Polyhydroxyalkanoates (PHAs) in Industrial Applications

116

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Contents

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Abstract

Polyhydroxyalcanoates (PHAs) are biodegradable polyesters produced by many bacteria that accumulate them as intracellular storage material in the cytoplasm. These polymers are potential candidate for substitution of petrochemical non-renewable plastics for their biodegradable and nontoxic properties.

Polyhydroxyalkanoate (PHA) can be synthesized by different strategies, such as microbial production by wild type or recombinant microorganisms, in vitro production via PHA synthase-mediated catalysis, or using genetically engineered plants.

PHA accumulation in natural strains is favored by high availability of carbon source and a limited amount of macrocomponents (nitrogen, phosphate, oxygen) or microcomponents (sulfate, magnesium ions, and other trace elements).

PHAs are applied in many fields, such as packaging, medicine, or agriculture, but the extensive application of the bioplastics is constrained by high production costs, especially for raw material, downstream processing, and polymer recovery.

In this chapter, the progresses in production of PHA in natural strains and in engineered *E. coli, Pseudomonas spp., Bacillus, Aeromonas*, and other bacteria, such as the halotolerant *Halomonas* spp., are presented.

In addition, the constrains on purification steps and the potential of high value applications are presented.

Abbreviations

PHBHHx	poly-3-hydroxybutyrate-co-hydroxyhexanoate
P(3/4HB)	poly(3-hydroxybutyrate-co-4-hydroxybutyrate)
PHB-co-HV	poly-3-hydroxybutyrate-co-3-hydroxyvalerate
PHBHV	poly(3-hydroxybutyrate-cohydroxyvalerate)

Introduction

Polyhydroxyalkanoates (PHAs) are biodegradable polyesters produced by bacteria such as *Cupriavidus necator* (*Ralstonia eutropha*), as well as other Gram-negative and Gram-positive bacteria. PHAs is accumulated in response to stress conditions. The carbon/nitrogen (C:N) ratio may be determinant for some species, while other bacterial species have growth-associated PHA production which is independent of C:N ratio. Poly(3-hydroxybutyrate) (PHB) is the prototype polymer for biodegradable polymers, appearing a tough, brittle plastic-like material, with good toughness and stiffness, which may be made more fluid by the addition of plasticizers, adapting its mechanical properties by the addition of other fibers and compounding materials. Applications of PHA are various, such as in coating materials and bioplastic components, packaging films and bottling, in drug delivery, and medical devices [1]. PHAs-based bioplastics possess good mechanical properties and are easily molded into various shapes and materials for bottles, inks, sealants, packaging for consumer goods and food films, and agriculture sheets.

Bioderived, biodegradable polymers are suitable for different kind of applications, in electronics, cosmetics, biomedical sector, aerospace, consumer goods, agriculture, packaging industries, and active packaging. It can also act as bioplastic material for soft biocomposition, conductive bioplastics, high-tech electronic devices, disposable tableware, toys, golf tees, bags, automotive components, lightweight structural composites for the buildings industry, textiles, elastomeric plastics, disposable materials and fibers, performance additives, transparent films, high-strength fibers, fertilizer mulches, and pellet for soil application.

Due to its biocompatibility and resorption qualities, PHA polymers have been exploited in devices for minimally invasive delivery, microparticles in drug delivery, for cardiac valves and surgical sutures, in regenerative medicine, artificial skin, tissue engineering such as scaffolds for tendon and fractured bones, artificial organ reconstruction, in matrices for nerve repair as support of cell growth [2, 3]. The FDA has delivered its approval for P4HB applied to clinics and human therapies; therefore, PHA applications in the medical fields will continue to grow.

PHA Industrial Production: Feed Costs

PHAs costs may also vary depending on the type of application, since materials for drug delivery and medical device components have high value [1]. The main problem is the cost of the feedstock, with incidence of 50% of the costs, in addition to biofermentor and personnel costs, the costs for extraction and purification.

As a raw material for the fermentation, in addition to carbohydrates and sugarcane molasses (Nikel et al. 2006; Naheed and Jamil 2014), vegetable oil or glycerol can be used (Kumar et al. 2015). With the aim to improve the sustainable use of feed materials, wastes and nonfood-competing sources have been used in production of PHA polymers. Therefore, methods to exploit by-products as feedstock have been developed (Kumar et al. 2016). High production of PHA in fermentation process has been obtained using C1 carbon sources (CH_4, syngas) [4], sugarcane molasses [5, 6, 7, 8, 9], soy molasses, agroindustrial wastes [10–12], glycerol from diesel waste [13, 14]; animal and vegetable fats [11, 15, 16, 17], and biorefinery byproducts [9, 14, 18, 19, 20, 132, 137]. New approaches of bioprocessing domestic kitchen waste, municipal solid waste (MSW), and organic biowastes [133, 137], using adapted bacterial strains, with possibility of hydrogen (H_2) and methane coproduction, have been shown feasible (Patel et al. 2015; Kumar et al. 2013, 2016), pushing forward the applications in the biorefineries sector [163–165].

Global demand for biodegradable plastics has reached the value of 700,000 tonnes. Envisioned trends of production sum up to 270,000 tonn/year. Table 1 describes the companies producing PHB and its copolymers, P2HB4HB, PHBHV, and PHBHHx. In 2016, Metabolix has closed its activities in the PHA field, and patents were acquired by Cheil Jedang, based in Seoul.

Table 1 Main type of PHA polymers produced industrially, with details on methods and feed type

Bacteria spp.	Polymer type	Capacity	Company	Trademark
R. eutropha + sugarcane molasses	PHBV	2000 tonn/ year	Tianan Biol. Mat., China	www. tianan-enmat.com Enmat
Chromobact phaC recomb *C. necator* + palm oil kernel effluents	PHBHV, PHB4HB	Automated system	SIRIM, Malaysia	
R. eutropha + sugarcane molasses	PHBV	10,000 tonn/ year	PHB Industrial, Brazil Copersucar/ Biocycle	www. biocycle. com.br Biocycle
P. putida + vegetable oil	PHBV copolymer PHBHHx	25,000 tonn/ year	Kaneka, Japan Kaneka/P&G	Nodax, other mixed PHAs
R. eutropha	–	–	Tepha	TephaElast
R. eutropha + methanol	PHB	–	Mitsubishi gas chemicals Japan	Biogreen
P. putida	PHBV	25,000 tonn/ year Automated system	Telles, USA ADM	Mirel, Mvera
–	–	10 tonn/year	Jiangsu Nantian, Tsinghua Univ., China	
R. eutropha + propionic acid	PHBV	50,000 tonn/ year	Cheil Jidang South Korea	Biopol
A. hydrophila 4AK4 + lauric acid	PHBHHx	20,000 tonn/ year	Procter & Gamble/ Meredian US, Tsinghua Univ. Jiangmen Biotech	
–	PHBHHx	Pilot scale	Tiangzhu	
–	PHBHHx	10,000 tonn/ year	Lianyi Biotech China	
A. hydrophila + lauric acid	PHBHHx	Pilot scale 3000 tonn/ year	ShanDong Lukang	
E. coli *P. putida*	P3HB4HB	10,000 tonn/ year	Tianjin Green Bio. Tepha, DSM Netherlands	GreenBio
–	PHBV, PHBH, PHBO	Pilot scale	Biomer Biotechnol., Germany	Biomer
–	–	10,000 tonn/ year	Bio-on, Italy/Europe	Minerv-PHA
–	PHBV copolymer		Polyferm, Canada	Versamer
–	–	100 tonn/ year	Biomatera, Canada	–

Types of PHA

While some species produce mainly 3PHB polymers, other species can synthesize PHAs, depending on availability of intermediate precursors, such as citrate [103]. PHA synthases can polymerize short-chain-length PHAs (scl-3PHAs), or medium-chain-length PHAs (mcl-3PHAs), depending on the class of enzymes and the species and genetic background [21]. Among the short-chain-length PHA (scl-PHA) types produced are copolymers containing hydroxypropionate (3HB-co-HP), 4-hydroxybutyrate (3HB-co-4HB), hydroxyvalerate (3HB-co-HV) [22], and 3-hydroxyhexanoate (3HB-co-HH), depending on the availability of precursors (propionate, valerate, hexanoate); as for the medium-chain-length PHA (mcl-PHA) types produced are copolymers containing hydroxyhexanoate, hydroxyeptanoate, hydroxyoctanoate, hydroxydecanoate, and hydroxydodecanoate [19, 23–27]. The synthesis of polyhydroxyalkanoate copolymers with controlled composition of hydroxyalkanes has been reported [25]. Until today, around 150 different compounds have been identified as the monomeric units within the PHA polymer. Randomly ordered copolymers, such as poly 3-hydroxybutyrate-co-3-hydroxyhexanoate (P3HB3HH) containing functional groups, i.e., olefin groups, branched alkyl chains, halogen atoms, aromatic groups, and cyano groups have been described [28]. New P(LA-co-3HB-co-3HP) terpolyesters incorporating polylactate have also been described [29]. The flexibility of PHA biosynthesis favors the design and production of biopolymers having particular physical properties, such as stiffness, elasticity, durability, resistance, or rubbery properties.

Homopolymers

Several homopolymers for bioplastics have been produced in bacteria, such as polylactic acid (PLA), and, for PHA, the reference polymer P3HB, 3PHB in sizes up to ultrahigh molecular weight (hmw-PHB) [30, 31], poly 4-hydroxybutyrate (P4HB) [143], poly 3-hydroxypropionate (P3HP) [13], poly 3-hydroxyvalerate (PHV) [32]; poly 3-hydroxy-4-pentenoate (P3H4P), poly hydroxyhexanoate (P3HH) [33, 34], poly 3-hydroxyheptanoate (P3HH), poly 3-hydroxyoctanoate (P3HO) [35], poly 3-hydroxydecanoate (P3HD) [36], poly 3-hydroxy-10-undecenoate (3H10U), poly 3-hydroxydodecanoate (P3HDD) [37], poly 3-hydroxytetradecanoate (P3HTD), poly 3-hydroxy-5-phenylvalerate P(3HPhV), 3-hydroxy-6-phenylhexanoate (3H6PhHs), and functionalized mcl-PHA [38, 142]. With the engineering of the β-oxidation pathway, additional homopolymers can be made available [39–41].

Random Copolymers

Poly(3HB-co-mcl-3HA) has been produced by P&G on industrial scale, under the NODEX trademark. The wide range of commercially produced PHA as random copolymers include poly(3HP-co-4HB), poly(3HB-co-3HP) [42], poly(3HB-co-3HV) (PHBV) [15, 43–46], poly(3HB-co-4HB) (P3HB4HB) [47, 143], and poly

(3HB-co-3HHx) (PHBHHx) [22, 48–51]. Using *Aeromonas hydrophila* expressing phaPCJ synthase, mutations in the enoyl coenzyme A hydratase enhanced the 3-hydroxyhexanoate availability for the synthesis of poly 3-hydroxybutyrate-co-3-hydroxyhexanoate (PHBHHs) [48, 49, 52]. Copolymers of P(3HHx-co-3HO-co-3HD-co-3HDD) can be produced using *Pseudomonas* spp. but possess a too soft consistence. Mixed polyesters such as poly-lactate-co-glycolate and poly(LA-co-3HB-co-3HP) have also been produced [29, 53]. Copolymers of poly(3HB-co-3MP) and poly(3HB-co-LA) have been produced using recombinant *Escherichia coli*; these copolymers demonstrated improved characteristics.

Block Copolymers

Block copolymerization allows to modify the thermodynamic property of polymers that resist ageing processes. PHA block copolymers of PHB-b-PHBV, a material more resistant to ageing process, were obtained by feeding alternated carbon sources during fermentation process [54]. Various researchers produced different diblock copolymers, such as PHB-b-P3HVHHp, PHB-b-P4HB [29]), PHB-b-PHHx [55], P3HB-b-P3HP [42], P3HP-b-P4HB, and P3HHx-b-P(3HD-co-3HDD) [37, 39, 40]. The addition in sequence of two carbon substrates can lead to the incorporation into the PHA of the block copolymer [42, 51]. By adding 1,3-propanediol followed by 1,4-butanediol, *E. coli* cells synthesized P3HP-b-P4HB block copolymers. These copolymers show optimized properties. Various diblock copolymers can be obtained regulating the availability of fed substrates.

Graft Polymers

Graft copolymers are synthesized by chemical modification, introducing a functional group into PHA chains, through insertion of small molecules (double bonds, triple bonds, epoxy groups, carbonyl, cyano, phenyl groups, or halogens) into the PHA side chain, with improved property and characteristics. Presently, PHA derivatives obtained are: poly(styrene peroxide)-g-PHA (PS-g-PHA), poly(methyl methacrylate peroxide)-g-PHA (PMMA-g-PHA), PHA-g-polyacrylic acid (PHA-g-PAA), PHA-g-cellulose, PHB-g-acrylic acid-starch (PHB-g-AA/starch), PHA-g-AA-chitosan (PHA-g-AA-COS), polyethylene glycol-g-PHA (PEG-g-PHA), monoacrylate-poly-ethylene glycol-g-PHO (PEGMA-g-PHO), polylactic acid-g-PHA (PLA-g-PHA), glycerol-1,3-diglycerol diacrylate-g-PHO (GDD-g-PHO), vinylimidazole-g-PHO (VI-g-PHO), PHBV-g-poly(phenyl vinyl ketone) (PHBV-g-PVK), PHBV-g-poly-acrylamide (PHBV-g-PA), among others.

Bacterial Species Producing PHAs

Many bacteria produce polyhydroxyalkanoates (PHAs) polymers and store them in intracellular organelles. Among the chemolytotrophic bacteria are *Cupriavidus necator* (*Ralstonia eutropha*), *Cupriavidus metallidurans*, and *Alcaligenes latus* [12, 56, 57, 58];

producing PHB from simple carbon sources. Among Gram-negative bacteria, *Pseudomonas* spp. [16, 156], has attracted the interest for their metabolism of oil wastes, such as *P. oleovorans* [15], *P. putida* [26, 59, 60, 151], *P. aeruginosa* [17], *P. pseudoflava* [61], *Thermus thermophilus*, *Azotobacter vinelandii* [62] a diazothroph bacterium, *Enterobacter* spp., *Burkholderia* spp. [61, 162] and halophilic, alkaliphilic, denitrifying species, such as *Halomonas campisalis* [43], that, being halophile, can be fed with fish industry wastes, with reduced needs to sterilize the feed and the biofermentors.

Among Gram-positive bacteria are *Bacillus* spp. [163–165], *B. subtilis* [7], *Bacillus thuringiensis* [7, 63, 163], and *B. cereus* [64, 65]. PHAs are produced by bacteria under stress conditions (pressure, N or P limitation) increasing the synthesis of PHAs [60, 66]; the bacteria perceive the signals, such as Guanosyl diphosphoguanosine (GppG) or dicyclic GTP alarmones and produce energy-storing polymers. Bacterial cells respond to environmental stress by PHA production has been found to be growth associated as well as nongrowth associated depending on the bacterial species and culture conditions [141, 146, 147]. In the production of PHA in biofermentors, one-stage culture [53, 67], two-stage batch culture [62, 67], fed-batch [16, 68]; high-cell density cultures [10, 18]; and mixed cultures [6, 134], as well as submerged and solid state fermentation processes [69] have shown potential to be exploited to produce PHAs [70]. Carbohydrates and feed stocks can be added in continuous or at determined time points (fed-batch), thus providing the required substrates for PHA polymers [8, 71, 72, 73].

Various factors (type of feed, aeration) influence the biomass growth, synthesis of PHA and its molecular weight [129, 149, 151, 152, 153]. Some author described higher PHA production in *E. coli* by increasing the oxygen dissolved into the medium [14], using high rate sparging and aeration [74]. Other authors choose to grow metabolically engineered *E. coli* in a microaerobic environment, exploiting metabolic pathways associated to anaerobic metabolism [75]. Several bacterial species have been genetically modified through gene engineering, in addition to the *E. coli* system; *Aeromonas hydrophila* and *Halomonas* spp. [76] can be genetically modified; mutants of *Pseudomonas putida* [77], *P. aeruginosa* [17], and *Bacillus* spp. have been obtained using biotechnology approaches [163]. In particular, interest has been linked to ability of bacteria to use as feeds vegetal oils and glycerol from biodiesel industry, or lignocellulose feedstock such as xylose [132], and halophytic species that can be repeatedly grown in high salt medium from one cycle to the next one with saving on the cost for sterilization of feed and biofermentor. Though genetic engineering helped a lot in broadening the substrate range to be metabolized into PHA as well as the polymer composition, it has been of limited success since mostly wild-type strains could produce higher PHA. Moreover, changing the culture conditions may enhance the overall PHA yield.

PHA Synthases and PhaCAB Operons

PHA synthases (PhaC) are grouped into four classes based on the kinetics and mechanisms of reaction. The PhaCs in *Alcaligenes eutrophus* synthesizes scl-3PHAs with monomer units oxidized at positions other than the third carbon; the

Pseudomonas oleovorans PhaCs synthesize mcl-3PHAs with monomer units oxidized at the third position [28], with few exceptions. The grouping of PhaC enzymes into four classes is dependent on substrate specificity, according to the preference in forming scl- or mcl-polymers: class I, class II, class III, and class IV PHA synthases have been characterized, determining catalysis properties, substrate recognition, and affinity [78]. Class I PhaC enzymes accept 3-hydroxyalkanes preferably forming short-chain-length PHAs utilizing CoA thioesters with a limited number of carbons [79]. Class I *Cupriavidus necator* PHA synthase structure of catalytic domain has been described. Class I phaC enzymes were N-terminally truncated, with different range of truncations, showing how the length of sequences affect the polymer length and product specificity [80, 81]. The enzymes belonging to class III, made of two 40 kDa subunits, PhaC and PhaE, have been recently reviewed [82]. The phaC catalytic site possesses a typical PhaC box sequence ([GS]-X-C-X-[GA]-G). Class III phaC are structured as tetramers, such as phaEC from *Allochromatium vinosum,* of phaE and phaC monomers of 40 kDa. Trapping of intermediates with substrate analogues showed that class III PHA synthases slow the rate of catalysis depending on cycling of reacylation and hydrolysis [39, 140]. In addition, class IV PHA synthases, in *Bacillus* spp., *B. megaterium* and *B. cereus* [82–84], are formed by the subunits PhaC and phaR (similar to phaE) [78].

Furthermore, chemically modified compounds have been developed as inhibitors of phaC, to study the synthesis mechanism and reaction kinetics [3]. The crystal structure of the catalytic domain of PhaC from *Chromobacterium* sp. USM2, Cs-CAT [83] was recently reported. *Chromobacterium* USM2 strain phaC(Cs) was found highly active, with fast polymerization rate, and preferring hydroxyvalerate, 3-hydroxyhexanoate (3HHx), in addition to 3HB [85]. The studies on PhaCs from *Chromobacterium* sp. USM2 were aimed to increase the activity and broaden substrate specificity of PhaC(Cs) [83]. PhaC(Cs) showed utilization of 3HB, 3HV, and 3HH, with high 3-hydroxybutyryl-coenzyme A activity.

Enzymatic activity has been determined on PhaC for PHA synthesis, studying the specificities for medium-chain-length monomers as 3-hydroxyhexanoate, on the enzymes from *Chromobacterium* sp., *Allochromatium vinosum*, and *Caulobacter crescentus* (PhaCCs, PhaCCc, A479S-PhaCCs, PhaECAv) [86], displaying varying preference for the alkyl side-chain length. Several point mutations, in particular at the position 479, are reported to increase PhaCCc substrate preference for 3HHx [86]. The *Chromobacterium* sp. PhaC synthase having a catalytic site Cs-CAT containing Cys291, Asp447, and His477 was studied, determining that the substrate-binding site is hidden by a partially disordered protein domain [85]. The structure has peculiar properties, differing from the catalytic domain from *Cupriavidus necator* (PhaC Cn-CAT). PhaC Cn-CAT adopts a partially open form maintaining a narrow substrate access to the active site, that needs PhaM for activation. Recently, studies have been focused on PHB synthases with mutations enabling the enzymes to accelerate the reaction kinetics [87, 88] and the catalytic site to accept bulk substrates as precursors for the production of mcl-PHAs and grafted

copolymers. *R. euthopha*, *Aeromonas caviae*, and *A. punctata* phaCs were modified by mutagenesis, for example, in S477X and Q481X, for efficient production of 3HB copolymers [50, 89–91].

Class IV phaCs, such as PHA synthase PhaC1 and PhaC2 from *Pseudomonas stutzeri* [92] has been exploited in polymerization of mcl-PHAs in engineered bacteria [82]. PhaC2P with four point mutations, at E130D, S325T, S477G, and Q481K [93] was used to accommodate substrates with various shapes and structures, to produce mcl-PHAs and block copolymers, and tolerate modifications of side chains, i.e., unsaturated bonds or azide groups [51]. These studies have the potential to enlarge the range of various copolymers and their larger physicochemical properties [27, 88, 94, 95]. Phasins, PhaPs are PHB granule-associated proteins, attached to the surface of PHA granules, with structural and regulatory functions, as reviewed in *Ralstonia eutropha* H16 [96]. Structure of PhaP from *Aeromonas hydrophila* has been described, while PhaM, the physiological activator of PHB synthase (PhaC1), has been analyzed in *Ralstonia eutropha* [97]. PhaM is a natural primer of phaC1 activity, decreasing the time of polymerization and increasing PhaC1 specific activity. Several authors reported that, rearranging the order of integration of PhaCAB operon genes, it is possible to synthesize P3HB-co-4HB [47], P3HP [13], P4HB [98], ultrahigh molecular weight P3HB [30, 163], PHA-containing hydroxyl groups [99], and copolyesters of 3-HB and mcl-3-PHA using an optimized PHA synthase gene [94].

Engineering Bacteria for Optimized PHA Synthesis

Through up-to-date gene engineering systems, researchers showed that PHB content and molecular weight are directly related to PhaC activity. PHA has been produced in *E. coli*, using genetic engineering [8, 13, 14, 30, 66, 100, 101] and through bacterial cell factories. The *E. coli* system overcomes fermentation problems [47, 73, 132], can grow rapidly, accumulate PHA up to 60% of dry weight [102, 103], and can be fed with various intermediate compounds [14, 103]. Chromosome integration of PHA synthase genes (phbCAB operon) and expression of metabolism regulating genes in recombinant *E. coli* was shown to increase the PHA yield [144]; for instance, NAD kinase gene *yfiB* was shown to improve PHB production for the efficiency to supply NADPH. *E. coli* engineered to synthesize various types of PHA have been obtained through sleeping beauty mutase, to modulate the synthesis of P(3HB-*co*-3HV) polymers [135, 161] through expression of β-ketothiolases, to condense acetyl-CoA or acetyl-CoA and propionyl-CoA to form acetoacetyl-CoA and 3-ketovaleryl-CoA, followed by expression of acetoacetyl-CoA reductase (PhaB) for thioester reduction and PHA synthase (PhaC) for copolymer synthesis [44].

E. coli was metabolically engineered to synthesize poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (3HB-co-3 HV) [45] through propionyl-CoA produced from 2-ketobutyrate, which is generated via 2-hydroxy-2-methylbutanedioate through an enzyme involved in isoleucine synthesis from acetyl-CoA and pyruvate. Two

approaches have been pursued for changing metabolic pathways to the synthesis of propionyl-CoA. In the first case, 2-ketobutyrate oxidase produces propionic acid, used to form propionyl-CoA. The other pathway relays on the conversion of 2-ketobutyrate into propionyl-CoA through pyruvate formate lyase. *E. coli* metabolic fluxes were modified to block succinate production and increase the carbon flux towards P4HB biosynthesis. The recombinant and metabolically improved *E. coli* can produce poly(3HB-co-4HB), and the presence of α-ketoglutarate or citrate can increase the content of 4HB up to 20% [47]. Also, threonine and serine metabolism can increase the availability of acetyl-CoA and utilization of the CO_2 derived from pyruvate dehydrogenase reaction [73]. The conversion of threonine into acetyl-CoA and glycine relies on threonine availability. During phosphoenolpyruvate carboxylase (PPC)-mediated carboxylation of phosphoenolpyruvate (PEP) into oxaloacetate (OAA), two Acetyl-CoA are produced through CO_2 fixation, with consumption of NADH and ATP.

In the Lin et al. study [73], enzymes for PHB synthesis were coexpressed with enzymes for threonine synthesis and degradation (Fig. 1).

Bacterial shape is a factor limiting the space and the potential accumulation of high amounts of PHA granules. Several scientists studied the suppression of filamentation to increase the synthesis of PHA. Subsequently, *E. coli* mutants in genes regulating cell division, *FtsL, FtsN, FtsQ, FtsW, FtsZ*, or the overexpression of SulA, an FtsZ inhibiting protein, have made possible an increase in yield and dry cell mass with increased recovery of PHA [104]. Multiple, dividing *E. coli* cells [105] with deleted genes *minC* and *minD*, and cell shape controlling, actin-like gene *mreB*, show formation of several fission rings and the elongated shaped cells divide into multiple daughter cells. The creation of new PHA synthesis pathways has been made possible by recent advancements and genetic modification in various species. Remarkably, the weakening of β-oxidation cycle in *Pseudomonas putida* and *Pseudomonas entomophila* has allowed production of different PHA polymers, with varying monomer ratios, forming either random and/or block copolymers when fatty acids (hexanoic, octanoic, and dodecanoic acids) are made available as PHA precursors [40]. When fatty acids containing functional groups are fed and taken up by the bacteria, PHA polymerization occurs with the functional groups incorporated into the PHA. The functional PHA polymer may be then processed with other reactive molecules to allow formation of grafted polymers.

Other factors influencing the heterogeneity of polymers are consequence of controlled synthesis of homopolymers, random copolymers, block copolymers, and grafted polymers.

Engineering Pathways for scl-PHA Synthesis

Metabolic engineering has been applied to microbial synthesis of PHAs. Synthetic biology approaches to regulate metabolic fluxes can be exploited to control PHA composition [130]. The PhaC recombinant *E. coli* inactivated in

the succinate semialdehyde dehydrogenase and expressing a more efficient succinate semialdehyde dehydrogenase, enhancing the carbon flux toward P4HB production.

Poly 3-hydroxypropionate (P3HP) was produced in *E. coli* containing four heterologous genes, propionyl-CoA ligase, dehydratase, aldehyde dehydrogenase, and 4-hydroxybutyrate-coenzyme A transferase, with bacteria producing 3HP4HB when fed by 1,3-propanediol and 1,4-butanediol added in sequence. The pathways were combined together, implementing the *E. coli* with glycerol dehydratase, propionaldehyde dehydrogenase, beta-ketothiolase A, and acetoacetyl-CoA reductase, the bacteria synthesized 3HP3HB with various content of 3HP, or block copolymers depending on the feeding method used.

Engineering the β-Oxidation Pathway mcl-PHA Synthesis

Many *Pseudomonas* spp. utilize fatty acids through β-oxidation to produce energy and feed to sustain their growth. The β-oxidation pathway produces alkanes with shortened carbon chain, that when incorporated into PHA lead to the production of random PHA copolymers. To make available fatty acid substrates of various length, several genes involved in β-oxidation were deleted to make ß-oxidation weakened, engineering a mutated *P. putida* KTQQ20 strain.

P. putida KTQQ20 strain was able to produce homopolymer PHD as well as the copolymer P(3HD-co-3HDD) by feeding sequentially decanoate and dodecanoate [39]. *P. putida* KTQQ20 grown on hexanoate plus decanoate, synthesized random copolymers of (HH-HD) with composition depending on hexanoate/decanoate ratio, or diblock copolymer -P(3HD-co-3HDD), when the two substrates were added in succession. The β-oxidation pathway was knocked down also in *P. entomophila,* to produce mcl-PHA. *P. entomophila* LAC26 strain produced PHA in high amounts. Homopolymers of 3HDD were synthesized, as well as other types of polymers, depending on the source of fatty acid added. The *P. putida* KTOY08DGC was able to produce the block copolymer P3HB-b-P4HB.

Recombinant *Aeromonas hydrophila* 4AK4 fatty acid β-oxidation impaired mutant expressing a bacterial hemoglobin and acyl-CoA synthase, produced PHBHV and PHBHHx copolyesters, using undecanoate as feedant, and supplying 3-hydroxyvalerate through threonine catabolism. Recently, the production of PHA containing 2-hydroxybutyrate and 3-hydroxypropionate was shown feasible. P3HP has been produced starting from 1,3-propandiol or from glycerol [39].

Engineering Pathways for Scl- and mcl-PHA Copolymers

P. putida KTOYO6 was mutated in the fatty acid β-oxidation enzymes 3-ketoacyl-CoA thiolase and 3-hydroxyacyl-CoA dehydrogenase, to increase PHA synthesis. When the strain was transformed with a less specific PHA synthase, the *P. putida* KTOYO6DC strain synthesized both scl- and mcl-PHAs, such as copolymers of PHB-b-PHVHHp,

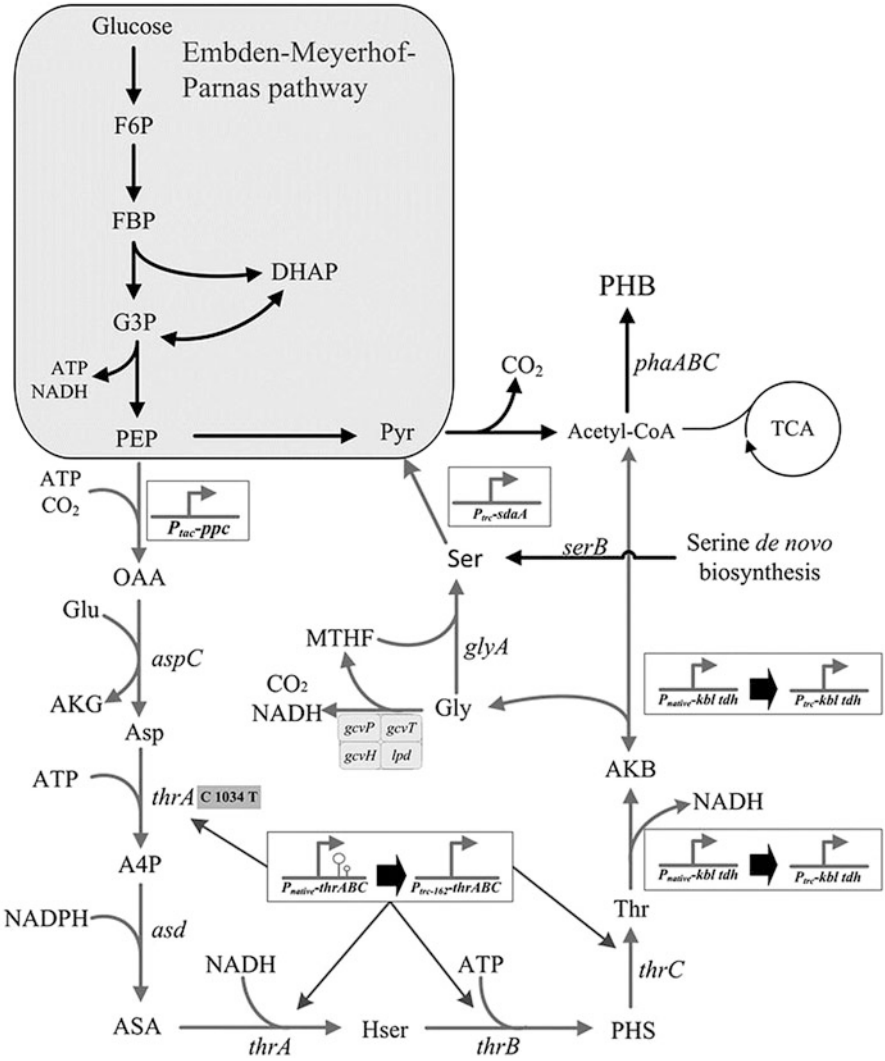

Fig. 1 Glycolysis flux and TCS cycle leading to Acetyl-CoA and threonine bypass scheme. Acronyms of metabolites: *AKB* 2-amino-3-ketobutyrate, *AKG* 2-oxoglutarate, *Asp* aspartate, *ASA* aspartate semi-aldehyde, *A4P* aspartyl-4-phosphate, *ATP* adenine trinucleotide phosphate, *DHAP* dihydroxyacetone phosphate, *F6P* fructose 6-phosphate, *FBP* fructose 1,6-bisphosphate, *GAP* d-Glyceraldehyde-3-phosphate, *Glu* glutamate, *Gly* glycine, *Hser* homoserine, *MTHF* methyltetrahydrofolate, *NADH* nicotinamide adenine dinucleotide, *OAA* oxaloacetate, *PEP* phosphoenolpyruvate, *PHS* phosphorylated homoserine, *Pyr* pyruvate, *Ser* serine, *Thr* threonine, *TCA* tricarboxylic acids cycle

obtained through feed regulation, at first by addition of sodium butyrate, followed by sodium heptanoate [55]. When mixtures of butyrate and hexanoate were added as feed, bacteria produced P(3HB-co-3HHx) polymers in which the percentage of monomer contents depended on C4/C6 feed [33, 48, 49, 106].

Engineering Pathways for Functional PHA

When *P. putida* KTQQ20 or *P. entomophila* LAC23 were cultivated in presence of fatty acids containing functional groups (double or triple bonds, epoxy groups, carbonyl, cyano, phenyl, and halogen groups), the resulting PHA contained the functional groups on side chains, exploited for chemical grafting on the reactive residues. Homopolymers, random copolymers, or a blend of both have been produced containing aromatic groups. PHAs containing alkoxy, acetoxy, or hydroxyl groups are important for their hydrophilicity, high solubility, and versatility of use.

P. entomophila LAC23 accumulated PHA containing phenyl groups on the side chain, while *P. putida* KT2442 accumulated diblock copolymer PHB-co-PHHx [55]. These strains, grown in presence of 5-phenylvaleric acid, accumulated poly(3-hydroxy-5-phenylvalerate) homopolymer. *P. entomophila* LAC23 cultured using phenylvaleric acid/dodecanoic acid as feed accumulated the copolymer containing 3-hydroxy-5-phenylvalerate (3HPhV) and 3-hydroxydodecanoate (3HDD). The content of 3HPhV in P(3HPhV-co-3HDD) was regulated by the ratio of dodecanoate/5-phenylvalerate [41, 106].

Fermentation in Biofermentors: Industrial Optimization of Costs/Yield

The principal bottleneck in production costs are the costs of feed substrates, operational cost of fermentors, extraction and purification costs. Therefore, producers have optimized bioreactor use and protocols for scaling up of the processing capacity of fermentors, the use of cheaper feeds, and high bacterial cell density to increase the yield of PHAs [157, 158, 159]. PHA determination in bacteria have relied on spectrophotometric techniques or on chromatographic methods. RAMAN spectra were acquired from marine bacteria mixed cultures [107]. Several dyes have been used, with most of them not specific for PHA, but binding also to membrane lipids. The most common methods are based on Nile Red (λ excitation: 543 nm, λ emission: 560–710) [108] and Nile Blue stain (Spiekermann et al. 1999; Oshiki et al. 2011; Weissgram et al. 2015). New quantitative methods have been based on fluorometry combined with a flow cell to evaluate PHB in bacteria stained with Nile Blue [56] [148, 166, 167], while other methods were based on fluorescence [108] and laser scanner quantification (λ_{exc} 460 nm/λ_{em} 550 nm) of bacteria stained with Nile Blue, determined end point PHA accumulation [103].

Biosensors and enzymatic methods for the evaluation of feed consumption have been described [109] and their applicability and usefulness validated [103]. Recently, a metabolic modeling system has been applied to control nutritional and aeration conditions for biomass and PHA production optimization. Also, sensors can determine bacterial concentration, and whole-cell bacterial detection has been shown feasible [102, 110]. A second critical point for the scale up of the process is the lysis of cells and the extraction of granules of PHAs. Several approaches have been proposed to make the process economically advantageous, from the single cell

protein method, to make fermentation and recovery of granules in a single passage [111], to various lysis systems [112, 113], to alkaline treatment for PHA recovery [114]. All these methods show economical advantage and are environmentally friendly, since they do not require the use of solvents such as chloroform.

Industrial Applications of PHA Polymers

Polyhydroxyalkanoates (PHAs) are a great opportunity for the polymer industry due to their property of high biodegradability and processing versatility, and the potential to replace fossil fuel-based plastics. Presently marketed PHAs originate from microbial cultures fed with renewable feedstocks (i.e., glucose) following sterilization [115]. The main properties of PHAs are: water insolubility and resistance to ultraviolet rays; low tendency to hydrolysis; degraded by acids and bases; solubility in chloroform and chlorinated solvents; biocompatibility with biological fluids and tissues; degradable anaerobically in sediments; nontoxic; and non"sticky" when melted in respect to other polymers. Polyhydroxybutyrate is brittle, fragile, and stiff, with low elongation ability, and a break point below 15%. Main problems from PHB ageing at room temperature is recrystallization and consequently mechanical properties changing with time. mcl-PHAs are elastomers, have low melting point, and a relatively low degree of crystallinity. PHB and other PHAs are degraded by exposure to temperatures above PHA melting point, that is 170 °C.

PHA tendency to thermal degradation is a serious problem in the PHB industry and its applications. An exposure to 180 °C induces PHB degradation with production of crotonic acid and shorter chain polymers. PHA is processed by extrusion, producing various rigid and flexible plastics for goods molded to the various shapes needed, coatings, fabrics, packaging films, films for agriculture, adhesives, additives, and medical applications. Based on the properties of the different types of PHAs, there are aspects related to the processing, commercial availability, challenges, and opportunities that need to be approached.

Compounding PHB

Plasticizers can be added to modify the thermal and mechanical properties of PHAs, to control and retard the crystallization process, and optimize flexibility and elongation ability of polymers.

Blending PHA polymers with plasticizers and nucleating agents modifies the physical properties of polymers, decreasing the processing temperature and lowering the crystallinity, for the formation of small and numerous crystallites.

During the processing, PHB may not tolerate high temperatures; therefore, a lubricant is added to prevent the degradation of the chains, and the process may be carried on at 170–180 °C. This leads to a decrease in the molecular weight and to a reduced melt viscosity. The temperature of crystallization (Tc) decreases and lowers, allowing crystallization to endure for longer times.

The mechanical properties of PHB are improved by its blending with P(3/4HB), increasing the elongation at break.

The plasticizers mostly used are cheap materials easily available on the market, such as glycerol, tributyrin, triacetin, acetyltriethylcitrate, acetyltributylcitrate, oxypropylated glycerol (or laprol), soybean oil, epoxidized soybean oil, fatty alcohols with glycerol fatty esters, triethyl citrate, triacetine, acetyl tributyl citrate, salicylic ester, acetylsalicylic acid ester, dioctyl sebacate (ATBC), polyethylene glycol (PEG), oligohydroxybutyrate, and triethylene glycol-bis-2-ethylhexanoate.

The use of glycerol, tributyrin, triacetin, acetyl triethyl citrate, acetyl tributyl citrate as plasticizers has been reported, and saccharin has been used as nucleation agent. Glycerol monostearate, various triglycerids, and 12-hydroxystearate have been used as lubricants.

Acetyl tributyl citrate has been used as PHB plasticizer. It influences PHB thermal properties during melting, while PHB needs to be rapidly cooled to reach the degree of crystallization required.

Blends obtained adding polyethylene glycol (2–5%) are compatible with PHB, with good miscibility, as shown by DSC analysis. PEG 400 is a good PHB plasticizer, able to reduce melting temperature of PHB. The blends of PHB with PEG 400 show that elongation at break is increased, but the tensile strength is reduced. The PEG plasticizing effect is ascribed to weakening of intermolecular force between the PHB chains, which leads to a change in free volume and to a decreased temperature of melting.

In the production of PHB composites with wood fibers, PEG 400 is added by extrusion and injection molding, with a lubricant effect on melted PHB/wood formulations, making the processing easier. However, PEG is not completely blended with PHB and leaches to the outside after some time, with a loss of plasticizing power.

The adhesion of PEG to the natural fibers is the main reason of its plasticizing effect in the manufacture of composites made of PHB and natural fibers. Organo-modified montmorillonite (OMMT) clay is a nanofiller used in PHB/V blends. The nanobiocomposites possess an intercalated/exfoliated structure and show good mechanical properties. The OMMT filler acts as a nucleating agent, enhancing the crystallization, and improves thermal stability of the polymer.

Blending of PHB with Other Polymers

Blending PHB with other polymers improve processability and reduce the brittleness of PHA bioplastics. Several blends containing PHA have been developed and various plasticizers have been used.

PHB-co-HV possesses higher temperature of crystallization (Tc). An increase in the HV fraction to 20 mol% in PHB-co-HV decreases the melting temperature (Tm) of the copolymer to 168.5 °C in respect to the initial value of 175.4 °C. Further increase in the HV fraction shows an isodimorphic relationship. The nucleating agent ULTRATALC 609 was used to obtain higher temperature of crystallization (Tc),

while reducing crystallization time required for injection molding [115]. Temperature of decomposition (Tdec) of homopolymer and copolymers mixed with ULTRA-TALC_609 resulted increased.

Poly(3-hydrobutyrate) mixed with dioctyl sebacate (ATBC) as plasticizer, shows a lowering of glass transition temperature (Tg), with improvement of thermal characteristics, without changes in mechanical properties. PHBHHx and/or P(3/4HB) with ATBC and antioxidant 1010 as stabilizer show improvement of PHB thermal stability and stabilization of the melt flow index (MFI), widening the application range of PHB processing methods.

Vinyl acetate polymer as well as polyvinyl alcohol have been used to strengthen PHAs blends. Different contents of the fillers have been shown to improve PHB tensile properties (modulus and strength).

Mixed PHA Copolymers

One approach to widen the properties of PHA-based bioplastics is the synthesis of copolymers, such as PHBHV or PHBHHx, with different molar ratios of hydroxycarboxylic acids, improving the mechanical properties and lowering the melting point, slowing down the degradation during processing. In addition, blends with other biodegradable polymers and composites are convenient as materials for industrial applications.

A largely used approach in PHA polymers with improved properties is blending the polymer with a second thermoplastic polymer. The degree of crystallinity is modified, and production costs can be lowered.

P(3HB) is miscible with poly(ethylene oxide), poly(epichlorohydrin), poly(vinyl acetate), highly substituted cellulose esters and trisubstituted cellulose butyrate and caprolactone, among others.

The blends with P(3HB) with immiscible polymers are important in the control of biodegradation profile. Binary blends, such as P(3HB)/poly(propiolactone), P(3HB)/poly(ethylene adipate), and P(3HB)/poly(3-hydroxybutyric acid-co-hydroxyvaleric acid) degrade more rapidly, and the acceleration depends on the phase separation of the two structures.

Wood flour and lignin have been used as fillers in composite materials. Fibers derived from renewable resources on conventional reinforcements such as glass and aramid fibers are convenient, cheap, recyclable, and competitive as strength per weight of material.

Processing of PHA Copolymers: Challenges and Opportunities

Depending on the molecular weight of the polymer and on the content of comonomer, different processing techniques have been developed.

Powdered PHB is blended with additives by mixing in a kneader at 170–180 °C in an extruder at temperatures in the range of 160 °C and 170 °C. The thread is cooled in water and a pelletizer allows the cutting into pieces.

Then, the granulates can be compressed and molded in a hydraulic press, heated at temperature of 170–180 °C between sheets of Teflon. After that, samples are cooled to room temperature.

Melting Behaviour of PHB

PHB is a linear polymer with elevated level of crystallinity (60–70%). The crystallization speed is slow below 60 °C and above 130 °C, behaving as an amorphous and sticky material. The rapid fluid/solid transition is exploited to obtain fast speed in the processing. The material is melted in proximity of the filling zone, lowering the temperature during the die-casting. The viscosity of PHB plastics is similar to polypropylene.

Processing Techniques and Conditions

PHAs can be extruded by injection molding and various types of extrusion protocols, into films and hollow bodies. The thermal, rheological, mechanical, and barrier properties of PHBV with different valerate contents and molecular weights have been characterized. The use of copolymers is very frequent, since they improve the plastics flexibility and lower the glass transition temperature (Tg) and the melting temperature (Tm).

The presence of HV in the copolymer enlarges and improves the processing parameters, thanks to higher melt stability at lower processing temperatures. The processing of copolymer below 160 °C is beneficial with low screw speed. PHBV shows good mechanical property, high elastic modulus and flexibility strength, low tensile strength, and low elongation at break. PHBV polymers are unstable at temperatures over 160 °C, possibly because of polymer breakdown due to random chain scission process, which leads to decreased molecular weight and lower viscosity. The PHBV plastics are brittle, elastic and with low tensile strength. P3HB4HB polymers and conventional thermoplastic used for packaging instead show high tensile strength and higher elongation at break. The PHBHV subjected to injection molding at temperatures from 135 to 160 °C, show low degradation and only for a small extent [115].

Although the incorporation of nanoclay improves the properties of PHB and its copolymers, thermo-mechanical degradation of the PHB and PHBV, in the presence of ammonium surfactants, used as clay organo-modifiers, has been reported. The surfactants affect polymer degradation.

The use of surfactants in PHBV processing is optimal. The surfactants improve the characteristics of PHBV-based nanocomposites with organomodified clays, exhibiting good thermo-mechanical properties, high shear rate, good level of exfoliation of layered silicates, and stabilization of the bioplastic products. The PHB blend with poly(vinyl acetate) (PVAc) in the amorphous state was characterized, showing that the two polymers are miscible. Overall, polymer blends containing PHA show good properties and high biodegradability [115].

Industrial Applications of PHAs

The bioplastics have a wide range of applications for the industry [27, 138, 139, 145, 154, 160] PHA is biodegradable, highly deformable, has high heat resistance and good resistance to hydrolysis, balancing both the degree of toughness and the degree of stiffness. PHA shows for many aspects similarity with linear low-density polyethylene (LLDPE). This makes PHA versatile enough to be made into a wide range of molded items, fiber and film. Manufacturers of hard-line consumer goods showed the potential many PHA uses due to its flexibility and its resemblance to LLDPE. In addition to furniture, tools, and sports equipment, PHA can be used in the molded parts of household appliances, such as covers, filters, housings, fasteners, and clips. The bioplastics have been used in cables, connectors, and housings of consumer electronic devices. The developing trend of use of bioplastics has been seen in electronics manufacturers: Samsung, NEC, Sony, Fujitsu, and Nokia make use of bioplastics in their goods. Consumers have shown increasing interest and support for green product content even at a higher, premium price, such as in packaging materials for cosmetics and personal care products. The products are marketed stressing their environmentally friendly qualities. PHA's flexibility makes it a natural bioplastic material for caps, bottles, blister packs, and other containers for the industry of consumer goods. It can also be used for packaging applications for articles in the food industry, such as bottles, laminated foils, fishnets, flowerpots, sanitary goods, fast foods, disposable cups, agricultural foils, and fibers in textiles.

The properties of various copolymers and block polymers, and their processability, enlarge the potential in their applications. Similarly to PVC and PET, PHA exhibits good barrier properties and can be used in the packaging industry as a bioplastic, contributing to solve environmental pollution problems. Due to these properties, PHB is a good candidate to substitute PP and PE but also PET. Poly(vinyl acetate)-based resins enhance the physical properties of the material containing PHB, which significantly simplifies the processing. This, combined with the high heat resistance of PHB, allows the possible use of this material in applications such as hot filling.

Compounders found PHA having great potential as a modifier for PVC as it can improve toughness and plasticization without affecting transparency or UV stability. As PHA has high miscibility with PVC, it is easy to handle and process in the same conditions as PVC.

Overall, PHA is a promising polymer for a wide range of applications. For example, it has better barrier properties and mechanical strength than other more widespread bioplastics such as polylactic acid. In spite of its intrinsic brittleness, a lot of progress has been made through the formulation of PHAs with tailored additives and blends, leading to greatly improved mechanical profiles, as well as suitable processability via extrusion or injection molding. This makes PHA versatile enough to be made into a wide range of molded items, fibers for textiles and biofilms. Blow-molded bottles and injection-molded hair caps are two main industrial products that benefited from the easiness to process the PHA polymer into a vast range of shapes. These advances will improve its capacity to penetrate markets such as packaging foils for the storage of food products.

PHA is a very versatile polymer, that is well suited in a wide range of applications. For example, it has good barrier properties, good oxygen transmission rate (OTR) and water vapor transmission rate (WVTR), and good mechanical strength, in respect to other bioplastics such as PLA. In spite of its brittleness, a lot of progress has been made through the formulation of PHB with tailored additives and blends leading to greatly improved mechanical profiles, as well as suitable processability via extrusion or injection molding. These advances will improve its capacity to penetrate markets and find new application at industrial level. Finally, further improvements (new block polymers, varying monomer percentage in copolymers, grafted polymers) could allow even more flexible grades of PHAs or transparent ones through the control of its crystallization.

PHA has a great potential for applications in agriculture, being a biodegradable plastic, having a controlled-release mechanism, and as fertilizer. Pellets made of PHA can be placed on field to the soil, gradually degraded and contents released over a prolonged period, reducing fertilizer and labor costs. The use of natural fillers with high availability and low cost will allow the production of biocomposite more suited for their application in packaging, such as consumer goods and in food packaging.

Automotive industry, under the recycle directive of plastic components, are driving the next development in components for cars and buildings. The EU End-of-Life Vehicle Directive requires reuse and recycling content rates of 85% in passenger vehicles and light commercial vehicles from 1 January 2015 onwards. As a consequence, automotive manufacturers are developing applications of bioplastics and other biobased materials in their products.

Presently, Hyundai and Toyota carmakers incorporate bioplastics in their vehicles. Because of its physical properties, PHA could serve as a more environmentally friendly material for automotive tubing, seat materials, interior panels, and trim parts.

However, in case the of PHA will be more competitive and affordable at cost level, and PHA will become comparable to the cheaper, nondegradable plastics, and similar in costs to polymers originated from petrochemicals (whose availability is estimated to decrease with time), it is envisaged that also the packaging industry will see an increase of the requests for PHA-based films and molded forms, and in fiber reinforced material for textiles, bioplastic composites for aerospace, automotive industries.

Sustainability of materials is a necessary aspect that need to be kept into account, needing to meet current and future regulatory requirements in many markets. The consumers will support a premium cost for products and services with positive social and environmental impact. Biobased products are set to capture an increasingly large share of the consume market.

PHA in Medical Applications

The most promising field of applications due to the high competitivity of products and high prize of the instruments is in the high-tech electronic devices and in the biomedical sector. PHA polymers, depending on the properties of the copolymer used, have been applied to development of medical devices, surgical sutures, stents,

cardiac valves, in tissue repair and regenerative medicine applications [116–118], bone and tendon repair [119–121], titanium bone implants with enhanced antibacterial power [122], tissue engineering [2, 123], artificial organ reconstruction such as artificial esophagus replacement, in drug delivery for nutritional/therapeutic uses, such as nanoparticles-releasing bioactive drugs. In pharmacology, PHB can be used as microcapsules in therapy or as materials for cell and tablet packaging, for encapsulation of Langerhans cells to restore insulin production and release [124, 125], and for coupling the bioplastics with arginine-glycine-aspartate (RGD) cell adhesion motif [126] or in microbeads for targeted drug release [127].

Antibacterial PHAs coating for titanium implants have been developed through the incorporation of antibiotics into the PHA polymer [122].

In biomedical applications, PHB is compatible with human cells and tissues. The hydroxybutyrate is present in the human body and is metabolized. Since PHB is reabsorbed in the body, it could be incorporated in implants in surgery, in sutures, in seam threads, as wound healing and blood vessel reconstruction. At Massachusetts Institute of Technology, several applications of bioplastics with slow release of chemotherapeutics implanted in the brain have been produced and patented. This, together with the effect of blood-brain barrier, encloses spatially and temporally the chemotherapeutics for a local and prolonged bioactivity.

Biomedical applications have been described in the last 5 years. Scaffold from poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) and poly-(3-hydroxybutyrate) mixed to type-I collagen have been tested for tissue engineering [45, 116].

Long bone fractures, bone fragility, and osteoporosis problems have been medicated using bioresorbable materials based on PHAs [128]. Osteoblast cells were shown to grow at high density on nanocomposite scaffold of hydroxyapatite/titania coated with poly hydroxybutyrate [121], making bone implants to be realized at high success rate.

Furthermore, PHA scaffolds for bone tissue engineering and orthotopic bone implants based on a hybrid construction from poly(3-Hydroxybutyrate) have been tested and applied in preclinical studies [3]. There are also studies to apply poly-3-hydroxyoctanoate as peripheral nerve graft. In general, the technological readiness level of these prototypes is at research and development level, so there is a need to push the advancements in this field to a preindustrial and demonstration level.

Conclusions

The PHAs have shown their potential as low-cost biodegradable plastics. Presently, there are still some bottlenecks in their wide use and applications, since the high costs of production, in respect to other nonbiodegradable plastics, and to the problems in controlling the ratio of monomers that influences the PHA properties. In order to lower costs of PHA production, agricultural byproducts, industrial wastes, and biorefinery byproducts have been successfully used as feedstock. One of the main bottlenecks in the application of PHB for the production of single-use items is based on its relatively high cost (7–10 Euro/kg) when compared to other

polymers. In this respect, the use of waste feedstock for the culture of the microorganisms accumulating PHAs is a way to lead to their greater economic viability and sustainability. Presently, different research studies are ongoing regarding the improvement of the yield of PHA by genetic modification of the bacteria or the use of waste for their growth. The β-oxidation weakened *Pseudomonas* strains newly developed have increased the range of polymers and copolymers that can be produced, allowing to obtain PHA with controlled mechanical and thermal properties. The possibility to introduce various functional groups into the PHA side chains and side chain grafting has made possible to enlarge the multiplicity of polymers available for industrial applications, with high value-added functionalities. Further investigations and efforts will allow reducing the production costs of PHA polymers, increasing the industrial sustainability and commercialization of PHAs.

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