Poly(3-Hydroxypropionate): a Promising Alternative to Fossil-Fuel-Based Materials

Björn Andreeßen,a Nicolas Taylor,a Alexander Steinbüchelab

Institut für Molekulare Mikrobiologie und Biotechnologie, Westfälische Wilhelms-Universität Münster, Münster, Germany; Environmental Sciences Department, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia

Polyhydroxyalkanoates (PHAs) are storage compounds synthesized by numerous microorganisms and have attracted the interest of industry since they are biobased and biodegradable alternatives to fossil fuel-derived plastics. Among PHAs, poly(3-hydroxypropionate) [poly(3HP)] has outstanding material characteristics and exhibits a large variety of applications. As it is not brittle like, e.g., the best-studied PHA, poly(3-hydroxybutyrate) [poly(3HB)], it can be used as a plasticizer in blends to improve their properties. Furthermore, 3-hydroxypropionic acid (3HP) is considered likely to become one of the new industrial building blocks, and it can be obtained from poly(3HP) by simple hydrolysis. Unfortunately, no natural organism is known to accumulate poly(3HP) so far. Thus, several efforts have been made to engineer genetically modified organisms capable of synthesizing the homopolymer or copolymers containing 3HP. In this review, the achievements made so far in efforts to obtain biomass which has accumulated poly(3HP) or 3HP-containing copolymers, as well as the properties of these polyesters and their applications, are compiled and evaluated.

Petroleum-based polymeric materials play a major role in our daily life. The increasing demand for such materials generates two major challenges: (i) diminishing oil reserves will no longer fulfill the high demand from society in the near future and (ii) as those plastics are not biodegradable, waste disposal becomes a key issue (1, 2). For several years, there has been a steadily rising interest in so-called bioplastics. Hence, the group of polyhydroxyalkanoates (PHAs) has attracted widespread attention (3, 4). Several microorganisms accumulate PHAs as intracellular granules when an excess of carbon is provided and when the depletion of at least one other macroelement such as nitrogen, oxygen, or phosphorus occurs simultaneously (5). PHAs are biodegradable, insoluble in water, nontoxic, biocompatible, piezoelectric, thermoplastic, and/or elastomeric (6). These features make PHAs suitable for several applications in the packaging industry and in the medicine, pharmacy, agriculture, and food industries and as raw materials for the production of enantioselectively pure chemicals and for the production of paints (7–12). More than 150 PHAs consisting of various monomers have already been discovered and can be divided into short-carbon-chain-length (scl [fewer than 6 carbon atoms]), medium-carbon-chain-length (mcl [6 to 14 carbon atoms]), and long-carbon-chain-length (lcl [more than 14 carbon atoms]) PHAs (4, 13, 14). Poly(3-hydroxybutyrate) [poly(3HB)] is the most prominent member of the PHA family and belongs to the scl subgroup (4, 15, 16). Since poly(3HB) is very brittle and highly crystalline and exhibits a low elongation at break factor and a high melting temperature, economic expectations with respect to the utility of poly(3HB) as a bioplastic have not yet become a reality. Nevertheless, some PHAs display material characteristics comparable to those of petrochemical-derived polyesters and are completely biodegradable, producing carbon dioxide and water (17).

**POLY(3HP)**

An interesting candidate among the scl PHAs is poly(3-hydroxypropionate) [poly(3HP)]. With a melting point of about 77°C, a glass transition temperature of −20°C, and a fusion enthalpy value of 64 J/g (16, 18–20), it combines a low melting temperature, flexibility, and good degradability with the high stability of, e.g., poly(3HB) (Table 1). Due to these favorable characteristics, several new metabolic pathways, processes, and hosts have been developed since the last review, which was published about 4 years ago (18). As there are still no microorganisms known to synthesize poly(3HP), all attempts rely on recombinant strains. Three different routes for poly(3HP) synthesis have evolved: (i) the propionaldehyde dehydrogenase route (PduP route), (ii) the 3-hydroxypropionate route (3HP route), and (iii) the malonyl-coenzyme A (malonyl-CoA) route.

**PduP ROUTE**

The first pathway for poly(3HP) synthesis was established in 2010 and comprises (i) dehydration of glycerol, (ii) oxidation and CoA ligation of 3-hydroxypropionaldehyde (3HPA) to 3-hydroxypropionyl-CoA (3HP-CoA), and (iii) polymerization of 3HP-CoA to poly(3HP) (21). These reactions are catalyzed by, for example, glycerol dehydratase (dhaB1) from *Clostridium butyricum* DSM2478, propionaldehyde dehydrogenase (*pduP*) from *Salmonella enterica* serovar Typhimurium LT2, and polyhydroxyalkanoate (PHA) synthase (*phaCl*) from *Ralstonia eutropha* H16 (Fig. 1). The respective genes were expressed heterologously in *Escherichia coli*. The recombinant strain was cultivated in a two-step fed-batch fermentation and accumulated up to a 12% ratio of PHA weight to cell dry weight (*wt*PHA/*wt*CDW) poly(3HP) after 92 h with glycerol as the sole carbon source (Table 2).

Wang et al. (22) modified this process by replacing glycerol dehydratase from *C. butyricum*, which is active only under strictly...
TABLE 1 Chemical and physical properties of poly(3HP) compared to other PHA and common fossil fuel-based plastics

<table>
<thead>
<tr>
<th>Fossil fuel-based plastic</th>
<th>$T_m$ (°C)</th>
<th>$\Delta H_m$ (J/g)</th>
<th>$T_c$ (°C)</th>
<th>Young’s modulus (GPa)</th>
<th>Tensile strength $\sigma_t$ (MPa)</th>
<th>Elongation at break $\varepsilon_b$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(3HP) (16, 28)</td>
<td>77</td>
<td>64</td>
<td>–20</td>
<td>0.3</td>
<td>27</td>
<td>634</td>
</tr>
<tr>
<td>Poly(3HB) (2, 16, 28, 36, 68)</td>
<td>177</td>
<td>88</td>
<td>3</td>
<td>3.5</td>
<td>37</td>
<td>6</td>
</tr>
<tr>
<td>Poly(3U) (2)</td>
<td>119</td>
<td>–16</td>
<td>–15</td>
<td>ND</td>
<td>31</td>
<td>14</td>
</tr>
<tr>
<td>Poly(4HB) (2, 69)</td>
<td>53</td>
<td>ND</td>
<td>–45</td>
<td>149.0</td>
<td>104</td>
<td>1,000</td>
</tr>
<tr>
<td>PLA (2, 28)</td>
<td>164</td>
<td>57</td>
<td>2.0</td>
<td>55</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>PP (2, 3, 28, 36)</td>
<td>174</td>
<td>148</td>
<td>–13</td>
<td>1.6</td>
<td>34</td>
<td>400</td>
</tr>
<tr>
<td>PS (36)</td>
<td>110</td>
<td>ND</td>
<td>100</td>
<td>3.7</td>
<td>54</td>
<td>4</td>
</tr>
<tr>
<td>PE (2)</td>
<td>130</td>
<td>293</td>
<td>–36</td>
<td>0.3</td>
<td>10</td>
<td>620</td>
</tr>
</tbody>
</table>

$^a$ $T_m$, melting temperature; PLA, poly-lactic acid; PP, polypropylene; PS, polystyrene; PE, polyethylene; ND, no data available.

anaerobic conditions (23, 24), with vitamin B$_{12}$-dependent glycerol dehydratase DhaB123 from *Klebsiella pneumoniae* ATCC 25955 (25). Furthermore, they coexpressed glycerol dehydratase reactivase (gdrAB) from *K. pneumoniae* to enhance glycerol dehydratase activity, yielding 46.4% (wtPHA/wtCDW) (Table 2) (26). As this process has to deal with low plasmid stability, Gao et al. (27) made several attempts to stabilize the artificial poly(3HP)-synthesizing pathway in *E. coli*. With chromosomal integration of gdrAB and dhaB123 and episomal copies of pduP and phaC1, the cells accumulated up to 67.9% (wtPHA/wtCDW). The productivity of this process is represented by a value of 337 mg PHA liter$^{-1}$ h$^{-1}$, the highest yield reported so far (Table 2).

3HP ROUTE

For poly(3HP) synthesis, 1,3-propanediol (1,3PD) can be used as the substrate, but, unfortunately, this substrate is more expensive than carbon sources from waste streams such as, e.g., glycerol. (i) First, 1,3PD is oxidized to 3HPA by 1,3PD dehydrogenase DhaT (from *Pseudomonas putida* KT2442). (ii) It is then subsequently oxidized to 3HP by aldehyde dehydrogenase AldD (also from *Pseudomonas putida* KT2442). (iii) Ligation to CoA is catalyzed by the Acs domain of propionyl-CoA ligase from *C. aurantiacus*. (iv) Finally, the monomers are polymerized by PhaCl from *R. eutropha* (28) (Fig. 1). The recombinant cells of *E. coli* and those from *Aeromonas hydrophila* contain up to 92% (wtPHA/wtCDW) when cultivated in lysogeny broth medium (LB) containing 10 g/liter 1,3PD. With Terrific broth medium (TB) and 10 g/liter 1,3PD, the polymer content dropped to 57% (wtPHA/wtCDW) but the cell mass increased (Table 2) (28).

To use a cheaper substrate and to avoid the addition of vitamin B$_{12}$, Heinrich et al. modified this pathway for poly(3HP) synthesis and introduced a new expression host, *Shimwellia blattae* (29). *S. blattae*, formerly known as *Escherichia blattae*, is a natural 1,3PD and vitamin B$_{12}$ producer and was isolated from the hindgut of the cockroach *Blatta orientalis* (30–32). For poly(3HP) synthesis, (i) glycerol is dehydrated to 3HPA and (ii) 3HPA is reduced to 1,3PD under anaerobic conditions. Under aerobic conditions, 1,3PD is (iii) first oxidized to 3HPA followed by (iv) a second oxidation step yielding 3-hydroxypropionyl-CoA (3HP). The final steps are (v) ligation to CoA and (vi) polymerization (Fig. 1). The recombinant strain harbors DhaT and AldD of *P. putida* KT2442, propionate-CoA transferase Pct of *Clostridium propionicum* X2, and PHA synthase PhaC1 of *R. eutropha* H16. Poly(3HP) was accumulated by these bacteria to 9.8% (wtPHA/wtCDW) (Table 2). To optimize productivity and poly(3HP) content, the artificial poly(3HP) operon was rearranged (33). With dhaT in the first position, pct in the second position, and aldD in the third position, the polymer content increased to 15% (wtPHA/wtCDW) (Table 2), whereas strains with pct in the first position accumulated only trace amounts of poly(3HP) (33).

FIG 1 Poly(3HP) synthesis. PduP route (---): DhaB and BhaBCE, glycerol dehydratase; PduP, propionaldehyde dehydrogenase; PhaC1, PHA synthase. 3HP route (---): poly(3HP) synthesis from glycerol and 1,3-propanediol. DhaB and BhaBCE, glycerol dehydratase; DhaT, 1,3-propanediol dehydrogenase; AldD, aldehyde dehydrogenase; Pcs’ (Acs), acetyl-CoA carboxylase; Pct, propionate-CoA transferase; PhaC1, PHA synthase. Malonyl-CoA route (---): poly(3HP) synthesis from acetyl-CoA. Acc, acetyl-CoA carboxylase; Mcr, malonyl-CoA reductase; PrpE, propionyl-CoA synthetase; Pcs’ (Acs), acyl-CoA synthase domain of propionyl-CoA ligase; PhaCl, PHA synthase.
TABLE 2 Synthesis of poly(3HP) by recombinant microorganisms*

<table>
<thead>
<tr>
<th>Host</th>
<th>Pathway</th>
<th>PHA content of cells [% (wtPHA/wtCDW)]</th>
<th>Cell density (g/liter)</th>
<th>Productivity (mg PHA liter⁻¹ h⁻¹)</th>
<th>Culture vol (liters)</th>
<th>Cultivation time (h)</th>
<th>Substrate</th>
<th>Cofactor/inducer/antibiotic(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>PduP</td>
<td>12</td>
<td>1.44</td>
<td>15.4</td>
<td>2</td>
<td>92</td>
<td>Glycerol</td>
<td>None/IPTG/Km</td>
<td>21</td>
</tr>
<tr>
<td>E. coli</td>
<td>PduP</td>
<td>46.4</td>
<td>21.8</td>
<td>120</td>
<td>3</td>
<td>84</td>
<td>Glycerol</td>
<td>VB₁₂/IPTG/Ap, Cm</td>
<td>22</td>
</tr>
<tr>
<td>E. coli</td>
<td>PduP</td>
<td>67.9</td>
<td>36</td>
<td>337</td>
<td>3</td>
<td>48</td>
<td>Glycerol</td>
<td>VB₁₂/IPTG/none</td>
<td>27</td>
</tr>
<tr>
<td>E. coli</td>
<td>3HP</td>
<td>92</td>
<td>5.5</td>
<td>115</td>
<td>3</td>
<td>48</td>
<td>1,3PD</td>
<td>None/none/Ap, Km</td>
<td>28</td>
</tr>
<tr>
<td>E. coli</td>
<td>3HP</td>
<td>57</td>
<td>20</td>
<td>260</td>
<td>3</td>
<td>28</td>
<td>1,3PD</td>
<td>None/none/Ap, Km</td>
<td>28</td>
</tr>
<tr>
<td>S. blattae</td>
<td>3HP</td>
<td>9.8</td>
<td>3</td>
<td>5.5</td>
<td>2</td>
<td>72</td>
<td>Glycerol</td>
<td>None/IPTG/Km</td>
<td>29</td>
</tr>
<tr>
<td>S. blattae</td>
<td>3HP</td>
<td>15</td>
<td>3.89</td>
<td>10.3</td>
<td>2</td>
<td>72</td>
<td>Glycerol</td>
<td>None/none/none</td>
<td>33</td>
</tr>
<tr>
<td>E. coli</td>
<td>M-CoA</td>
<td>0.98</td>
<td>1.32</td>
<td>0.18</td>
<td>ND</td>
<td>72</td>
<td>Glucose</td>
<td>Biotin/IPTG/Ap, Cm</td>
<td>34</td>
</tr>
</tbody>
</table>

* 1,3PD, 1,3-propanediol; Ap, ampicillin; Cm, chloramphenicol; IPTG, isopropyl β-D-thiogalactosyl phosphate; Km, kanamycin; ND, no data available; VB₁₂, vitamin B₁₂; 3HP, 3-hydroxypropionate route; M-CoA, malonyl-CoA route; PduP, propionylaldehyde dehydrogenase route.

MALONYL-CoA ROUTE

The only attempt at synthesizing poly(3HP) from an unrelated carbon source starts with acetyl-CoA. As acetyl-CoA is an intermediate of the central metabolism, there is no restriction with respect to the use of a specific carbon source. The pathway exhibits (i) carboxylation of acetyl-CoA to malonyl-CoA, (ii) reduction of malonyl-CoA to 3HP, (iii) ligation to CoA, and (iv) polymerization (Fig. 1). Wang et al. (34) engineered an E. coli strain harboring the genes for malonyl-CoA reductase (mcr) of Chloroflexus aurantiacus, propionyl-CoA synthetase (prpE) and acetyl-CoA carboxylase (accABCED) of E. coli, and PHA synthase (phaC1) of R. eutropha. Due to the high optimum temperature of malonyl-CoA reductase and the loss of one or both expression plasmids, the polymer content was marginal (0.98%, wtPHA/wtCDW) (Table 2).

3HP-CONTAINING COPOLYMERS

As poly(3HP) has several beneficial chemical and physical properties, incorporation of 3HP monomers improves the qualities of other PHAs. As we previously reported, the 3HP present in the polyester lowers glass transition temperature, crystallinity, fusion enthalpy, and melting temperature (18). Due to the limitations in the physical characteristics of poly(3HB), several copolymers have been developed to improve the performance of poly(3HB) (35–37). To date, several new 3HP-containing copolymers and block polymers have also been synthesized and analyzed regarding their material properties. Many 3HP-containing copolymers have been synthesized by feeding substrates such as 3HP or different diols (18).

The most prominent 3HP-containing PHA is poly(3HP-co-3HB). The first synthesized copolymer harboring 3HP moieties was reported by Nakamura et al. in 1991 (38). As there was no artificial pathway available to form 3HP from a cheap substrate like glycerol in those days, 3HP, 1,5-pentanediol (1,5PD), or 1,7-heptanediol (1,7HD) was used. R. eutropha degrades 1,5PD and 1,7HD to 3HP-CoA via β-oxidation and polymerizes together with 3-hydroxybutyryl-CoA (3HB-CoA) different poly(3HP-co-3HB) with 3HP moieties between 3 and 7 mol% depending on the substrate. R. eutropha accumulates under those conditions to a level of between 30% and 40% (wtPHA/wtCDW) (Table 3) (38). Higher fractions (4.3 and 10.5 mol%) of 3HP moieties were incorporated by Methyllobacterium sp. KCTC 0048 when the strain was fed with 3HP (Table 3) (39). For a fully controlled 3HP to 3-hydroxybutyrate (3HB) composition process, Azohydromonas lata (formerly Alcaligenes latus) (40) was cultivated in the presence of various amounts of 3HP and 3HB and accumulated poly(3HP-co-3HB)s with 3HP fractions of between 7 and 88 mol% (16). Due to decreasing PHA content and lower cell densities along with increasing 3HP contents, the productivities of those processes dropped (Table 3). Besides feeding strategies for different wild-type strains, poly(3HP-co-3HB) could also be synthesized by recombinant E. coli strains. Valentin et al. coexpressed the phaC1AB operon of R. eutropha together with different propionyl-CoA synthetases and used 3HP as the substrate (41). Expression of acetyl-CoA synthetase (Acce) from R. eutropha resulted in 31 mol% incorporated 3HP. By using acetyl-CoA:4-hydroxybutyrate-CoA transferase (OrfZ) from Clostridium kluveri, catalyzing the same reaction, the proportion of 3HP increased to 91 mol%. The third candidate, the propionyl-CoA synthetase (PrpE) from S. enterica serovar Typhimurium, was able to incorporate 89 mol% from 1% (vol/vol) 3HP. Unfortunately, the total PHA amount ranged only from 12% to 19% (wtPHA/wtCDW) (Table 3) (41).

Fukui et al. described the synthesis of poly(3HP-co-3HB) from low-cost sugars or fatty acids (42). The 3HP moiety was built when employing the malonyl-CoA route expressed heterologously in R. eutropha. As R. eutropha is a natural poly(3HB) producer, 39% to 76% (wtPHA/wtCDW) poly(3HP-co-3HB) were accumulated with fractions of 3HP between 0.2 and 2.1 mol% (Table 3). Poly(3HP-co-3HB) synthesis in E. coli was established by Wang et al. by combining the PduP route with β-ketothiolase (PhaA) and acetoacetyl-CoA (PhaB) from R. eutropha, with glycerol as the sole carbon source (43). To control the monomer composition, both pathways were equipped with individual promoters. With various inducer concentrations, poly(3HP-co-3HB)s consisting of 3HP moieties ranging from 11.5 mol% to 94.6 mol% were synthesized. The total PHA content increased from 30.3% (wtPHA/wtCDW) to 44% (wtPHA/wtCDW) with decreasing 3HP fractions (Table 3).

Besides the homopolymer poly(3HP), Zhou et al. also synthesized poly(3HP-co-4-hydroxybutyrate) [poly(3HP-co-4HB)], employing the same strain (E. coli S17-1/pQZQ01/pQZQ03) (28). To combine the already-discussed properties of poly(3HP) with those of poly(4-hydroxybutyrate) [poly(4HB)], which has the highest elasticity among all known PHAs (44), the recombinant E. coli strain was fed with 1,3PD and 1,4-butanediol (1,4BD). Depending on the medium and substrate supply, the achieved poly(3HP-co-4HB) content ranged between 24.7% and 41.7% (wtPHA/wtCDW) with 4HB fractions from 0.5 to 2.0 mol% (Table 3) (28). To improve 4HB incorporation, a specific 4-hydroxybutyrate-CoA transferase (OrfZ) from C. kluveri was coexpressed in the aforementioned strain (45). When the recombinant strain was cultivated in the presence of 1,3PD and 1,4BD, levels of poly(3HP-
Poly(3HP-co-3HB) was the 3HP-containing copolymer synthesized in these experiments. Poly(3HP-co-4HB) was the 3HP-containing copolymer synthesized in these experiments. Available; VB12, vitamin B12; 3HP, 3-hydroxypropionate route; M-CoA, malonyl-CoA route; PduP, propionaldehyde dehydrogenase route; wt, weight; WT, wild type (no genetic modifications were performed).

- D-1-thiogalactopyranoside; FA, fatty acids; Km, kanamycin; ND, no data

1,5PD, 1,5-pentanediol; 1,7HD, 1,7-heptanediol; 3HP, 3-hydroxypropionate; Ap, ampicillin; Ara, L-arabinose; Cm, chloramphenicol; IPTG, isopropyl-

**TABLE 3. Synthesis of 3HP-containing copolymers by different microorganisms**

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Host</th>
<th>Cofactor(s)/inducer(s)/antibiotic(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3HP</td>
<td>E. coli</td>
<td>Cofactor(s)/inducer(s)/antibiotic(s)</td>
<td>Reference</td>
</tr>
<tr>
<td>M-CoA</td>
<td>WT</td>
<td>1.0–3.1 7–30 0.1 72 Glucose/FA None/Ara/Km42</td>
<td>Reference</td>
</tr>
<tr>
<td>3HP</td>
<td>None</td>
<td>ND ND ND 48 3HP None/IPTG/Ap41</td>
<td>Reference</td>
</tr>
<tr>
<td>WT</td>
<td>1.9–4.0 0.45–44 0.1 48 3HP None/none/none16</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>sp. WT</td>
<td>1.1–1.9 0.6–2.7 1.2 72 3HP None/none/none39</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>3.4–6.8 0.8–36.8 1 48–96 3HP/1,5PD/1,7HD None/none/none38</td>
<td>Reference</td>
<td></td>
</tr>
</tbody>
</table>

3HP-containing ter- and tetrapolymers

Examples of 3HP incorporation in polyesters consisting of three or more different monomers are rare. The biosynthesis of such polyesters is mainly based upon feeding structurally related carbon sources. Only *Vibrio* sp. KN01 was able to synthesize the terpolymer consisting of 14 mol% 3HP, 83 mol% 3HB, and 3 mol% 5HV from soybean oil (productivity $P_{PHA}$, 3.3 mg PHA liter$^{-1}$ h$^{-1}$) under aerobic or anaerobic conditions (46).

The first 3HP-containing tetrapolymer was described by Green et al. (47). In the presence of acrylic acid and sodium octanoate, *R. eutropha* accumulates poly(3HP-co-3HB-co-3-hydroxyhexanoate-co-3-hydroxyoctanoate) [poly(3HP-co-3HB-co-3HH-co-3HO)]. Due to the inhibition of 3HP-oxidation by acrylic acid, octanoate degradation was incomplete and thus mlc and scl monomers were incorporated in addition to 3HB. The ratios of 3HP, 3HH, and 3HO in the copolymer increased as the concentration of acrylic acid was increased. In the presence of 10.6 mM acrylic acid, *R. eutropha* synthesizes a tetrapolymer consisting of 1.4 mol% 3HP, 95.9 mol% 3HB, 2.1 mol% 3HH, and 0.6 mol% 3HO. A further increase of the concentration of acrylic acid to 29.3 mM resulted in a tetrapolymer consisting of 6.5 mol% 3HP, 81.7 mol% 3HB, 10.23 mol% 3-hydroxyhexanoate (3HH), and 1.6 mol% 3-hydroxyoctanoate (3HO).

Furthermore, ter- and tetrapolymers were synthesized by cultivation of cells of a recombinant *E. coli* strain harboring propionyl-CoA transferase (Pct) from *C. propionicum*, phosphate butyryltransferase (Pbt) and butyrate kinase (Buk) from *Clostridium acetobutyricum*, and PHA synthase from *Pseudomonas* sp. strain 6-19 in the presence of lactate (LA), 3HP and 4HB, or LA, 3HP, 3HB, and 4HB. The authors obtained the terpolymer poly(3HP-co-4HB-co-La), as well as the tetrapolymer poly(3HP-co-3HB-co-4HB-co-La) (48). Unfortunately, no data were provided regarding PHA content, molar composition, and cultivation time.

When *R. eutropha* H16 or *R. eutropha* PHB-4 harboring an episomal copy of *phaC* from *Aeromonas caviae* or *R. eutropha* was fed with sodium-5-hydroxovalerate (Na5HV) or ω-pentadecalcitonne (ω-PDL), the bacterium accumulated a terpolymer consisting of 3HP, 3HB, and 5-hydroxyvalerate (5HV). The resulting poly(3HP-co-3HB-co-5HV)s contained 3HP fractions between 1 and 18 mol% and 5HV fractions ranging from 1 to 10 mol% (49). The productivities associated with synthesizing such polyesters are between 0.04 to 3.6 mg PHA liter$^{-1}$ h$^{-1}$.
3HP-CONTAINING BLOCK POLYMERS

Although they consist of the same monomers as the corresponding random copolymers, block polymers have different properties and have attracted more attention in recent years (50, 51). It has been shown that PHB-b-PHBV synthesized by R. eutropha prevents polymer aging (51). Meng et al. cultivated their recombinant E. coli strain in the presence of 1,3PD and 1,4BD to accumulate poly(3HP-co-4HB) (45). When the substrates were provided consecutively in the medium beginning with 1,4BD, cells synthesized at levels of 16% (wtPHA/wtCDW) poly(3HP-b-36.7 mol% 4HB) (P_{PHA} 23.9 mg PHA liter$^{-1}$ h$^{-1}$) or 21% (wtPHA/wtCDW) poly(3HP-b-28.5 mol% 4HB) (P_{PHA} 15.8 mg PHA liter$^{-1}$ h$^{-1}$), depending on the substrate concentration (52). If 1,3PD was fed first, only a mixture of the two homopolymers poly(3HP) and poly(4HB) or only poly(3HP) was accumulated. Wang et al. also modified their cultivation strategy to synthesize poly(3HP-b-3HB) block polymer instead of the poly(3HP-co-3HB) copolymer (53). For copolymer accumulation, they induced the two synthetic pathways concurrently (43). The first block contained 3HB monomers and was formed after L-arabinose induction. Later, glucose, glycerol, and IPTG (isopropyl-$\beta$-d-thiogalactopyranoside) were added to suppress arabinose induction and induce 3HP monomer synthesis from glycerol. By adjusting the inducer concentrations, poly(3HP-b-3HB)s with 3HB moieties of between 25 and 92 mol% were synthesized (19% to 28% [wtPHA/wtCDW]; P_{PHA} 12.2 to 26.7 mg PHA liter$^{-1}$ h$^{-1}$).

PROCESS GUIDANCE

Some already-mentioned routes synthesizing poly(3HP) and copolymers containing 3HP are dealing with obvious problems: (i) low plasmid stability and addition of cost-intensive (ii) substrates, (iii) cofactors, and (iv) inducers. For scaling up of production, it is important to overcome these drawbacks.

To stabilize the artificial pathways, plasmid addiction systems (27, 33) and chromosomal integrations (27) were developed and applied here. The plasmid addiction systems that have been established so far are metabolism based. Gao et al. generated phenylalanine- and/or tyrosine-auxotrophic E. coli mutants and added copies of pheA and/or tyrA to their expression plasmids. Without antibiotics, the plasmid stability was calculated to be 80.8% ± 1.7% (27). In the case of poly(3HP) synthesis in S. blattae, a carbon source-dependent plasmid addiction system was applied. For growth on glycerol as the sole carbon source, triosephosphate isomerase is essential (54). As a result of knocking out the chromosomal copy of tpiA of S. blattae and inserting an intact E. coli tpiA into the expression vector, no loss of plasmid was observed after 72 h (33).

To compete with fossil fuel-based materials, poly(3HP) synthesis must be cheap. Most petrochemical-derived plastics have production costs below 1 €/kg, whereas the costs for poly(3HB)-based materials are between 1.50 €/kg and 5 €/kg (55). To establish a feasible production process, cost-intensive substrates or cofactors should be avoided. Hitherto, the highest productivities for poly(3HP) synthesis were achieved from substrates such as 3HP and 1,3PD (28) and from addition of cofactors such as vitamin B$_{12}$ and inducers (22, 27), respectively. Until now, productivities of processes avoiding the addition of vitamin B$_{12}$ were about 5- to 10-fold lower (29, 33). For low-cost production, those processes are more promising and should be further optimized.

CHEMICAL AND PHYSICAL PROPERTIES

As already mentioned, poly(3HP) has very promising properties. For industrial processing, a low melting temperature and a broad process range between the melting point and thermal degradation as well as stability at low temperatures ($T_m - 20^\circ$C) combined with high crystallization speed ($\Delta H_{m}$, 64 J/g) are advantageous and make such materials competitive with fossil fuel-based materials such as polyethylene (PE) (Table 1). In this review, we introduced several methods for synthesis of poly(3HP-co-3HB). We now focus on the impact of various levels of 3HP content on chemical and physical characteristics with regard to industrial applications. The $T_m$ decreases linearly with increasing 3HP moieties (16, 20, 43, 56).
melting temperature is lowest in poly(3HP-co-3HB)s with 3HP content of between 50 and 80 mol%. In the same range of content, fusion enthalpy is also lowest (19, 20, 38, 43, 56). As industrial applications such as injection molding need materials with low melting temperatures as well as fast crystallization behavior (57), poly(3HP-co-3HB)s with a 3HB content below 30 mol% are favorable. Although the $M_w$ values differ with regard to their synthesizing process, a general trend toward lower $M_w$ for 3HP-rich poly(3HP-co-3HB)s caused by the lower $M_w$ values of 3HP monomer ($M_w$ of 3HP, 89; $M_w$ of 3HB, 103) is noticeable. The polydispersity index is influenced only marginally (Fig. 2).

Although the characteristics of poly(3HP) and poly(4HB) differ (Table 1), the different poly(3HP-co-4HB)s are quite similar with regard to their material properties (Fig. 3) (45). Remarkably, poly(3HP-co-4HB) films become more transparent, with increases in 4HB content up to 67 mol%, whereas higher 4HB amounts lowered the transparency (45).

Furthermore, terpolymers (48, 49) and tetrapolymers (47, 48) with incorporated 3HP monomers have beneficial properties. But due to the different characteristics of the diverse monomers, properties of the ter- and tetrapolymers are poorly predictable. In terpolymers consisting of more than 70 mol% 3HB, incorporated 3HP and 5HV lowers the melting and glass transition temperatures as well as fusion enthalpy compared to the poly(3HB) homopolymer (49). In comparisons of the terpolymers to poly(3HP-co-3HB)s with equal 3HB contents, a decrease of glass transition

FIG 3  Influence of 4HB moieties on physical properties of poly(3HP-co-4HB) based upon the data published so far (45). Melting temperature (●), tensile strength (△), glass transition temperature (□), Young’s modulus (■), and elongation at the breaking point (○) are plotted against 4HB content.

FIG 4  Comparison of poly(3HP-co-3HB) and poly(3HP-co-3HB-co-5HV) based upon the data published so far (16, 18–20, 38, 39, 42, 43, 49, 56). The melting temperature (●), fusion enthalpy (△), and glass transition temperature (□) are plotted against 3HB content. Values for terpolymer poly(3HP-co-3HB-co-5HV) are highlighted in gray, and molar ratios of 3HP and 5HV are indicated.
temperature and fusion enthalpy can be observed, whereas the melting temperature is influenced only marginally (Fig. 4). The influence of each type of constituent in a tetrapolymer consisting of a high level (81 to 96 mol%) of 3HB contents is only negligible (47). Melting and glass transition temperatures were in the same range as those of poly(3HB-co-3HP) copolymers consisting of the same molar 3HB fraction.

PERSPECTIVES FOR POLY(3HP) AND THE COPOLYMERS

As poly(3HP) and 3HP copolymers are very ductile and flexible, they can be used for film blowing, injection and blow molding, and thermoforming, as well as for breathable cast films, coatings, and laminations (58). For medical applications, good degradability as well as biocompatibility is needed for slow-release preparations (12, 59); thus, poly(3HP) can be used for drug capsules. In addition, as poly(3HP) fulfills the key properties, employment as a scaffold for tissue engineering is conceivable (60). Furthermore, poly(3HP) might act as a plasticizer to soften brittle materials such as poly(3HB) in blends. For those applications, the production costs must be low to be competitive with petrochemical-derived materials such as polypropylene (PP) and PE. Glycerol is a cheap and abundantly available carbon source, as it is a byproduct of still-growing biodiesel production (61, 62). Hence, the most promising approaches synthesizing poly(3HP) and its copolymers use glycerol as the sole source of carbon and energy. Glycerol dehydratases convert glycerol to 3HPA, one component of reuterin. Reuterin is a known antimicrobial agent and is often used in food preservation (63, 64). Hence, accumulation of 3HPA during poly(3HP) synthesis results in growth inhibition and lower cell dry masses, which are crucial for intracellular products. Thus, 3HP formation rates should be avoided during poly(3HP) synthesis, e.g., by increasing PduP or AldD activities (Fig. 1).

Furthermore, there are two types of glycerol dehydratases known, vitamin B 12-independent DhaB1 from C. butyricum, active only under strictly anaerobic conditions (23, 24), and vitamin B 12-dependent DhaBCE of several Enterobacteriaceae species (65). The benefit of the glycerol dehydratase of C. butyricum is that no expensive cofactors are needed to maintain enzyme activity. Under anaerobic conditions, however, cell growth of most expression hosts is diminished. Therefore, and as aerobic processes give higher cell densities, vitamin B 12-dependent glycerol dehydratases should be employed and expressed in natural vitamin B 12-producing hosts to overcome the need for addition of expensive cofactors (29, 33).

Besides the mentioned applications of the 3HP homopolymer and 3HP copolymers, poly(3HP) can be used as a source for 3HP, which is considered one of the building blocks from renewable resources (66). For example, 3HP can be oxidized to malonic acid, reduced to 1,3PD or 3HPA, and cyclized to propiolactone as well as dehydrated to acrylic acid, one of the most appealing compounds (12, 59); thus, poly(3HP) can be used for drug capsules. In conclusion, poly(3HP) is a promising material for several applications.

Until now, the maximal relevant culture volumes ranged between 1.5 and 3 liters (20–22, 27–29, 33). Since chromosomal integrations or plasmid addiction systems stabilize the poly(3HP) pathways, addition of antibiotics is no longer needed (27, 33). Furthermore, employing natural vitamin B 12 producers as hosts can overcome the need for cost-intensive cofactors (29, 33). Hence, most processes use pure glycerol. To reduce costs more, research should focus on conversion of crude glycerol derived from, e.g., the biodiesel industry (21). To bring this promising polymer to the next stage of development, it is necessary to combine those benefits with the pathways yielding high polymer content (20, 22, 28) to establish a cheap and scalable process for efficient poly(3HP) production.

ACKNOWLEDGMENT

Financial support from BMELV/FNR (FKZ 22005211) is gratefully acknowledged.

REFERENCES


6580 aem.asm.org Applied and Environmental Microbiology


32. Guests and other contributors from the Institute of Biochemistry and Biotechnology, University of Tokyo, Japan.


