (1986)
Haloferax volcanii	Yeast extract/glucose	PHB	Fernandez-Castillo et al. (1986)
Haloferax gibbonsii	Yeast extract/glucose	PHB	Fernandez-Castillo et al. (1986)
Haloarcula hispanica	Yeast extract/glucose	PHB	Fernandez-Castillo et al. (1986)
Haloferax mediterranei ATCC 33500	Starch	PHB	Lillo and Rodriguez-Valera (1990)
Haloferax mediterranei DSM 1411	Hydrolyzed whey	PHA	Koller et al. (2007)
Haloferax mediterranei DSM 1411T	Glucose	PHB/ PHBV	Legat et al. (2010)
Haloarcula hispanica DSM 4426T	Glucose	PHB/ PHBV	Legat et al. (2010)
Halococcus dombrowskii DSM 14522T	Yeast extract/hy-case	PHB/ PHBV	Legat et al. (2010)
Halococcus salifodinae DSM 8989T	Yeast extract/hy-case	PHB/ PHBV	Legat et al. (2010)
Halobacterium noricense DSM 9758T	Yeast extract/tryptone	PHB/ PHBV	Legat et al. (2010)
Natronobacterium gregoryi 2189T	Yeast extract/ casamino acids	PHB/ PHBV	Legat et al. (2010)
Haloarcula marismortui	Raw vinasse	PHB	Pramanik et al. (2012)
Haloarcula japonica T5	Molasses	PHB	Nicolaus et al. (1999)
Haloarcula marismortui ATCC 43049	Glucose	PHA	Han et al. (2007)
Halalkalicoccus tibetensis CGMCC 1.3240	Glucose	PHA	Han et al. (2010)
Haloferax mediterranei CGMCC 1.2087	Glucose	PHA	Han et al. (2010)
Haloarcula amylolytica 26–3	Glucose	PHA	Han et al. (2010)
Haloarcula argentinensis CGMCC 1.7094	Glucose	PHA	Han et al. (2010)
Halobacterium cutirubrum CGMCC 1.1962	Glycerol	PHA	Han et al. (2010)
Halobiforma nitratireducens CGMCC 1.1980	Fructose	PHA	Han et al. (2010)
Halobacterium halobium PM CGMCC 1.1952	Glycerol	PHA	Han et al. (2010)
Halococcus morrhuae CGMCC 1.2153	Glucose	PHA	Han et al. (2010)
Haloferax gibbonsii CGMCC 1.2148	Glucose	PHA	Han et al. (2010)
Halorubrum litoreum 12–2	Glucose	PHA	Han et al. (2010)

(continued)

Table 11.1 (continued)

Extremely halophilic archaea	Carbon sources	Biopolymer	References
Halorubrum trapanicum CGMCC 1.2201	Glucose	PHA	Han et al. (2010)
Halostagnicola larsenii 24–25	Glucose	PHA	Han et al. (2010)
Haloterrigena turkmenica CGMCC 1.2364	Glucose	PHA	Han et al. (2010)
Haloterrigena hispanica	Carrot waste	PHB	Di Donato et al. (2011)
Natrinema altunense CGMCC 1.3731	Glucose	PHA	Han et al. (2010)
Natrinema pallidum JCM 8980	Glucose	PHA	Han et al. (2010)
Natrinema pellirubrum JCM 10476	Glucose	PHA	Han et al. (2010)
Natronobacterium gregoryi CGMCC 1.1967	Glucose	PHA	Han et al. (2010)
Natronorubrum tibetense CGMCC 1.2123	Glucose	PHA	Han et al. (2010)
Natrinema sp. XA3–1	Glucose	PHA	Han et al. (2010)
Halogeometricum borinquense strain TN9	Glucose	PHA	Salgaonkar et al. (2013)
Natrinema pallidum (KYS1)	Starch	PHA	Danis et al. (2015)
Haloferax mediterranei ATCC 33500	Extruded rice bran and corn starch	PHA	Huang et al. (2006)

PHA polyhydroxyalkanoates, *PHB* poly (3-hydroxybutyrate), *PHBV* poly (3-hydroxybutyrate-co-3-hydroxyvalerate)

without addition of 3-hydroxyvalerate (3HV) precursors (Koller et al. 2007). Han et al. (2007) found that *Haloarcula marismortui* accumulated 21% (CDW) PHB in the test medium containing 2% glucose. Moreover, investigators examined the PHA accumulation of 28 haloarchaeal strains belonging to genera of *Halalkalicoccus*, *Halobacterium*, *Haloarcula*, *Halobiforma*, *Halostagnicola*, *Haloterrigena*, *Halococcus*, *Halorubrum*, *Haloferax*, *Natrinema*, *Natronococcus*, *Natronobacterium*, *Natronomonas*, *Natronorubrum*, and *Natrialba* in the test media containing, separately, different carbon sources such as glucose, glycerol, fructose, and acetate (Table 11.1). Eighteen of the twenty-eight tested strains accumulated PHA at levels in the range of 0.8% to 22.9% (w/w) of CDW (Han et al. 2010), and the highest PHA production (22.9% (w/w) of CDW) was detected in *Natrinema pallidum* JCM 8980 grown in glucose medium (Han et al. 2010).

Previous experiments clearly show that extremely halophilic archaeal species may be potentially important resources for industrial production of PHAs. Production of PHA by halophilic archaea may have several advantages. As it is known, extremely halophilic archaea grow in high-salt concentrations (2.0–5.2 M) wherein other non-halophiles cannot survive (Lillo and Rodriguez-Valera 1990). PHA produced from nonpathogenic haloarchaeal species reduces the risk of microbial contamination during cultivation (Koller et al. 2007; Quillaguamán et al. 2010). Researchers suggested that a production system such as open pond, which is used

for sewage treatment, may be used for PHA production of haloarchaeal strains (Lillo and Rodriguez-Valera 1990). These microorganisms can be continuously grown in unsterile conditions without risk of contamination (Yin et al. 2015). Due to low microbial contamination during cultivation, energy can be very efficiently conserved as sterility is irrelevant (Koller et al. 2007). Industrial and household waste products or cheap materials can be used as carbon sources by extremely halophilic archaea to produce PHA (Koller et al. 2007; Pramanik et al. 2012; Danis et al. 2015). Using industrial waste for PHA production is also a promising solution to the waste disposal problem and its huge expense (Koller et al. 2007). Hence, production of PHA from extremely halophilic archaea may be implemented for cost-effectiveness. Furthermore, high-quality polyesters are derived from some strains of halophilic archaea (Koller et al. 2007). Isolation of PHA from extremely halophilic *Archaea* is easier than that of *Bacteria* (Koller et al. 2007) because of disintegration of haloarchaeal cells in water (Ventosa and Nieto 1995). Additionally, investigators mentioned that genome sequence of *H. mediterranei* was determined, and biotechnological applications could be applied for PHA accumulation (Bhattacharyya et al. 2015).

11.2.1 Biochemistry of PHA Synthesis

The key components for the biosynthesis of PHA are acetyl-CoA produced from carbohydrates and acyl-CoA generated by the β-oxidation of fatty acids. For the synthesis of scl-PHA, many bacterial and some archaeal species use a three-step reaction: production of acetoacetyl-CoA from two molecules of acetyl-CoA by the activity of β-ketothiolase, reduction of the product to 3-hydroxybutyrl-CoA by NADPH-dependent acetoacetyl-CoA reductase, and finally polymerization of PHB catalyzed by the action of PHA synthase. 3-Hydroxyvaleryl-CoA is synthesized by the reaction of acetyl-CoA and propionyl-CoA and used for polymerization of PHBV (Shirastav et al. 2013). Mcl-PHAs or copolymers of scl and mcl PHAs may be synthesized from the acyl-CoA produced by the β-oxidation of fatty acids in certain bacteria. Bacteria such as *Pseudomonas aeruginosa* and *Aeromonas caviae* have PHA synthase enzymes with broad substrate specificity; therefore, they can accumulate PHAs with different monomer lengths (C6–C12) (Insomphun et al. 2014). In another pathway, the production of mcl PHAs is achieved by the polymerization of (*R*)-3-hydroxyacyl intermediates of de novo fatty acid biosynthesis from structurally unrelated substrates such as glycerol, glucose, and sucrose (Philip et al. 2007). This pathway is especially important for the utilization of industrial and agricultural by-products and wastes for the synthesis of PHAs.

PHA biosynthesis is regulated by both high level of NAD(P)H and the ratio of NAD(P)H to $NAD(P)^+$. β-ketothiolase, the first enzyme of the PHA synthesis, is inhibited by the high intracellular CoA levels. However, when a nutrient is limited, $NADH/NAD^+$ ratio increases, and subsequently, as a result of the inhibition of the

enzymes of TCA cycle, CoA levels fall. This phenomenon in turn cancels the inhibition of β-ketothiolase (Shirastav et al. 2013).

11.2.2 Chemical and Mechanical Properties of PHA

Since PHAs are semicrystalline in structure, their thermal and mechanical properties are generally defined with their melting temperatures (T_m), glass transition temperatures (T_g), and thermodegradation temperatures (T_d) (Anderson and Dawes 1990). Due to their diverse structural varieties, PHAs have variable T_m, T_g, and T_d within these respective ranges: non-observable to 177 °C, −52 to 4 °C, and 227 to 256 °C (Tan et al. 2014). They also possess a varying degree of Young's modulus, elongation at break, and tensile strength values within these respective ranges: 0.008 to 3.5×10^3 MPa, 2% to 1000%, and 8.8 to 104 MPa (Chen 2010; Tan et al. 2014).

Due to the stereoregular structure of PHB, it has a high degree of crystallinity, and therefore its use as a polymeric material is limited. In order to overcome this mechanical weakness, researchers usually employed chemical modifications such as copolymerization with alternative PHA monomers or blending with other polymers. Incorporation of PHB with 3HV resulted in the production of PHBV, which has more suitable physical properties—lower T_m, less stiffness, and increased toughness—for commercial applications (Anderson and Dawes 1990).

Mcl-PHAs such as 3-hydroxyoctanoate and 3-hydroxydecanoate have significantly lower T_m and crystallinity. These qualities make them more elastomeric in structure when compared to scl-PHAs and, therefore, broaden the range of applications for PHA (Davis et al. 2013).

11.2.3 Biodegradability and Biocompatibility of PHA

Most of the plastics produced in each year are used in a short period of time and usually discarded, either to be recycled, incarcerated, or sent to landfill. One of the unique advantages of biological PHA materials over traditional petroleum-based polymers is their biodegradability in various natural environments such as soil and water by a number of bacteria and fungi (Shirastav et al. 2013). The rate of biodegradability depends on the various properties of the environment such as temperature, pH, and microbial population and composition of PHA.

Since PHB and its degradation product 3-hydroxybutyrate naturally exist in the human body as a normal blood constituent, PHB and other PHAs in general considered as biocompatible, which means they are not toxic and their existence does not elicit an immunological tissue response (Yang et al. 2014).

11.2.4 Applications of PHA

Just like conventional petroleum-based plastics, PHAs can be used in a broad range of applications. Furthermore, because of their biodegradability, biocompatibility, and other superior properties, they are attractive candidates to replace petrochemicals (Chee et al. 2010).

In accordance with the growing environmental awareness and demands from the consumers for the use of sustainable and eco-friendly materials in packaging, bioplastics, especially PHAs, are attracting renewed attention. PHAs are utilized as shopping bags, personal hygiene products, and disposable tableware (Chen 2010).

PHAs, due to their biocompatibility and biodegradability, have more expedient attributes when compared to conventional petroleum-based polymers with biomedical applications. PHAs have been used to develop devices including cardiovascular and nerve repair devices, sutures, bone plates, stents, bone marrow scaffolds, skin substitutes, wound dressings, and other medical purposes (Koller et al. 2010).

One important application area of PHAs is as controlled drug carriers. By inserting biodegradable PHAs impregnated with a drug into tissue, it is possible to achieve that drug's controlled release over a period of time. The use of PHA-based vehicles for the delivery of drugs such as anticancer agents, anesthetics, and antibiotics has been previously reported (Bonartsev et al. 2007). Our group reported the use of PHBV films as a carrier for the antituberculosis drug rifampicin (Danis et al. 2015).

PHA monomers also find application area as drugs themselves. 3-Hydroxybutyrate and its derivatives were found to be effective in decreasing cell apoptosis of mouse glial cells (Xiao et al. 2007). O'Connor et al. (2013) describe the purification of 3-hydroxydodecanoic acid, and they demonstrated that upon conjugation, it improved the anti-proliferation activity of peptide DP18L. Moreover, PHAs are also used in agriculture as controlled release vehicles for substances such as insecticides (Philip et al. 2007).

11.3 The Importance of Reutilization of Renewable and Waste Materials

Bio-based and biodegradable polymers are viable replacements for petroleum-derived plastics (Steinbüchel and Valentin 1995; Braunegg et al. 1998). As mentioned before, PHAs are produced by a variety of prokaryotic strains from such sources as starch, glucose, sucrose, lipids, vegetable oils, alcohols (e.g., glycerol), and organic acids when a nutrient is insufficient in the growth medium. Usage of edible raw materials is not economically profitable; starch, which is the most used raw material for industrial PHA production, is relatively expensive.

Today, despite the numerous advantages of using biodegradable plastics and interest in biotechnological production of PHAs from renewable resources, PHA production has made notable progress as a profitable enterprise (Ciesielski et al. 2015).

The high production cost of PHAs is primarily due to the significant reliance on carbon substrate. Efficient bacterial strains, fermentation, and recovery processes have been developed in a number of studies, but low-cost, waste-based substrates have only recently been employed for PHAs production (Koller et al. 2007; Han et al. 2010). Thus, the selection of proper carbon substrates is very demanding and influences the overall performance of the bacterial fermentation and the cost of PHA. Hence, for large-scale production of PHAs and microbial growth, the selection of renewable, cheap, and most readily available carbon sources should be considered as potential renewable feedstock (Chee et al. 2010). The cost of complex nitrogen nutrients is another financial consideration in phosphate-limited biosynthesis of PHAs. Both cheap carbon and nitrogen sources can be extracted from many sorts of industrial and agricultural wastes. Utilizing these waste materials for PHA production as a carbon source does not only reduces the substrate cost but also reduces the expense of waste disposal and cuts the pollution load (Braunegg et al. 1998).

Waste streams can be grouped as waste streams from biofuel production, surplus whey from the dairy industry, wastes from sugar industry (molasses and bagasses), lignocellulosic materials and their waste by-products, and municipal solid and wastewater treatment plants.

11.3.1 Glycerol from Biofuel Production

Crude glycerol is generated in large quantities from the transesterification of vegetable oils as a coproduct mainly from biodiesel plants. With the increase in production of global biodiesel, large quantities of glycerol are discharged. It is very costly to refine the crude glycerol to a pharmaceutical grade for use in foods, pharmaceuticals, and cosmetics. Many methods for disposal and utilization of this crude glycerol have been attempted (Koller et al. 2005). One of the innovative utilization of crude glycerol is PHA production. Different species of microorganisms metabolize glycerol as a sole carbon and energy source due to the plentiful occurrence of glycerol in nature (Posada et al. 2011). Cui et al. (2015) showed that using glycerol at low concentrations as the only carbon source, with the addition of mixed nitrate (NH_4Cl, yeast extract, and tryptone), stimulated PHB accumulation in thermophilic *C. daeguensis* TAD1, while excess glycerol inhibited PHB accumulation.

Working with cells of native *Lactobacillus reuteri* in an early stage, Linares-Pastén et al. (2015) designed a production system for the conversion of glycerol to 3-hydroxypropionaldehyde (3HPA) by transformation of the 3HPA to poly (3-hydroxypropionate) (PHP) using recombinant *Escherichia coli* strain co-expressing highly active coenzyme A-acylating propionaldehyde dehydrogenase

from *L. reuteri* and polyhydroxyalkanoate synthase (PhaCs) from *Chromobacterium* sp. PHP content was found up to 40% CDW using crude glycerol as a waste substrate together with organic matter in the form of nonvolatile fatty acids. Moita et al. (2014) investigated the feasibility of manufacturing PHA production by a mixed microbial culture (MMC) capable of consuming both glycerol and methanol present in the crude. The aerobic mixed culture generated a maximum PHB content of 47% (CDW) and a productivity of 0.27 g PHB/Ld. A moderate halophile *Yangia* sp. ND199, isolated from mangrove soil sample in Vietnam, was found to accumulate PHBV when cultivated in a medium containing 4.5% (w/v) NaCl, with glycerol as carbon and yeast extract as nitrogen source. The content of value-added polymer PHBV and productivity were found as 40.6% (CDW) and 0.25 g/Lh, respectively (Van-Thuoc et al. 2015). *Cupriavidus necator* and *Burkholderia sacchari* were used by Rodríguez-Contreras et al. (2015) to produce value-added PHB biopolymers with low molecular masses. When glycerol was used together with glucose in fermentation with *C. necator*, high cell dry mass and growth rate were obtained. However *B. sacchari* used only glycerol as a sole carbon source and accumulated low-molecular-weight PHB.

Hermann-Krauss et al. (2013) compared the accumulation of PHA co- and terpolyesters of *Haloferax mediterranei* from inexpensive crude glycerol phase (1%, w/v) obtained from biodiesel production and from pure glycerol (1%, w/v). When the pure glycerol and crude glycerol phase were used, 13.4% and 16.2% (g/L) PHA accumulated, respectively, in the test strain.

11.3.2 Crude and Waste Plant Oils and Oil Mill Effluents

Bacterial polyesters from crude and waste plant oils, which can be difficult to dispose of, can be recovered and used as disposable crude, and waste plant oils can be used to grow bacterial polyesters (Ciesielski et al. 2015).

Acidic oil cake of *Calophyllum inophyllum* which is nonedible was examined under dark and photo fermentation conditions by using a coculture composed of a dark fermentative (*E. aerogenes*) and a photo fermentative (*R. sphaeroides*) bacteria for biohydrogen and PHA production. With the use of a minimal salt media and alternate dark-photo fermentative periods, cost of production has reduced (Arumugam et al. 2014). Mozejko and Ciesielski (2013) investigated the synthesis of mcl-PHAs from *Pseudomonas* sp. Gl01 cultivated in a biofermentor containing saponified waste palm oil as the only carbon source. Martino et al. (2014) used cooking oil as the only carbon source for production of PHB by *C. necator* DSM 428. The resultant biomass was used for extraction of the PHB granules with a solvent-free approach using sodium dodecyl sulfate, ethylenediaminetetraacetic acid, and the enzyme alcalase in an aqueous medium. In another study, *C. necator* DSM 7237 strain was grown in crude glycerol, sunflower meal hydrolysates, and levulinic acid as the only fermentation feedstock in a bioreactor. Levulinic acid

could be combined with biodiesel industry by-products for the generation of high content, industrially useful PHB and PHBV (Kachrimanidou et al. 2014).

Taking into account of their higher volatile fatty acids (VFA) concentration, fermented olive oil effluents might be a potential source for PHA production (Dionisi et al. 2005). The feasibility of producing PHAs by feeding a pure culture of *C. necator* with dephenolized and fermented olive mill wastewater as the carbon source for PHAs generation was demonstrated at shaken-flask scale (Martinez et al. 2015). Alpecin, which is an effluent of olive oil wastewater, is a harmful environmental contaminant due to its considerably high phenolic content concentration. *Pseudomonas putida* KT2442, containing plasmid harboring *C. necator*, grew in high concentrations of alpecin and accumulated considerable amounts of PHA (Ribera et al. 2001). In another study, researchers demonstrated that *A. chroococcum* H23 was capable of accumulating substantial amounts of PHA in the media with high alpecin content (Koller et al. 2010).

11.3.3 Surplus Whey from the Dairy Industry

Whey is a by-product of cheese or casein manufacture which is regarded as a waste and surplus material. It constitutes about 80–90% of the volume of processed milk. The amount of whey that is produced globally is about 120 million tons per year. Roughly half of all whey is turned into whey beverages, additives for food processing (e.g., meat products and ice cream), and animal feed, and the remainder is disposed of as waste material (Koller et al. 2010).

Lactose in whey when discharged into the environment becomes a hazard because of its high biochemical oxygen demand. Hence, many studies have aimed to recover and find suitable uses for lactose (Braunegg et al. 2007; Koller et al. 2007; Nikodinovic-Runic et al. 2013; Koller 2015). While the higher-grade lactose obtained from whey is used in infant formulae and as an excipient for pharmaceutical products, the amounts of purified lactose required for these uses make up only 5–10% of what can be potentially derived (Nikodinovic-Runic et al. 2013). While the direct application of recovered lactose may not be possible for all bacteria strains of interest, one technique is to hydrolyze lactose into glucose and galactose, each of which is readily and efficiently fermented. The glycosidic bond can be enzymatically hydrolyzed by β-galactosidase or by acid hydrolysis. Fermentations using concentrated, high lactose content whey solution and recombinant *E. coli* CGSC 4401 having *Alcaligenes* PHA synthase genes were performed in laboratory-scale bioreactors, reaching a final PHB concentration of 96.2 g/L (Nikodinovic-Runic et al. 2013). Wong and Lee (1998) also reported 50 g/L PHA with *E. coli* CGSC 6576 containing plasmids of *C. necator* PHA synthase genes.

The potential of various microorganisms to convert whey lactose to PHAs has been studied by Koller et al. (2007) with *H. mediterranei*, *Hydrogenophaga pseudoflava*, and *Pseudomonas hydrogenovora*, as industrial scale PHAs producers from the hydrolyzed whey feedstock. Among the strains only *H. mediterranei* has

significantly accumulated (50%, CDW) of PHBV from hydrolyzed whey without necessity of expensive propionic or valeric acids and 3-hydroxyvalerate (3HV) precursors.

Koller (2015) examined the reutilization of waste fermentation broth, obtained from previous PHA production of *H. mediterranei* in whey, for a second PHA production using the same microorganism. It was observed that the waste fermentation broth may be used instead of fresh saline fermentation medium to produce PHA. However, he mentioned that 29% of yeast extract can be substituted by cell debris from the previous fermentation broth.

11.3.4 Wastes from the Sugar Industry

Molasses is a by-product of sugarcane or sugar beet processing containing high amount of sugar and has been commonly used as a carbon source in industrial-scale fermentations due to its low price and abundance (Du et al. 2012). PHA production was reported in 1992 by *Azotobacter vinelandii* UWD using sugar beet molasses. *Bacillus* sp. JMa5 which was isolated from molasses contaminated soil was osmotolerant and able to grow at high temperatures. This strain produced 70 g/L PHB (25–35%, CDW) using sugar beet molasses (Wu et al. 2001). In another study, *Bacillus subtilis* and *E. coli* isolated from industrial contaminated soil samples were grown on cane molasses yielding a maximum PHA production of 54.1% and 47.16%, respectively (Gomaa 2014). Albuquerque et al. (2007) developed a three-stage PHA production process from sugarcane molasses using MMC. This culture reached a maximum PHA content of 74.6%. In a Brazilian company manufacturing sucrose and ethanol from sugarcane, economically competitive PHA was produced by *C. necator* DSM 545. This process employed the factory's two main waste streams: bagasse and fusel alcohols. Bagasse was used for energy generation, while fusel alcohols (mainly isopentyl alcohol), which are less harmful than chloroform, are used for the PHA extraction (Koller et al. 2010).

11.3.5 Lignocellulosic Wastes

Lignocellulosic material, containing lignin, cellulosic, and hemicellulosic fibers, constitutes the most abundant renewable resource on the earth. With the development of optimization methods for the bioconversion of cellulose and hemicellulose into microbially usable carbon sources such as monosaccharides, it will be possible to use lignocellulose and cellulose wastes for the production of high-value biopolymers and other materials (Braunegg et al. 1998).

Davis et al. (2013) investigated the potential of delignified and hydrolyzed grass biomass as a feedstock for *Pseudomonas* strains such as *P. putida* W619, *P. putida* KT2440, and *P. fluorescens* 555. Tested strains accumulated 20–34% of CDW.

Cesário et al. (2014) utilized wheat straw hydrolysates and *B. sacchari* cells accumulated PHB (70%, CDW). The PHB volumetric productivities attained were the highest ever achieved on agricultural waste hydrolysates. Extruded rice bran and extruded cornstarch (1:8 w/w) were utilized as carbon sources to produce PHA by *H. mediterranei*, and PHA amount was found as 77.8 g/L (Huang et al. 2006). Researchers examined the usage of vegetable wastes as growth media for extremophilic microorganisms that produce biopolymers. They found that biomass fermentation provides a cheaper way to produce PHA (Di Donato et al. 2011). The *Haloterrigena hispanica* strain FP1 cells grown on carrot wastes as sole carbon source were able to produce a comparable amount of PHB (0.13%, CDW) with respect to that produced when cells were cultivated on complex standard media (0.14%, CDW). This result suggests an alternative and low environmental impacting method for vegetable waste management (Poli et al. 2011). In our previous study, whey, melon, apple, and tomato wastes and sucrose as carbon source were evaluated on PHA production from Natrinema 1KYS1 (Danis et al. 2015), and the strain was able to grow and produced 19.9%, 10.5%, 3.1% and 12.1% of CDW PHBV, respectively.

Wine lees, pretreated with crude enzymes of *Aspergillus oryzae*, were converted into a fermentation nutrient for the strain *C. necator* DSM 7237. This process maintained 30.1 g/L of PHB concentration (71.3%, CDW) and a productivity of 0.56 g/Lh during fed-batch fermentation (Dimou et al. 2015). The synthesis of PHAs by activated sludge with aerobic dynamic feeding process was conducted in a sequencing batch reactor by using food wastes and excess sludge fermentation liquid as the carbon source (Zhang et al. 2014).

Hardwood spent sulfite liquor, a complex feedstock originating from the pulp industry, was tested as a substrate for a MMC identified as α-(72.7%), β-(11.1%), and γ-proteobacteria (10.3%), and maximum PHA content of 67.6% CDW was achieved under aerobic dynamic feeding conditions (Queirós et al. 2014).

H. mediterranei, with its supreme advantages mentioned before, can utilize waste stillage from the rice-based ethanol industry for PHA production. This strain accumulated PHBV (63%, CDW) in the simple plug-flow reactor configuration of the activated sludge process (Bhattacharyya et al. 2015).

Rice bran treatment process for the production of hydrolysate solution containing 24.41 g/L of glucose and small amount of fructose was developed and used to produce PHAs. Recombinant *E. coli* expressing *R. eutropha* phaCAB genes and *R. eutropha* were found to produce PHB with the polymer contents of 90.1% and 97.2% of CDW, respectively (Oh et al. 2015). Utilization of hydrolyzed hemicellulose and cellulose fractions of bagasse as carbon source for PHA production was examined using *B. cepacia* and *Burkholderia sacchari* IPT 101 on a laboratory scale (Silva et al. 2004). Promising results were reported for the latter; accumulation of 62% PHA CDW was accomplished. Production of PHA by *Haloarcula marismortui* from vinasse, which is a waste of the ethanol industry, was evaluated. This microorganism accumulated PHB (23%, CDW) in the test medium containing raw vinasse (10%, v/v) (Pramanik et al. 2012).

11.3.6 Municipal Wastes

Municipal waste consists of waste originated from households, offices, and various sources and collected by municipal authorities. Especially in the developing countries, municipal wastes are not well managed and pose a serious threat to the environment. The use of municipal waste as a resource could be useful to cut greenhouse gas emissions (Nikodinovic-Runic et al. 2013). Waste-based PHA production by bacterial enrichments generally follows a strategy in which the wastewater is converted into a volatile fatty acid rich stream. A non-fermented substrate was studied by Moralejo-Gárate et al. (2014), supporting the development of pure culture-based PHA production as a replacement for bacterial enrichment. Pittmann and Steinmetz (2014) investigated the production of PHAs as a side stream process on a municipal wastewater treatment plant. He also studied the effect of substrate concentration, pH, temperature, and cycle of installed feast/famine regime on PHA production. High PHA production up to 28.4% of CDW was maintained with optimal of lower substrate concentration, neutral pH value, 20 °C, and a 24 h cycle time.

The biosynthesis of PHB directly from carbon dioxide (CO_2) is a sustainable alternative for nonrenewable, petroleum-based polymer production. The conversion of CO_2 implies a reduction of greenhouse gas emissions. Through an autotrophic conversion, hydrogen-oxidizing bacteria such as *C. necator* have the ability to store PHB using CO_2 as a carbon source. A mathematical model based on mass balances was simulated taking into account the stoichiometry and kinetics of biomass growth and PHB formation, as well as physical transfer from the gas phase to the liquid fermentation broth. The developed model was calibrated and validated for *C. necator* based on independent experimental datasets from literature. The obtained simulation results accurately described the dynamics of autotrophic biomass growth and PHB production. The effect of oxygen (O_2) and/or nitrogen stress conditions, as well as the effect of the gas mixture composition in terms of O_2 and hydrogen, was investigated through scenario analysis (Mozumder et al. 2015).

11.4 Concluding Remarks and Future Perspectives

With the increasing demand for healthy and natural products and awareness of environmental issues, biopolymers have emerged as attractive candidates. Being both biocompatible and biodegradable, PHAs are the most promising and most extensively studied biopolymers. Besides these excellent properties, it is possible to produce various PHA formulations with distinct physical and chemical properties for a variety of applications. However, the expensive carbon sources for the bio-based PHA production and the lack of efficient large-scale processes make PHAs uncompetitive with petroleum-based plastics. PHAs therefore have a limited

market share. In order to reduce the need for expensive carbon sources which constitute the major part of the production cost, it is possible to use waste-based substrates. This decision also has a positive impact in so far as it reduces environmental pollution and carbon dioxide emissions.

The production cost is remarkably reduced in processes in which halophilic archaea are used. Expenses for sterile conditions are not needed, and expensive organic solvents are not utilized for PHA extraction. Also, for the production of monomers like 3-hydroxyvalerate from bacterial sources, addition of substrates such as propionate is required, but archaeal strains can produce this monomer solely from waste materials.

Future work should focus on discovering new PHA-producing microorganisms as well as improving the production processes depending on minimum energy and solvent requirements.

Take-Home Messages

- Polyhydroxyalkanoates are produced by more than 300 microorganisms including various species of *Bacteria* and *Archaea* for carbon and energy storage in an environment that is carbon-rich but poor in certain nutrients such as phosphorus and nitrogen.
- More than eight different pathways for the synthesis of PHAs have been discovered to date in various organisms; however, many species use a three-step reaction: production of acetoacetyl-CoA, its reduction to 3-hydroxybutyrl-CoA, and finally polymerization of PHB.
- Due to their diverse structural varieties, PHAs have variable chemical and mechanical properties such as glass transition temperatures, thermodegradation temperatures, Young's modulus, and tensile strength.
- Being biocompatible and biodegradable, PHAs have superior attributes when compared to conventional petroleum-based polymers in biomedical applications such as cardiovascular repair devices, sutures, and skin substitutes.
- PHA production cost is not compatible to petroleum-based polymers, primarily due to the significant reliance on expensive carbon substrates.
- It is critically important to develop cost-effective and technically performing PHAs by utilizing renewable and waste materials to meet the market demand for green polymers.

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