REVIEW

Pseudomonas Species as Producers of Eco-friendly Polyhydroxyalkanoates

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Abstract

Polyhydroxyalkanoates (PHAs) have been produced by various bacteria as natural polymers stored in bacterial cells as a source of carbon and energy. They are currently preferred biomaterials for use in many industrial fields instead of conventional non-degradable plastics. Due to their unique properties they can reduce pollution caused by the increasing global polymer demand. *Pseudomonas* species have been chosen as PHAs producers in many recent studies. Being metabolically versatile and possessing a remarkable tolerance to a wide range of carbon sources, these bacteria have become an efficient cell factory for PHAs production. Currently, attention is focused on the design of *Pseudomonas* strains to increase their ability to accumulate PHAs in the cell and modifying their biosynthetic pathways to obtain strains with modified compositions and improved properties. This article discusses the current state of knowledge of polyhydroxyalkanoates synthesized by *Pseudomonas* species which are industrially important microorganisms. This review provides an overview of recent trends towards PHA production, focusing on the utilization of low-cost carbon sources, fermentation strategies, PHAs properties and their uses as valuable bioproducts.

Keywords Biodegradable polymers · Bioproducts · Polyhydroxyalkanoates · *Pseudomonas* sp.

Introduction

Due to their excellent properties, petroleum-based plastics are intensively used in everyday life and are an essential part of modern society. Because of their stability and durability they are replacing glass, wood, metal in many industrial and medical applications. It is estimated that over 300 million tons of plastics are produced in the world annually and about 50% of this volume is for disposal applications - products that are discarded within a year of their purchase [1]. Plastic waste management has become a global issue. However, being non-biodegradable, synthetic plastics are persistent and accumulate in the natural environment resulting in a significant burden on waste management. Furthermore, it has become evident that fossil fuel reserves are finite and will be depleted soon. Therefore, there is a need to develop new technologies for producing materials that have polymer-like properties and degrade after being discarded [2]. Among the various types of biodegradable polymers polyhydroxyalkanoates (PHAs) are especially attractive because they have useful properties due to the fact that they are biodegradable, non-toxic and biocompatible. PHAs are stored intracellularly by many microorganisms (gram-positive and gram-negative bacteria) as carbon and energy reserves [3]. For the first time, they were obtained from *Bacillus megaterium* in 1926 by French researcher Lemoigne [4]. Later, it was found that PHAs are accumulated by microorganisms as the reserve materials of carbon and energy. Since then, many companies have been set up to commercialize polyhydroxyalkanoates. Large scale PHA producers sell them as raw materials in the form of pellets or powder. PHAs are also marketed as the end-product with a defined applications. They are developed as additives for PVC and PLA, micropowder for cosmetics, sutures or films for medical applications [5]. However, the global PHAs market is still small compared to petroleum-based polymer production. According to the latest market data published by European Bioplastics [6], global biopolymers production reached 2.11 millions tonnes in 2018, including 1.4% of PHAs. Therefore, both

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industry and research put great effort into the screening of host producers used for fermentative PHAs production and to optimize the bioprocess towards the ultrahigh productivity of these biopolymers.

Many studies provide data on several PHA producers. It is well known that microorganisms capable of synthesizing PHAs can be divided into two groups. Bacteria belong to the first group are able to synthesize polyhydroxyalkanoates under nutrient limitation such as oxygen, nitrogen or phosphorus and they do not accumulate PHAs in their growth phase. The second group is presented by microorganisms that accumulate biopolymers during the growth phase and do not need to limit the nutrients [7].

Pseudomonas strains are especially extensively studied as metabolically versatile bacteria which have become efficient cell factories for the production of value-added compounds [8]. Due to their versatility, degradative potential and capacity to use a wide range of carbon sources, they are considered to be attractive candidates in a number of biotechnological applications. Biopolymers extracted from these bacteria can be used in various sectors from consumable goods to medicine [9, 10].

This review gives insights into recent challenges and progress in PHAs synthesis by *Pseudomonas* species. It provides an overview of recent trends towards PHAs production focusing on the development of fermentation strategies, utilization of different carbon sources, properties of PHAs and their use as valuable environmentally-friendly bioproducts.

Structure, Classification and Biochemical Pathways

Polyhydroxyalkanoates are synthesized in the bacterial cells as insoluble granules that form primarily a reservoir of carbon and energy. It has been proven that the biological function of PHAs is far more comprehensive. The involvement of PHA in stress resistance of microbial cells has been recently discussed and summarized by Obruca et al. [11]. Recent studies revealed that the presence of PHA in bacterial cells can help bacteria to preserve cell integrity when exposed to sudden osmotic imbalances [12]. In addition, PHA granules can serve as a potent cryoprotectant against the adverse effects of freezing-thawing cycles [13]. Moreover, 3-hydroxybutyrate was evaluated to be an enzyme-protective agent against heating and oxidative damage [14]. Intracellular PHA granules measure a diameter of 0.2 to 0.5 mm. Cytoplasmic PHA granules consist of an amorphous PHAs fraction that forms a hydrophobic core surrounded by proteins bound to the PHA granule. The formation process of the polyhydroxyalkanoate granules is shown in Fig. 1. These biopolyester surfaces are covered by numerous phospholipids and proteins that play an important role in the synthesis, degradation and regulation of their synthesis process [15]. One of the most numerous proteins that coat the surface of the granules are phasins, and it has been shown that the levels of coverage range from 27 to 54% [16]. Cytoplasmic granules can be visualized by phase contrast microscopy due to their high refractivity or by means of dyes such as Nile red and Sudan Black B [17]. It has been reported that under conditions corresponding to the biopolymer biosynthesis, some bacteria are able to produce as much as 90% (w/w) PHAs of cell dry weight [18]. Recently, it has been shown that bacterial cultures lengthen the cells in order to control the PHA volume portion so that it does not exceed about 40% of the cell volume [19].

PHAs are polyesters that consist of hydroxylic acid (HA) monomers linked by an ester bond. The formation of the bond occurs by combining the carboxyl group of the monomer with the hydroxyl group of the adjacent group [21]. The polyhydroxyalkanoates may have a diversified structural composition depending on the bacterial strain used and the carbon source provided during cultivation. Biopolymers can be divided into two groups due to the number of carbon atoms in the monomer units: short-chain-length polyhydroxyalkanoates (scl-PHAs) and medium-chain-length polyhydroxyalkanoates (mcl-PHAs) [22]. Scl-PHAs contain from 3 to 5 carbon atoms in each monomer units and
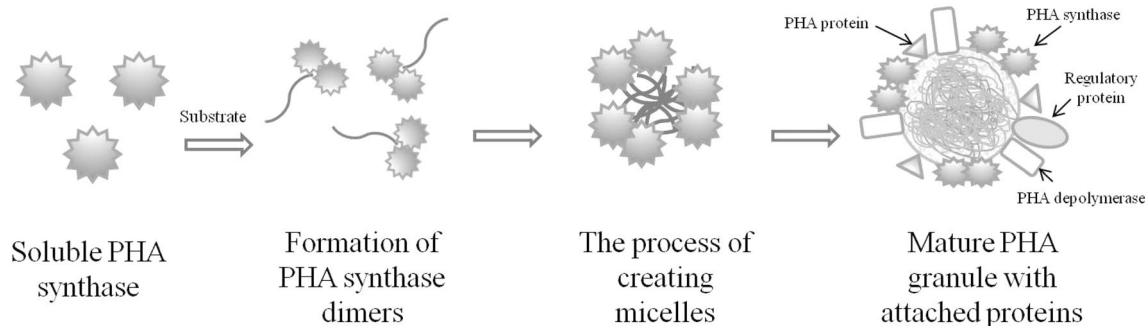

Fig. 1 PHAs granule formation process. Modified according to Rehm [20]

mcl-PHAs consist of 6–14 carbon atoms in each unit. The most commonly synthesized scl-PHAs are poly-3-hydroxybutyrate [P(3HB)]. It is a crystalline polymer with a fragile, rigid structure that has a high melting point and a low glass transition temperature. P(3HB) is produced by numerous microorganisms, including *Cupriavidus necator* and *Alcaligenes latus*. Mcl-PHAs like poly-3-hydroxyoctanoate [P(3HO)] or poly-3-hydroxyhexanoate [P(3HHx)] are more desirable than scl-PHAs because of high elasticity with low crystallinity and tensile strength as well as a high melting point, as shown in Table 1 [21]. They are synthesized mainly by *Pseudomonas* species. In addition, some bacterial strains are able to scl-, mcl- and scl-mcl-copolymers. To date, at least 150 different monomer possessing various structures have been found. Different functional groups on

Table 1 Comparison of properties of scl-PHAs with mcl-PHAs

Properties	Homopolymer scl-PHAs	Homopolymer mcl-PHAs
Crystallinity (%)	80	40
Melting temperature (°C)	179	86
Glass transition temperature (°C)	4	− 40
Tensile strength (Mpa)	5	20
Young's modulus (Gpa)	3.5	1–2
Elongation to break (%)	40	300
Resistance to UV light	Good	Good
Resistance to solvents	Weak	Weak
Biodegradability	Good	Good

scl-PHAs short-chain-length polyhydroxyalkanoates, *mcl-PHAs* medium-chain-length polyhydroxyalkanoates

the monomer chain (such as halogen, epoxy-, hydroxy-, carboxyl- and esterified carboxyl) have also been discovered [23].

The type of polyhydroxyalkanoates produced depends on the metabolic pathway of the bacterium being used. Carbon sources are metabolized differently in bacteria. Bacteria belonging to *Pseudomonas* species involve two metabolic pathways to generate precursors for mcl-PHAs synthesis (Fig. 2). Each pathway uses a different substrate to provide 3-hydroxyacyl (3HA) precursors, which are then used to synthesize mcl-PHAs. Aliphatic carbon sources, such as fatty acids, are degraded by pseudomonads via the β-oxidation process. This pathway is involved in the oxidation of fatty acids into enoyl-CoA, (S)-3-hydroxyacyl-CoA and (R)-3-ketoacyl-CoA. They are then converted into (R)-3-hydroxyacyl-CoA. *Pseudomonas* species are also able to use the de novo synthesis fatty acid pathway to synthesize mcl-PHA monomers from unrelated carbon sources, such as glucose, gluconate or ethanol [24]. In this process, these substrates are oxidized into acetyl-CoA and then converted by a series of reactions into malonyl-CoA and activating by transacylation to (R)-3HA-acyl carrier protein (ACP). The resulting acyl-ACP and malonyl-ACP intermediates are transformed into (R)-3-hydroxyacyl-ACP being further elongated by successive two-carbon units. In most pseudomonads, the specific transacylase PhaG transforms acyl-ACP intermediates into (R)-3-hydroxyacyl-CoA [25].

The synthesis of polyhydroxyalkanoates by microorganisms is possible due to the substrate specificity of the PHA polymerase, i.e. α-/β-hydrolase family enzymes catalysing the polymerization of (R)-3-hydroxyacyl-CoA thioester monomers. PHA synthases are divided into four groups
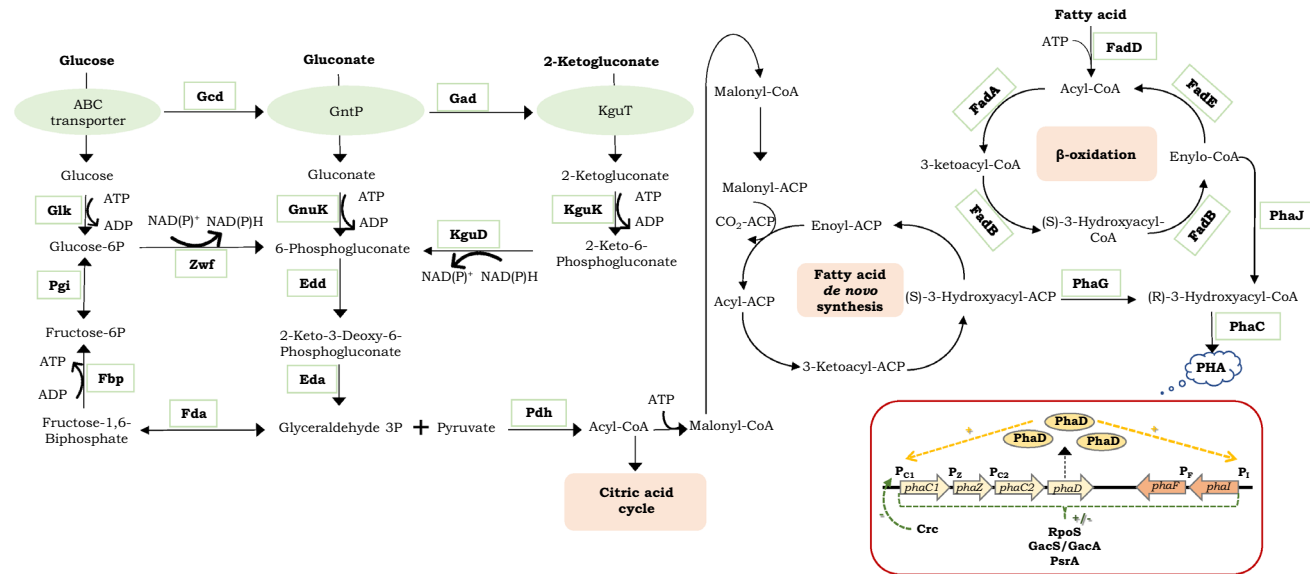

Fig. 2 Metabolic pathways and regulatory network of PHAs metabolism in *Pseudomonas* species

based on their substrate specificity and subunit composition [26]. Class I and II synthases consist of one PhaC subunit, whose molecular mass is in the range of 61–73 kDa [27]. Class III and IV synthases contain two different subunits. Class III synthase has PhaC and PhaE subunits with a similar molecular mass − 40 kDa. Class IV synthases consists of two subunits: PhaC and PhaR. Classes I, III, and IV use short-chain-length monomers, while class II uses medium-chain-length monomers being clearly different than other classes [28]. The class II of PHA synthase was found in *Pseudomonas* species.

In pseudomonads six proteins have been characterized as being essential for the accumulation and synthesis of polyhydroxyalkanotes: two polymerases (PhaC1, PhaC2) depolymerase (PhaZ), two phasins (PhaI, PhaF) and two regulatory proteins (PhaD, PhaG). In *Pseudomonas* species the *pha* cluster is very well-conserved and is organized into two operons *phaC1ZC2D* and *phaFI*. The PhaC1, PhaC2, PhaZ and PhaD proteins are encoded by genes transcribed in the same direction, whereas the PhaF and PhaI proteins are transcribed in the opposite direction [29]. It is well-known that *phaC1* and *phaC2* genes are responsible for mcl-PHAs synthesis, while the *phaZ* gene allows the hydrolysis of accumulated biopolymers. PhaD is the only known protein that is not bound to PHA granule surface. It encodes the putative transcription regulator from the TetR family, which plays an essential role in the PHA biosynthesis. The mechanisms of action of *phaD* gene are still unknown, however it was postulated that it prevents expression or binding to major granule proteins leading to mcl-PHA accumulation [30]. PhaF and PhaI are associated with granule formation. They are believed to facilitate a segregation of the granules during cell division serving as an interface between the granule and the cytoplasm [31]. It was recently reported that the PhaF phasin plays a role in the control of *Pseudomonas putida* shape and length [32]. Sierro [33] revealed that the activity of the *phaF* gene is reinforced by the presence of PhaD, resulting in the production of higher amounts of PhaF and PhaI phasins. That author also showed that PhaI appears to block the Pc1, Pc2 and Pi promoter-driven expression, while PhaD is able to induce both phasins (PhaF and PhaI). The expression of the *pha* genes is under the control of global transcriptional regulators such as the Crc, RpoS, PsrA and GacS/GacA systems (Fig. 2). The regulatory network that could drive the PHA cycle in pseudomonads has been reviewed in detail by Prieto et al. [34].

It has been suggested that PhaG encoded transacylase, which is not co-localised with the PHA biosynthesis gene cluster, could also be involved in PHA biosynthesis from non-related carbon sources, such as gluconate and glucose, linking fatty acid *de novo* biosynthesis with PHA biosynthesis. It is known that *Pseudomonas putida* GPo1 is incapable of synthesizing mcl-PHAs out of a structurally-unrelated carbon source, because of the lack of PhaG transcription [35]. However, Możejko-Ciesielska et al. [36] recently added to these findings by showing that *phaG* expression is associated with mcl-PHA synthesis on oleic acid (a structurally-related carbon source), suggesting that PhaG may play a role in this process as well. An association between increased *phaG* expression and mcl-PHAs synthesis on oleic acid was also found using the *P. putida* KT2440 *relA/spoT* mutant [37]. All the above-mentioned interactions show that the regulation of the PHA accumulation process seems to be complex. It is likely that this complexity does not only result from the effects of PhaD, PhaF or PhaI but also from other, yet-undiscovered proteins which affect the activity of promoters of the whole *pha* gene cluster.

Fermentation Strategies

The productivity of polyhydroxyalkanoates by bacteria is dependent on many factors, including the carbon source-to-nitrogen source ratio, cultivation period, temperature, pH and the presence of macro and microelements [38]. However, the most important factor is a species of bacteria that has the ability to accumulate biopolymers. Many microorganisms have been studied for PHA synthesis but most of them do not have a promising capacity to synthesize and accumulate these biopolyesters. The PHA industrial producer should meet several criteria, such as the ability to metabolize raw carbon sources, to achieve a good growth rate and a high biopolymer content in bacterial cells. A number of *Pseudomonas* species have been reported to synthesize polyhydroxyalkanoates in satisfactory amounts and at a low cost [39]. The PHA productivity in bacteria belonging to *Pseudomonas* genus could be so high as during bioprocesses using other bacterial genera such as *Cupriavidus, Burkholderia, Haloferax*. The fermentations processes conducted with the above-mentioned prokaryotes were comprehensively reviewed by Możejko-Ciesielska and Kiewisz [40].

To optimize PHA yield, various types of fermentation strategies have been used, including batch, fed-batch and continuous processes. To enhance polymer productivity by the bacteria within a short time, cultivations can be carried out under varied conditions. Table 2 gives an overview of the results obtained using various cultivation strategies and employing different *Pseudomnas* species as PHA producers.

Batch Cultivations

Due to the reasonable overall production costs, batch fermentation is widely used in industrial fermentation processes. It is a closed cultivation strategy based on the supplementation of the culture medium with a carbon source at the beginning of the process and the removal of the

Table 2 Summary of results of different strategies fermentation for PHAs production by *Pseudomonas* sp.

Microbial strain	Carbon sources	Biomass (g l⁻¹)	PHA content (%)	M_w (kDa)	M_n (kDa)	T_m (°C)	T_g (°C)	References
Batch fermentation								
Ps. putida GO16	Terephthalic acid	1.0	27.0	7.4	3.7	35	− 53	[41]
Ps. putida GO19			23.0	12.3	5.2	34	− 53	
Ps. frederiksbergensis GO23			24.0	9.3	4.4	35	− 53	
Ps. oleovorans ATCC 29347	Jatropha curcas oil	–	26.06	166.8	131.8	150–160	NO	[42]
Ps. aeruginosa ATCC 27853	Oleic acid	0.38	15	57	26	50	− 52	[43]
	Erucic acid	0.866	9.3	122	63	50	− 46	
	Nervonic acid	0.416	10	114	56	50	− 43	
Ps. putida KT2442	Decanoic acid	5.78	15.2	ND	ND	ND	ND	[44]
	Dodecanoic acid	6.96	14.9	100.0	80.0	53	− 44	
	Tetradecanoic acid	2.74	21.82	ND	ND	ND	ND	
Ps. putida KT2442	Hexanoate	3.01	13.5	272.0	206.0	ND	− 28.19	[45]
	Heptanoate	4.11	28.2	455.0	252.0	no	− 32.13	
	Octanoate	4.64	28.9	180.0	148.0	66.06	− 38.38	
	Nonanoate	3.90	57.4	ND	ND	ND	ND	
Pseudomonas sp.	Corn oil	12.53	35.63	ND	ND	ND	ND	[46]
Pseudomonas sp. TN301	Naphthalene	0.48	23.0	ND	ND	ND	ND	[47]
Ps. putida CA-3	Volatile fatty acids	1.56	39	ND	ND	ND	ND	[48]
Ps. putida Bet001	Fatty acids (C₈:₀ to C₁₈:₁)	9.8–15.5	49.7–68.9	60.06–77.66	13.61–38.69	43.3–66.5	− 1.0 – 1.0	[49]
Fed-batch fermentation								
Ps. putida KT2440	Glucose	2.0	1.7	ND	ND	ND	ND	[50]
	Glycerol	1.4	1.6	ND	ND	ND	ND	
	Citrate	1.8	0.4	ND	ND	ND	ND	
Ps. puitda KTQQ20	Decanoic acid	0.94	4.61	361.4	248.6	72.2	− 37.21	[44]
	Dodecanoic acid	1.03	9.19	155.5	119.4	77.62	− 32.49	
Pseudomonas sp. SGP4502	Acetate	0.57	10.1	158.5	73.4	ND	ND	[51]
	Octanoate	1.80	28.0	260.01	107.0	ND	ND	
	Dodecanoate	0.30	36.1	169.5	76.7	ND	ND	
Ps. aeruginosa BP C1	glucose	7	14.7	5.9	5.6	139/227	34.3	[52]
Ps. mosselii TO7	Octanoic acid	1.70	45.20	218.3	98.7	ND	ND	[53]
	Palm kernel oil	4.31	47.10	ND	ND	37.2/55.7	− 42.8	
	Soybean oil	3.76	49.82	ND	ND	46	no	
Ps. putida	Glucose	61.8	67.1	ND	ND	ND	ND	[8]
Ps. putida KT2440	Glucose, Nonanoic acid	2.85	32.0	ND	ND	ND	ND	[54]
Ps. aeruginosa ATCC27853	Heptadecanoic	1.6	9.81	77	48.1	52	− 45	[55]
	Nonadecanoic	2.37	5.36	97	48.5	48	− 43	
	Heneicosanoic	2.74	0.255	188	110.6	49	− 39	
Continuous fermentation								
Ps. oleovorans ATCC 29347	Citrate	1.68	6	ND	ND	ND	ND	[56]
	n-Octane	1.75	37	ND	ND	ND	ND	
Ps. putida GPo1	5-phenylvalerate, octanoate, and 10-undecenoate	0.98–1.06	16–29	ND	ND	ND	ND	[57]
Ps. putida KT2440	Potassium octanoate, Potassium 10-undecenoate	ND	42	ND	ND	ND	ND	[58]

M_w weight avaerage molecular weight, M_n number avaerage molecular weight, T_g glass transition temperature, T_m melting temperature, *ND* not determined

biopolymer at its end. The entire process is maintained in a constant and undisturbed condition [59]. In batch fermentation, two approaches can be conducted: one-stage and two-stage cultivation [60]. In the one-stage process, the cell growth, synthesis and accumulation of the biopolymer take place simultaneously. The two-stage process involves two phases: microbial growth and biopolymer accumulation [61]. In the first stage the cultivation is carried out to ensure sufficient bacterial cell concentration, while in the second stage (nutrient limitation stage) the bacterial growth rate remains constant but the cells begin to store PHAs intracellularly [62]. The batch fermentation process could also be associated with a low yield of polyhydroxyalkanoates due to the degradation of accumulated PHAs as carbon and energy materials after complete use of the substrate, resulting in a reduced amount of the final product [63].

Batch fermentation has been employed in the investigation and optimization of mcl-PHA homopolymers synthesized by *Pseudomonas* species [64] investigated the batch production of 3-hydroxyoctanoate [P(3HO)]. The authors reported that 31.38% of this homopolymer can be produced by *Pseudomonas mendocina* grown on sodium octanoate. It was reported that *Pseudomonas* sp. could also utilize aromatic hydrocarbons as a feedstock towards mcl-PHAs with different monomeric compositions. Investigations applying batch fermentations of monoaromatic substrates have shown that the strain was able to synthesize 19.2% of CDW grown on o-xylene and 3-methylbenzene. The produced mcl-PHAs contained 3-hydroxyoctanoate (3HO), 3-hydroxydecanoate (3HD) and 3-hydroxydodecanoate (3HDD) as the major components and a small amount of 3-hydroxyhexanoate (3HHx). Furthermore, the same monomeric composition was found when *Pseudomonas* sp. TN301 were grown on naphthalene and achieved higher PHA productivity (22.9 mg g CDW^{-1}) [47]. Lower mcl-PHA concentration was achieved during two-stage batch cultivation of *Pseudomonas aeruginosa* ATCC 27853 using oleic, erucic and nervonic acids. In these cases, the PHAs consisted of five monomers, with 3-hydroxyoctanoate and 3-hydroxydecanoate in greater amounts, and 3-hydroxyhexanoate, 3-hydroxydodecanoate, 3-hydroxy-5-tetradecenoate in lesser amounts [43].

Also, extremophiles belong to *Pseudomonas* genus were employed towards PHA synthesis. *Pseudomonas* sp. SG4502, a thermophilic bacterium, was reported to produce mcl-PHA up to 40% of cell dry weight from a biodiesel fuel by-product as a carbon source [65]. Goh and Tan [66] demonstrated that *Pseudomonas* strains isolated from Antarctic soils were able to synthesize mcl-PHAs. The highest PHAs content (48% of CDW) was achieved in the cultivation of *Pseudomonas* sp. UMAB-40 growing on octanoate. The accumulated biopolyesters contained mainly 3-hydroxyhexanoate and 3-hydroxyoctanoate and a trace amount of 3-hydroxydecanoate. *Pseudomonas extremaustralis* 14-3b,

a highly stress resistant bacterium isolated from Antarctica, has been extensively studied for its potential in synthesizing PHA. This strain was able to accumulate large quantities of PHB and minor amounts of mcl-PHAs when grown with octanoate or glucose as carbon source [67].

Fed-Batch Cultivation

A fed-batch culture is a more efficient process than batch cultivation, because it achieves a high bacterial cell density and the highest possible bioproduct concentration [68, 69]. During fed-batch fermentation, the growth medium is supplemented with an additional portion of a substrate. This bioprocess strategy ensures constant regulation of nutrients, avoids carbon limitations and enables the effective growth of microorganisms and bioproduct accumulation [69]. This type of cultivation is the best method for industrial PHA production. It has been demonstrated that co-feeding in fed-batch cultivations could improve PHA production. According to Jiang et al. [70] *Pseudomonas putida* KT2440 is able to accumulate more mcl-PHAs using acrylic acid together with nonanoic acid and glucose as co-substrates in a fed-batch culture. The authors found that this bacteria synthesized 75.5% of PHA with 89 mol% HN at a nonanoic acid: glucose: acrylic acid feed mass ratio of 1.25: 1: 0.05 and a specific growth rate of 0.15 h^{-1}. Lower mcl-PHA content (32% of CDW) was reported by Davis et al. [54] when nonanoic acid was supplied to the glucose grown cells of the above-mentioned strain without the supply of pure oxygen or oxygen enriched air. Promising results were also published by Chen et al. [53] who demonstrated that a newly-isolated strain TO7 belonging to *Pseudomonas mosseli* cultivated on palm kernel oil was able to accumulate mcl-PHAs in the concentration of 47.1% of CDW. However, the feeding of soybean oil lowered the biopolymer content in these bacterial cells up to 28.6% of CDW [71].

Continuous Cultivations

Continuous cultivation is recognized as a process that enhances productivity and offers a high consistency and uniformity of product quality, providing long-term genetic stability of the organism [72]. The active biomass concentration, PHA content and substrates are kept constant as soon as steady-state conditions are reached. Cell harvest also occurs continuously under these conditions [73]. However, the use of this cultivation strategy for PHA production using *Pseudomonas* sp. has been limited with only a few examples. A major disadvantage of this technique seems to be the risk of microbial contamination that can endanger whole fermentation batches and, consequently, cause extensive economic loss [74]. The first continuous mcl-PHA biosynthesis was conducted using *Pseudomonas putida* GPo1 [75]. The

authors confirmed that continuous cultivation was a way to provide the bacterial cells with sufficient substrate without an inhibition of substrate concentrations in the culture medium. At the dilution rate of 0.24 h^{-1} and an increasing carbon/nitrogen ratio, the percentage of PHAs in the biomass was constant (13% of CDW). When the specific growth rate was below the maximum specific growth rate of 0.3 h^{-1}, the extracted PHAs consist of a constant proportion of monomer composition with a 3HB/3HHx/3HO/3HD ratio of 0.1:1.7:20.7:1.0. Due to the effects of higher concentrations of unmetabolised octanoate in the fermentor an increase of μ_{max} was observed. It causes a decrease in the percentage of 3HO with a concomitant increase in 3HD monomers. *Pseudomonas oleovorans* ATCC 29347 grown on gaseous octane also forms mcl-PHAs most effectively at growth rates below the maximum specific growth rate in a two-stage continuous system. The connection of two biofermentors in a series was confirmed to be a useful approach to achieve a specific growth rate of bacteria in the first compartment, leading to the generation of PHAs at higher rates in the second compartment. Under the applied conditions, *Pseudomonas oleovorans* cells synthesized 63% PHA of CDW in the effluent of the second fermentor. Due to the completely different kinetics of cell growth and mcl-PHA accumulation the above-mentioned two-stage bioprocess seems to be more efficient than a single stage setup. A maximum mcl-PHA accumulation is reached when the cells grow at a μ_{max} of 0.21 h^{-1}, whereas μ_{max} of *Pseudomonas oleovorans* is 0.48 h^{-1} as demonstrated in single-stage chemostat cultures. Recently, Follonier et al. [58] conducted a 3-stage continuous cultivation for the production of mcl-PHAs using *Pseudomonas putida* KT2440 under elevated pressure. The data indicated that the mcl-PHA content, yield on substrate and volumetric productivity were all found to increase at elevated pressure. It was estimated that the oxygen provided to the culture broth under elevated pressure would be sufficient to triple mcl-PHA productivity in the chemostat system from 3.4 (at 1 bar) to 11 g l^{-1} h^{-1} (at 3.2 bar).

Fermentation Process Using Recombinant Pseudomonads

In addition to the wild strains, efforts have been made to use genetically recombined *Pseudomonas* strains for enhanced PHA biosynthesis. These bacteria are recognized as being easy to use for molecular manipulations. It is known that to synthesize PHAs containing non-3-hydroxybutyrate monomers the bacterial cultivations should be supplemented with fatty acids as carbon sources [76]. These substrates are metabolized during the β-oxidation cycle, leading to shortened n-alkanoic acid monomers in the final biopolymer. Therefore, to improve the efficiency of the substrate-to-PHA

conversion, β-oxidation impaired mutants have been constructed. Using lysogeny broth and dodecanoate as co-substrates β-oxidation inhibiting mutant of *Pseudomonas putida* KT2442 was able to form a copolymer containing 4 mol% 3HHx, 37 mol% 3HO, 20 mol% 3HD and 39 mol% 3HDD [77]. Additional deletion of the gene encoding the 3-hydroxyacyl-CoA dehydrogenase increased 3HDD content to 56 mol% [78]. Liu et al. [44] weakened the β-oxidation pathway by deleting fadB (PP2136), fadA (PP2137), fadB2x (PP2214), fadAx (PP2215) and genes coding 3-hydroxyacyl-CoA dehydrogenase (PP2047) and acyl-CoA dehydrogenase (PP2048). The recombinant *P. puitda* KT2442 synthesized homopolymer poly-3-hydroxydecanoate [P(3HD)] or copolymer P(3HD-co-84 mol% 3HDD) growing on decanoate or dodecanoate, respectively. Recently, this recombinant was applied as a random and block co-polymer producer. Tripathi et al. [79] proved that co-monomer compositions could be easily controlled by a predefined ratio of the fatty acids used as carbon sources. According to the authors, *Pseudomonas putida* KTOYO6ΔC (*pha*PCJ$_{A,c}$) cultivated on mixtures of sodium butyrate and sodium hexanoate accumulated random copolymers of P(3HB-co-3HHx) with a monomer fraction of 3HHx content ranging from 19 to 75 mol%. While the *P. putida* KTQQ20 mutant was grown on mixtures of sodium hexanoate and decanoic acid, the recombinant was able to produce random copolymers of P(3HHx-co-3HD) with 3HHx ranging from 16 to 63 mol%. The authors also demonstrated the capability of the recombinants to synthesize a novel diblock copolymer P3HHx-b-P(3HD-co-3HDD) consisting of 49 mol% P3HHx and 51 mol% P(3HD-co-3HDD). In addition, a β-oxidation-deleted *Pseudomonas entomophila* was used to successfully synthesize biopolyesters. Li et al. [80] proved that stabilization of the PHA monomer structures is possible. The authors used a *Pseudomonas entomophila* mutant as a platform for the biosynthesis of polyhydroxyalkanoates with adjustable monomer content and composition. They achieved random copolymers of 3-hydroxydodecanoate (3HDD) and 3-hydroxy-9-decenoate (3H9D) confirming that their compositions can be adjusted by ratios of dodecanoic acid (DDA) to 9-decenol (9DEO) fed to the *Ps. entomophila* culture. Furthermore, it was demonstrated that random copolymers P(3HDD-co-3H9D) could be crossed-linked under UV-radiation due to the presence of the unsaturated bonds. A stable synthesis of homopolymers of 3-hydroxyvalerate to 4-hydroxytetradecanoate was also applied in the cultivations of *Ps. entomophila* LAC23 mutant grown on different fatty acids [81].

The genes from other microorganisms have been introduced into *Pseudomonas* sp. to improve PHA biosynthesis. Le Meur et al. [82] proved that introduction of the genes encoding xylose isomerase (XylA) and xylulokinase (XylB) from *E. coli* W3110 into *Ps. putida* KT2440 was sufficient to allow the recombinant to efficiently utilize xylose as the

sole carbon source. Experiments performed in bioreactors showed that XylA and XylB were active in *Ps. putida* KT2440. The recombinants produce mcl-PHA up to 20% of CDW by sequential-feeding strategy using xylose as the growth substrate and octanoic acid as the precursor for mcl-PHA production. The PHA content of the *Ps. putida* KT2440 mutant increased by 100% compared to the parent strain after the single deletion of the *gcd* gene encoding glucose dehydrogenase. The engineered strain could almost maintain a growth rate at the same level as the wild type [83]. These mutants were also cultured into a high cell density fed-batch with carbon-limiting exponential feeding followed by three different feeding approaches during the PHA accumulation phase (nitrogen-limitation) in the presence of glucose. This approach resulted in 23-fold higher mcl-PHA productivity than with the batch cultivations [84]. The authors successfully deleted *pgl* gene coding for 6-phosphoglucolactonase. In terms of mcl-PHAs synthesis, the *Ps. putida Δgcd-pgl* mutant strain accumulated 33% more biopolymers than the wild type KT2440.

PHA Synthesis by *Pseudomonas* sp. Using Cheap Carbon sources

Microbial PHA production can not compete with synthetic plastics for large-scale production because of the high cost of bacterial fermentation. The cost of the carbon substrate accounts for about 50% of the overall production costs. Therefore, there is a growing need for the development of novel microbial processes using inexpensive carbon sources. Such substrates should meet several basic requirements such as an availability in constant amounts and quality throughout the year and should also have the same composition, purity, stability and resistance to deterioration. There are a number of publications which have reviewed the selection of suitable substrates for efficient PHA synthesis and for making this bioprocess economically reasonable [39, 85]. A detailed overview of microbial PHA synthesis by bacteria belonging to the *Pseudomonas s*pecies is presented in Table 3.

Many waste plant oils have been tested towards polyhydroxyalkanoates synthesis by *Pseudomonas* sp. They are not recovered and may cause serious environmental problems. Moreover, the use of plant oil as one of the most efficiently consumed products in the world for biopolymer production could decrease costs and, as a consequence of PHA adoption, may become more widespread. Generally, this bioprocess is associated with lipase activity. However, it was reported that some pseudomonads capable of producing lipase were unable to convert plant oils into biopolymers. Therefore, in some cases, an additional saponification step was conducted [88, 101]. The composition of the monomer fraction of PHAs reflects the composition of plant oils used.

It was confirmed that *Pseudomonas* sp. cultivated on long fatty acids consisting of from 13-carbon to 18-carbon are able to synthesize PHAs containing 8-carbon and 10-carbon monomers. Możejko et al. [86] demonstrated the ability of *Pseudomonas* sp. Gl01 to accumulate 21% CDW of mcl-PHAs grown on waste rapeseed oil. The authors reported that 3-hydroxyoctanoate and 3-hydroxydecanoate were the major components of the extracted mcl-PHAs. A much higher mcl-PHA content in the same bacterial strain has been achieved in the fed-batch fermentations using saponified waste palm oil. It has been shown that bacteria are able to synthesize up to 48% of mcl-PHAs consisting of monomers such as 3-HHx, 3-HDD, 3-HTD. Additionally, up to 63 mol% of 3-HN monomer was identified in the extracted biopolymer indicating the presence of uneven fatty acids in the waste palm oil used [88]. According to Sharma et al. [92], the PA23 strain of *Pseudomonas chlororaphilis* is able to synthesize mcl-PHAs at the lower level of 10.5% of CDW with a biomass accumulation of 4.6 g l^{-1} when grown on waste frying oil. The authors detected 3-hydroxyoctanoate followed by 3-hydroxydecanoate and 3-hydroxydodecanoate as the dominant subunits in the extracted biopolymers.

Recently, it was revealed that other wastes from the food industry like fruit pomace could replace the costly sugars and fatty acids typically used as carbon substrates for the *Pseudomonas* sp. fermentations. Follonier et al. [90] conducted a two-step fermentation process with *Pseudomonas resinovorans*, hydrolysed pomace as a growth substrate and waste frying oil as a mcl-PHA precursor. The final biomass concentration was 10.2 g l^{-1} and 6.1 g l^{-1} for apricots and Solaris grapes pomace, respectively. Interestingly, Solaris grapes proved to be a very promising growth substrate, resulting in the accumulation of 21.3 g PHA l^{-1} pomace compared to 1.4 g PHA l^{-1} pomace for apricots, corresponding to a volumetric productivity of 0.05 and 0.03 g mcl-PHA l^{-1} h^{-1}, respectively. The mcl-PHA composition was very similar in both cases and consisted of a mixture of 3-hydroxyhexanoate, 3-hydroxyoctanoate, 3-hydroxydecanoate, 3-hydroxydodecanoate and 3-hydroxytetradecenoate which is consistent with the fact that these biopolymers were derived from the same precursor substrate.

The increasing demand for biodiesel production worldwide has led to a surplus of crude glycerol as a main byproduct. Disposal of biodiesel-derived glycerol has created problems with waste management and water pollution [102]. Thus, microbial fermentation to convert crude glycerol to value-added products like polyhydroxyalkanoates is an attractive solution. Recently, it was revealed that partially-refined glycerol could be utilized towards mcl-PHA synthesis by strain 9.1 belonging to *Ps. mediterranea*. However, this strain was capable of reaching a cell dry mass of 3 g l^{-1} with 21% of mcl-PHA content after 72 h of the cultivation [94]. Chanasit et al. [95] demonstrated that a maximum

Table 3 An overview of microbial synthesis of PHAs by *Pseudomonas* sp. grown on renewable carbon sources

Strain	Cultivation mode	Carbon sources	Biomass (g l^{-1})	PHA content (%)	M_w (kDa)	M_n (kDa)	T_m (°C)	T_g (°C)	References
Food industry wastes									
Pseudomonas sp.	Batch	Waste corn oil	12.53	35.63	ND	ND	ND	ND	[46]
Pseudomonas sp. Gl01	Fed-batch	Waste rapeseed oil	5.5	21.0	ND	ND	ND	ND	[86]
Pseudomonas sp. Gl06	Fed-batch	Waste rapeseed oil	4.8	19.3	ND	ND	ND	ND	[86]
Pseudomonas sp. Gl01	Two-step fed-batch	Waste rapeseed oil	3.0	20	ND	ND	ND	ND	[87]
Pseudomonas sp. Gl01	Fed-batch	Waste palm oil	3.8	43.0	109.0	65.0	86	−55	[88]
Pseudomonas sp. Gl01	Fed-batch	Waste rapeseed oil	4.4	44.0	144.0	58.0	46	−38	[89]
Ps. resinovorans	Two-step fed batch	I°—Apricot pomace II°—waste frying oil	10.2	12.4	ND	ND	ND	ND	[90]
Ps. resinovorans	Two-step fed batch	I°—Solaris grape pomace II°—waste frying oil	6.1	23.3	ND	ND	ND	ND	
Ps. putida KT2440	Two-step fed batch	I°—grapes pomace II°—octanoic acid, 10-undecenoic acid	14.2	41.1	139.0	70.6	NO	−45.5	[91]
Ps. chlororaphilis PA23	Batch	Waste canola fryer oil	4.88	10.56	ND	ND	ND	ND	[92]
Biodiesel derived-glycerol									
Ps. oleovorans NRRL B-14682	Batch	Crude glycerol	2.9	1.1	85.0	31.0	ND	ND	[93]
Ps. mediterranea	Batch	Partially refined glycerol	3.0	21	55.0	41.0	40.6	−40.6	[94]
Ps. mendocina	Batch	Biodiesel derived liquid	3.65	77	ND	ND	ND	ND	[95]
Ps. mediterranea 9.1	Batch	Crude glycerol	4.77	61.4	ND	ND	ND	ND	[96]
Ps. corrugata A1	Batch	Crude glycerol	4.72	41.9	ND	ND	ND	ND	
Ps. corrugata 388	Batch	Crude glycerol	4.25	28.2	ND	ND	ND	ND	
Ps. citronellolis	Fed-batch	Tallow-based biodiesel	42	26.6	78.0	19.6	53.6	−43.5	[97]
Pseudomonas sp. ASC2	Batch	Crude glycerol	32.3	61.8	6.5	3.6	ND	ND	[98]
Ps. chlororaphis PA23	Batch	Biodiesel-derived free fatty acids	4.03	24.17	ND	ND	ND	ND	[92]
Ps. mosselii TO7	Fed-batch	Crude glycerol	1.31	48.4	9.7	7.5	49	NO	[71]
Sugar refinary wastes									
Ps. fluorescens A2a5	Batch	Sugarcane liquor	32.0	70	ND	ND	ND	ND	[99]

Table 3 (continued)

Strain	Cultivation mode	Carbon sources	Biomass (g l^{-1})	PHA content (%)	M_w (kDa)	M_n (kDa)	T_m (°C)	T_g (°C)	References
Pseudomonas sp.	Batch	Spent wash	8.56	25.46	ND	ND	ND	ND	[46]
Pseudomonas sp.	Batch	Fermented wash	7.02	23.56	ND	ND	ND	ND	
Pseudomonas sp.	Batch	Molasses	10.54	20.63	ND	ND	ND	ND	
Ps. aeruginosa NCIM 2948	Batch	Cane Molasses	6.63	62.44	ND	ND	ND	ND	[100]

M_w weight avaerage molecular weight, M_n number avaerage molecular weight, T_g glass transition temperature, T_m melting temperature, *ND* not determined, *NO* not observed

biomass concentration of 3.65 g l^{-1} with a biopolymer content of 77% CDW was accumulated by *Pseudomonas mendocina* PSU when grown with 20 g l^{-1} of a biodiesel liquid waste. The authors determined that a higher concentration of the substrate from 30 to 50 g l^{-1} caused a reduction in the biomass and PHA content. Other batch cultures of *Pseudomonas* species have also been employed in the investigation and optimization of mcl-PHA synthesis using biodiesel-derived glycerol. Palmeri et al. [96] extracted 2.93, 1.98 and 1.28 g PHA l^{-1} from *Ps. mediterranea* 9.1, *Ps. corrugata* 388 and A1 growing on crude glycerol, respectively. The chromatographic profile of PHAs obtained by *Ps. corrugata* A1 and 388 strains showed peaks referring to methyl 9-do-decenoate (C12:1), methyl hexadecanoate (C16:0) and methyl 9-hexadecenoate (C16:1). Interestingly, *Ps. mediterranea* 9.1 chromatogram of PHAs also revealed oleic acid methyl ester (C18:1) and linoleic acid methyl ester (C19:3). Recently, promising results have been published by Liu et al. [71] who cultivated *Ps. mosseli* TO7 to 1.31 g CDW l^{-1} on waste glycerol. The PHA concentration reached 48% of CDW with a productivity of 13.16 mg PHA l^{-1} h^{-1}. Tallow-based biodiesel was used by Muhr et al. [97] as a substrate in mcl-PHA production by *Ps. citronellolis*. In this work, final intracellular PHA contents calculated from the sum of active biomass and PHA reached 26.6% of CDW. A GC-FID analysis showed that the obtained biopolyester predominantly consists of 3-hydroxyoctanoate and 3-hydroxydecanoate, and lower amounts of 3-hydroxydodecanoate, 3-hydroxynonanoate, 3-hydroxyhexanoate, and 3-hydroxyheptanoate monomers. However, Muangwong et al. [98] reported that *Pseudomonas* sp. ACS2 utilized crude glycerol to synthesize up to 61.8% of CDW of a copolymer poly (5 mol% 3-hydroxyoctanoate-co-95 mol% 3-hydroxy-5-cis-dedecanoate).

Molasses, a common industrial by-product of sugar production, is widely available and is much cheaper than glucose. Since it consists of about 50% sucrose along with a significant amount of fructose and glucose, it seems to be a good carbon source for PHA production in the biological

processes. However, only a few studies have been conducted into the utilization of molasses for biopolymer synthesis. Jiang et al. [99] observed the production of 70% of polyhydroxyalkanoates with the highest cell concentration of 32 g l^{-1} when the cultivation was supplemented with sugarcane liquor. In addition, other by-products from the sugar industry have been found to be good substrates for the growth of *Pseudomonas* sp. and for subsequent PHA production. A maximum PHA content was observed in the first 24 h of the cultivations, resulting in 25.46%, 23.56% and 20.65% of biopolymers when *Pseudomonas* sp. grown on spent wash, fermented mash and molasses, respectively [46]. Furthermore, Tripathi et al. [103] successfully utilized cane molasses for the PHA production using *Pseudomonas aeruginosa* NCIM 2948. A cell density of 6.6 g l^{-1} and a total PHA content of 62.4% CDW were determined after 60 h of the fermentation process.

Material Properties and Applications of Polyhydroxyalkanoates Synthesized by *Pseudomonas* sp.

Polyhydroxyalkanoates have similar properties to synthetic polymers like polypropylene or polystyrene. PHAs vary considerably in their chemical, mechanical and physical properties. The properties of mcl-PHAs are different from scl-PHAs. The characteristics of the final biopolymer depend on PHA producer, the fermentation mode and conditions used during the bioprocess. Scl-PHAs are highly crystalline, brittle and stiff. Their melting temperature (T_m) ranges from 173 to 180 °C and the glass transition temperature (T_g) lies between 5 and 9 °C. However, mcl-PHAs are thermoelastomers with low Tm ranging between 40 and 86 °C and Tg values between −50 and −25 °C [104].

Generally, glass and melting transition temperature are important parameters that determine in-service application of polyhydroxyalkanoates. They evaluate the temperature

limits for potential applications, especially for semicrystalline polymers. The thermal properties of mcl-PHAs accumulated by *Pseudomonas citronelloli* showed a Tm value in the range of 53.6 °C with a ΔHm of about 20 J/g. These values indicate that the samples are not totally amorphous but partially crystalline with classical rubber-to-latex-like characteristics [83]. This might be due to an assembling of both the main and the side chains of the polymer in a layered order as frequently observed for other polymers harbouring long side chains [105]. Higher thermal values were determined for scl-copolymer by Allen et al. [42] who cultivated *Pseudomonas oleovorans* ATCC29347 on Jatropha curcas oil. DSC thermograms showed a poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer melting temperature of up to 160 °C. However, so far the highest reported T_m value to date of the mcl-PHAs isolated from *Pseudomonas* sp. grown on waste substrates was 86 °C [88].

The molecular weight of polyhydroxyalkanoates is regarded as a parameter affecting their properties. Number (M_n) and weight (M_w) average molecular weights of mcl-PHAs are lower compared to those of scl-PHAs. Phukon et al. [52] reported that M_w and M_n for poly(3-hydroxy-valerate-co-5-hydroxydecenoate) copolymer synthesizing by *Pseudomonas aeruginosa* BP C1 grown on glucose were 5.986 and 5.648 Da, respectively. The polydispersity index ($I = M_w/M_n$) bears a narrow value of 1.059, which suggests possession of uniform chain length what is essential for the food and pharmaceutical industries as it does not require further processing for polymer chain uniformity. In contrast, Impallomeni et al. [55] demonstrated that supplementation of long odd-chain fatty acids led to the higher molecular weight values (M_w) of mcl-PHAs from *Pseudomonas aeruginosa* ATCC 27853 which ranged from 77 to 188 kg mol^{-1}.

PHAs have useful properties such as: biodegradability, biocompatibility, non-toxicity, thermoplasticity. In view of their properties they are promising biopolymers in the medical field. Their applications have focused on drug delivery carrier matrix [106], bone tissue engineering [107, 108] and cartilage tissue engineering [109]. In recent years, polyhydroxyalkanoates and their copolymers, and composites have been considered as polymers suitable to develop bone marrow scaffolds, cardiovascular patches, orthopaedic pins, stents, guided tissue repair or regeneration devices, articular cartilage repair devices, nerve guides, tendon repair devices, sutures and wound dressings [110].

Recently, it has been reported that polyhydroxyalkanoates synthesized by *Pseudomonas* sp. could be promising biopolymers in pharmacy. Chung et al. [111] cultivated genetically modified *Pseudomonas entomophila* to produce 3-hydroxyalkanoates as precursors for the synthesis of value-added chemicals including antibiotics, pharmaceuticals, vitamins. Furthermore, the biodegradable mcl-PHAs produced by *Pseudomonas putida* CA-3 was tested to enhance the anti-cancer activity of peptides. O'Connor et al. [112] purified from the mixture of 3-HHx, 3-HO, 3-HD and 3-HDD the dominant monomer 3-hydroxydecanoic acid (R10). This monomer was conjugated to DP18 peptide containing D-amino acids and its derivatives. It was revealed that the conjugated peptides (R10DP18L) improved anti-proliferation activity that appears to be mediated through grater uptake into the cells.

Furthermore, high immunotolerance and low toxicity are the benefits associated with PHAs in tissue engineering. In the study conducted by Naveen et al. [10] the suitability of an unmodified/raw mcl-PHAs (uMCL-PHA) extracted from *Pseudomonas putida* grown on saponified palm kernel oil as a novel scaffold for a future tissue-engineered products was investigated. The authors revealed that uMCL-PHA supports mesenchymal stromal cell attachment. It was confirmed that the cell viability was not affected in mcl-PHA film, a significant increase in cells proliferation was evaluated in day 6 compared to the initial time point (day 3). Also, P(3HB-co-3HHx) was tested as a scaffold using human embryonic stem cells (hESCs), spontaneously differentiated hESCs (SDhESCs) and mesenchymal stem cells (hMSCs). The results showed that undifferentiated hESCs are not viable after 20 days of culture within a PHBHHx/collagen gel hybrid scaffold, whereas hMSCs and SDhESCs demonstrated good viability over the long-term with an overall lack of stimulus-independent differentiation [113]. Lukasiewicz et al. [114] describe an approach to tuning the poor mechanical properties of P(3HB) using mcl-PHAs, as a plasticizer precursor, synthesized by *Pseudomonas mendocina* CH50 during a two-step fed batch cultivation supplemented with waste frying oil as a carbon source. The authors confirmed that an addition of oligomeric bacterial mcl-PHA to P(3HB) resulted in softer and more flexible material with a dominant amorphous phase, giving it potential for application in unique soft tissue engineering. Furthermore, this novel binary PHA polymer was proven to be biocompatible without any cytotoxic effects. It showed high viability and proliferation of C2C12 myoblast cells relevant to skeletal muscle and cardiac tissue engineering.

In addition, polyhydroxyalkanoates synthesized by *Pseudomonas* sp. have been studied as potential biomaterials for stent development. Basnett et al. [115] studied the composite of P(3HO)/bacterial cellulose which was prepared by solvent casting. The P(3HO) was produced by *Pseudomonas mendocina* grown on sodium octanoate as the sole carbon source. The mechanical properties of the prepared matrix film were significantly improved e.g. the tensile strength was found to be higher compared to the neat P(3HO) film. Thermal characterization of the neat as well as the composite films using DSC showed that there was an increase in the glass transition temperature while the melting temperature of the composite films decreased in comparison to the homopolymer

films. Furthermore, an *in vitro* degradation study confirmed that the inclusion of the acetylated cellulose microcrystals in the P(3HO) films increased the degradation rate of the composite films in comparison to the neat P(3HO) film in both PBS and DMEM media. In addition, on the composite film, increased cell growth and proliferation of MEC-1 cells compared to the neat P(3HO) film was observed. The same P(3HO) homopolymer was used to develop a novel blend with P(3HB). In addition, in this case, the blended films exhibited better mechanical and thermal properties. They were found to be highly biocompatible with significantly higher overall protein absorption and cell viability compared to the neat P(3HO) film [116].

Concluding Remarks

Due to current problems with the harmful effects of synthetic plastic materials and increasing concerns about oil shortages, governments and industries put much effort into the development of natural and biodegradable polymers. Polyhydroxyalkanoates are one of the most promising biopolymers that could replace petroleum-based plastics in the future. During more than 90 years of PHA-related research, great progress has been made regarding its biosynthesis, metabolic and genetic regulation and applications. Because of its biodegradability, biocompatibility and nontoxicity, PHAs can be used in many industrial fields ranging from everyday products to medical applications. However, the high manufacturing costs hamper their synthesis on a large scale.

Many bacteria are able to synthesize PHAs, but the *Pseudomonas* species seems to be a potential cell factory for the production of these biopolyesters. *Pseudomonas* sp. without any doubt could become a platform bacterium that is capable of accumulating defined PHAs structures and constant biopolymer properties. Many studies have been carried out so far into improving their biomass concentration, PHA formation and properties. Advances in molecular biology have also helped to improve PHA yields through genetic engineering of bioproducers belonging to the *Pseudomonas* species. However, a greater understanding of genetic and metabolic regulation could lead to the construction of a suitable bacterial platform that will be able to provide a consistent PHA molecular structure to ensure constant material properties which determine their applications. Metabolically-engineered *Pseudomonas* sp. seems to be capable of synthesizing PHAs at the high concentration. The above-mentioned data confirm that genetic manipulation of PHA biosynthetic pathways could considerably enhance the final productivity of the biofermentations. Furthermore, a better understanding of the regulation of genes involved in PHAs synthesis and accumulation will be helpful to improve their

content in bacterial cells and help to isolate new biopolymer films useful in many applications.

One of the major limitations of the industrial production of PHAs is the final cost mainly depending on the price of carbon sources added for microbial growth. Research into the biological synthesis of PHAs should aim at improving the cost-effectiveness and the efficiency of their recovery. The limited knowledge of the PHAs central biochemical network when using low-cost carbon sources hampers the use of *Pseudomonas* in such endeavours. To make the PHAs synthesis process by *Pseudomonas* sp. more feasible, it is essential to put greater effort into the high-throughput screening of potentially new bacterial producers and to optimize the fermentation process using renewable carbon sources. Discontinuous fed-batch cultivations are mainly used for large scale production of PHA. Low volumetric rate or unpredictable bioproduct quality are some of the drawbacks that should be taken into account employing such processes [117]. Therefore, to improve the PHAs productivity, the optimization processes using continuous mode of operation needs to be implemented. Furthermore, the development of a multistage bioreactor cascade could result in production of novel block-co-polyesters with unique properties.

Compliance with Ethical Standards

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