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REVIEW PAPER

# Recent Advances in the Production, Recovery and Applications of Polyhydroxyalkanoates

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**Abstract** Polyhydroxyalkanoates (PHAs) are biodegradable and biocompatible polyesters that can potentially replace certain plastics derived from petroleum. PHAs can be produced using a combination of renewable feedstocks and biological methods. Native and recombinant microorganisms have been generally used for making PHAs via fermentation processes. As much as 90 % of the microbial dry mass may accumulate as PHAs. A range of PHAs has been produced using fermentation methods, including copolymers and block copolymers. Alternative production schemes based on genetically modified plants are becoming established and may become the preferred route for producing certain PHAs. Production in plants is likely to be inexpensive compared to production by fermentation, but it does not appear to be as versatile as microbial synthesis in terms of the range of products that may be generated. Cell-free enzymatic production of PHAs in vitro is receiving increasing attention and may become the preferred route to some specialty products. This review discusses the recent advances in production of polyhydroxyalkanoates by the various methods. Methods of recovering the polymer from microbial biomass are reviewed. Established and emerging applications of PHAs are discussed.

**Keywords** Biopolymers · Bioplastics · Polyhydroxyalkanoates · Polymerization · Applications

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**Abbreviations**

| | |
|---|---|
| ATRP | Atom transfer radical polymerization |
| CALB | *Candida antarctica* lipase B |
| CSTR | Continuous stirred tank reactor |
| DO | Dissolved oxygen |
| DNA | Deoxyribonucleic acid |
| DW | Dry weight |
| *c*PHB | Complexed poly-(*R*)-3-hydroxybutyrate |
| EDTA | Ethylenediaminetetraacetic acid |
| FNL | *Fervidobacterium nodosum* lipase (FNL) |
| HACoA | HydroxyalkanoylCoA |
| HB | Hydroxybutyrate |
| 3HB | 3-Hydroxybutyrate, or 3-hydroxybutyric acid |
| 4-HB | 4-Hydroxybutyrate |
| HEC | Hydroxyethyl cellulose |
| HEMA | 2-Hydroxyethyl methacrylate |
| HHx | Hydroxyhexanoate |
| HOPG | Highly oriented pyrolytic graphite |
| HV | Hydroxyvelarate |
| mcl-PHA | Medium-chain-length PHA |
| $NAD^+$ | Nicotinamide adenine dinucleotide |
| NADH | Reduced form of nicotinamide adenine dinucleotide |
| P3HB3HV | Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) |
| PANi | Polyalanine |
| PCL | ε-Caprolactone, or polycaprolactone |
| PDH | Pyruvate dehydrogenase |
| PDL | ω-Pentadecalactone |
| PEG | Polyethylene glycol |
| PEO | Polyethylene oxide |
| PHA | Polyhydroxyalkanoates |
| PHB | Polyhydroxybutyric acid |
| PHBHHx | Poly-3-hydroxybutyrate-*co*-3-hydroxyhexanoate |

| | |
|---|---|
| PHBV | Polyhydroxybutyrate-*co*-valerate |
| PHBVHHx | Poly-3-hydroxybutyrate-*b*-3-hydroxyvalerate-*b*-3-hydroxyhexanoate |
| PHF | Polyhistidine |
| PHO | Poly-3-hydroxyoctanoate |
| PLA | Polylactide |
| PNIPAAm | Poly(N-isopropyl acrylamide) |
| RAFT | Reversible addition fragmentation chain transfer |
| (*R*)-LATP | Thiophenyl (*R*)-lactate |
| (*R*)-3HBTP | Thiophenyl (*R*)-3-hydroxylbutyrate |
| $scCO_2$ | Supercritical carbon dioxide |
| SDS | Sodium dodecylsulfate |
| TMC | Trimethylene carbonate |

## Introduction

In 2011, nearly 280 million tons of petrochemicals-based polymers were produced with expected increased in 4 % per annum to 2016 [1]. Production of synthetic polymers is expected to increase to around 810 million tons by 2050 [2]. A strong interest exists in attempting to replace petrochemicals-derived plastics with biologically produced alternatives. Here we review the production, recovery and applications of polyhydroxyalkanoate (PHA) biopolymers.

PHAs are a class of biopolymers with useful physicochemical properties for diverse industrial and biomedical applications. PHAs are biocompatible and biodegradable. PHAs can be produced sustainably using renewable resources and biological methods. Accumulation of PHAs in the bacterium *Bacillus megaterium* was first reported in 1926 [3]. Since then, many microorganisms have been shown to accumulate PHAs as intracellular granules [4–6] or secrete the polymer extracellularly [3, 7, 8]. Good yields of PHAs have been reported from certain genetically engineered plants [9–12].

Synthetically produced polymers are generally inexpensive, but their persistence in the environment poses a significant problem. Furthermore, production of fossil-based polymers has a significant environmental impact [13] as a net contributor to the level of atmospheric carbon dioxide. Processes for making synthetic polymers often use hazardous materials. In contrast to most petropolymers, biologically produced polymers are generally biodegradable, biocompatible [14, 15] and may be produced sustainably using processes with a reduced environmental impact. Technology for producing various biopolymers is developing rapidly, but because of their relatively high cost, they are used mostly in specialty applications. By 2018, the production of biopolymers is expected to grow by nearly 35 % and around $5 billion worth of biopolymers are expected to be produced [16, 17].

Continuing advances in genetic engineering, metabolic engineering and enzymology are improving accessibility of an increasing number of biopolymers. In addition to production in microorganisms and plants, in vitro enzymatic production of biopolymers is attracting much attention [18–23]. PHAs are commercially produced and are perhaps the best studied of the biopolymers [24, 25]. Nevertheless, they are still relatively expensive and this hinders their wider commercial use.

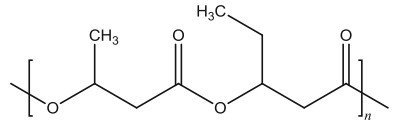
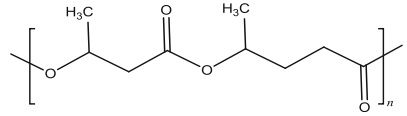
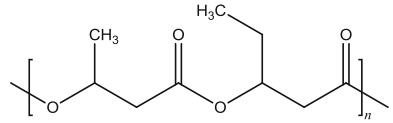
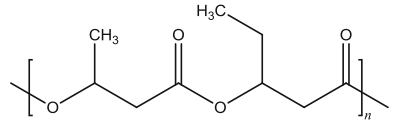
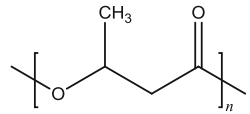
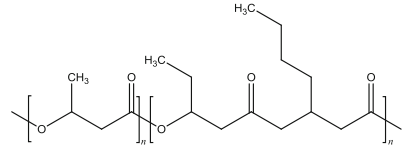
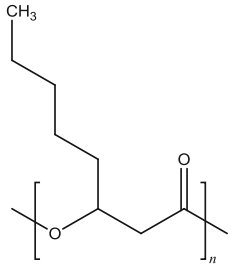
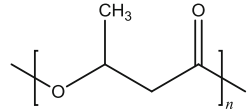
## PHA Production

### Production by Microbial Fermentation

In bacteria, PHAs accumulate in the presence of an excess of a carbon source coupled to a deprivation of nutrients such as nitrogen [26]. All metabolizable carbon sources can be used for the production of PHAs, including fatty acids and carbohydrates. PHA polymers accumulate as intracellular inclusions in bacteria of the genera such as *Alcaligenes*, *Pseudomonas*, *Enterobacter*, *Necator*, *Rhodobacter*, *Ralstonia* and *Cupriavidus* [27–32]. Literature on PHA production in bacteria is extensive [24, 25, 33, 34]. Some of the most recent studies are summarized in Table 1. Certain cyanobacteria also accumulate PHAs under suitable environmental conditions [35, 36] and so do some halophiles [33].

Random copolymers of PHA have been successfully produced in monoculture fermentations by controlling the type of carbon feed and composition [37]. This has been shown to be possible using both native and recombinant bacteria [38]. Using *Cuprividus necator* in fed-batch fermentations to produce the copolymer poly-3-hydroxybutyrate-*co*-4-hydroxybutyrate, Chanprateep et al. [27] attained a polymer level of 77 % (w/w) in the biomass. This was achieved with a C:N mole ratio of 200:1 with fructose as the precursor for 3-hydroxybutyrate (3HB) and 1,4-butanediol as the precursor for 4-hydroxybutyrate (4HB). Ammonium sulfate was the nitrogen source. The composition of the copolymer, i.e., the ratio of 3HB to 4HB in it, was affected by the molar ratio of the two carbon substrates in the culture medium. For example, if the carbon source contained 25 % (w/w) 1,4-butanediol, a copolymer with a 30 % mole fraction of 4HB was produced, but this could be increased to 80 % by increasing the 1,4-butandiol level to 75 % (w/w). Another commonly used precursor for 4HB is the sodium salt of $\gamma$-hyroxybutyrate, but this is more expensive than 1,4-butanediol. Although using too high a concentration of 1,4-butanediol has been found to be toxic to cells [27]. In the production of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)

**Table 1** PHA fermentation processes

| Fermentation conditions | Microorganism | Carbon source | Product | PHA yield (%) | References |
|---|---|---|---|---|---|
| Fed batch: 30 °C, 200 rpm, DO 20 %, 72 h | *Cupriavidus* sp. USMAA2-4 | Oleic acid (0.82 % v/v), 1-pentanol (011 % v/v) |  | 29 | [31] |
| Continuous: 30 °C, 500 rpm, pH 7, air flow 2.5 mL $min^{-1}$ | *Cuprividus necator* A-04 | Brown sugar, rock sugar, toddy palm sugar, etc. |  | 77 | [27] |
| Two-stage CSTR: 30 °C, pH 6, 400 rpm | Mixed culture | Molasses |  | 77 | [42] |
| SBR: 30 °C, pH 6, 500 rpm | Mixed culture | Volatile fatty acids |  | 77 | [43] |
| Fed batch: 30 °C, 400 rpm, pH 6.8, DO 50 % | *Ralstonia eutropha* DSM428, ATCC 17699, NCIB 10442 | Fructose |  poly(3-hydroxybutyrate) | 45 | [28] |
| Two-stage continuous fed-batch: 30 °C, 200 rpm, 48 h, pH 6.5, DO 30 % | *Pseudomonas putida* KTOY06ΔC (*phaPCJ*$_{ac}$) | Mixed fatty acids |  | – | [51] |
| Batch shake flask: 30 °C, 200 rpm, pH 7 | *P. mendocina* CH50 | Sodium octanoate |  | 31 | [38] |
| Two-phase partition reactor: air flow 0.42 L $min^{-1}$, 500 rpm | *Methylobacterium organophilum* CZ-2 | Methane |  poly(3-hydroxybutyrate) | 57 | [45] |

*Abbreviations CSTR* continuously stirred tank reactor, *SBR* sequencing batch reactor, *DO* dissolved oxygen

(P3HB3HV) by a *Cupriavidus* sp. changes in culture pH were reported to affect the composition of the copolymer produced [31].

Accumulation of poly-3-hydroxyoctanoate (PHO) homopolymer has been recently shown to occur in a wild-type *Pseudomonas mendocina* [39]. Up to 31 % of the cell dry weight consisted of PHO, but the biomass concentration in this preliminary study was relatively low [39]. A two-stage approach was proposed for accumulating the polymer. This consisted of a first stage tailored to achieving a high concentration of the biomass, followed by a second stage tailored to accumulation of the polymer within the cells [39]. Using a controlled feeding strategy based on a metabolic flux balance analysis, Ramalingam et al. [40] achieved a PHA content in the biomass of 35.6 % (w/w) with a PHA concentration in the fermentation broth of 1.14 g $L^{-1}$ in a continuous fermentation involving *Pseudomonas putida* MTCC 102 (Type B). Linoleic acid was used as the carbon source.

In some cases at least, the culture temperature can alter whether PHA or some other metabolite is produced preferentially. For example, using *Pseudomonas aeruginosa* IFO3924 both medium-chain-length PHA (mcl-PHA) and rhamnolipids were produced simultaneously [41]. Production of rhamnolipids was favored by changing the temperature from 30 to 28 °C [41]. *P. aeruginosa* IFO3924 fed with fatty acids having an even-number of carbons led to the production of 3-hydroxyalkanoates containing only an even-number of carbons in the in the polymer. In contrast, feeding of fatty acids with an odd-number of carbons resulted in 3-hydroxyalkanoates containing both odd- and even-numbered carbon chains. Of the different fatty acids fed, C11 and C12 fatty acids proved to be the best carbon sources for this microorganism [41]. The feeding of C11 substrate resulted in 504 mg $L^{-1}$ PHA.

Recombinant *Escherichia coli* harboring *pha*ABC and *pha*P of *Azotobacter* sp. has been reported to accumulate PHB (polyhydroxybutyrate) when fed with glycerol [42], a relatively inexpensive carbon source. Accumulation of PHB decreased with an improved supply of oxygen. Changing the carbon source to glucose also led to PHB accumulation, but in this case accumulation was enhanced by an improved supply of oxygen. Presumably, therefore, differences in the oxidation state of the cells differently affect PHB accumulation from carbon sources with different oxidation state [42]. Glycerol has a lower oxidation state of −2 compared to glucose (0). Depending on the oxidation state, catabolism of a carbon source would produce different ratios of NADH/$NAD^+$ and this will determine how much of the carbon flows to the synthesis of a more reduced product [42].

The utilization of complex low cost carbon substrates and the elimination of sterilization energy cost made mixed microbial culture (MMC) to be a cost-effective PHA production process [43]. In fact, it has been suggested that based on life cycle analysis (LCA), PHA production by MMC could be more favorable compared to using pure cultures in both economic and environmental perspectives [44]. MMC have been successfully used in producing PHAs [43]. In such an operation with a 2-stage stirred tank reactor, the use of molasses as the carbon source provided a PHA content in the biomass of 61 % (w/w) [43]. In similar studies with continuous stirred tank reactors and sequencing batch reactors, the fermentation conditions were found to greatly influence the accumulation of PHA in the microbial cells growing on volatile fatty acids [44]. For example, a feast–famine operational regimen affected PHA content in the biomass [44]. The composition of the biopolymer formed in the mixed culture could be manipulated by changing the composition of the carbon source and by whether it was fed pulse-wise or continuously [44]. Such changes could be used to alter the ratio of hydroxybutyrate (HB) and hydroxyvelarate (HV) in the PHA copolymer to change its average molecular weight, degree of crystallinity and other physical properties [44].

The production economics of PHAs depend very much on the cost of the carbon feedstock and whether aseptic (monoculture) or open mixed culture production methods are used. In some applications, the use of mixed carbon sources and microbial cultures as found in municipal wastewaters, for example, may be acceptable for making PHAs. Use of palm oil for producing PHAs has been discussed [34].

In attempts to use a cheap feedstock for producing PHA, ruthenium (Ru) was used for catalytic hydrolysis of inexpensive cellulose to glucose [45]. The hydrolysis occurred at 220 °C and converted 15–20 % of cellulose to glucose and other products. The crude hydrolysate was then used to culture a recombinant *E. coli*. The bacterium accumulated 42 % (w/w) of its biomass as PHB in a 72 h fermentation at 30 °C, but the concentration of biomass was low because of Ru toxicity. The polymer produced had a number averaged molecular weight of $3.1 \times 10^5$ Da. For otherwise the same fermentation conditions, the use of pure analytical grade glucose as the feed yielded a polymer with a somewhat higher number averaged molecular weight of $4.3 \times 10^5$ Da.

Methane has been used as a carbon source for producing PHAs using consortia of methanotrophic bacteria as well as single strains. In one such study, *Methylobacterium organophilum* pure culture was used in a two-phase partitioning reactor [46]. The specific methane consumption rate of the isolate was 100 mg $CH_4$ $g^{-1}$ $h^{-1}$ compared to much lower rates reported for consortia of methanotrophic bacteria. The isolate accumulated nearly 57 % (w/w) PHB in the biomass under nitrogen limiting conditions.

Exposure of the activated sludge bacteria to a 7 mT static magnetic field has been claimed to enhance PHB accumulation by the cells under conditions of elevated concentrations of acetate [47]. This effect is said to be a consequence of a reduced uptake of acetate which is toxic to cells in high concentration [47]. The imposed magnetic field alters acetate uptake by modifying the net-charge on the surface of the cell membrane [47].

Certain photosynthesizing microorganisms are known to accumulate PHAs [35, 36]. In cyanobacteria such as *Spirulina subsalsa* [48], accumulation of PHA has been shown to be influenced by the salinity of the culture medium. A two-fold increase in NaCl concentration enhanced PHA accumulation relative to control, although the PHA yield was relatively low. The mechanism of this ionic strength effect is not entirely clear. PHAs accumulate also in certain halophilic bacteria as reviewed elsewhere [33].

### *Metabolic Engineering*

In bacteria, the synthesis of polyhydroxyalkanoates from sugars normally begins with glycolysis of the sugar to pyruvate. The latter is converted to acetyl-CoA via the pyruvate dehydrogenase (PDH) oxidation pathway. Two molecules of acetyl-CoA are then condensed to form acetoacetyl-CoA through the action of *β*-ketothiolase, an enzyme encoded by the *phaA* gene. Acetoacetyl-CoA is reduced by acetoacetyl-CoA reductase (*phaB*) to form the monomer of (*R*)-3-hydroxyacyl-CoA, the building block of PHAs. PHA synthase (*phaC*) finally polymerizes the monomers to PHA (Fig. 1).

Metabolic engineering of PHA biosynthesis pathways (Fig. 1) has been used to produce PHAs of different types and properties in various bacterial species. For example, *Escherichia coli* strains have been metabolically engineered to regulate the expression of short chain fatty acid catabolism operon to significantly enhance the expression of short chain complexed poly-(*R*)-3-hydroxybutyrate (*c*PHB) [49, 50]. Theodorou et al. [50] reported a 1.7-fold increase in accumulation of *c*PHB in a mutant *E. coli* compared to the wild-type.

Acetoacetate induction was used to regulate expression of the product [49–51]. Reducing the production of acetic acid capability is reported to improve carbon channeling to

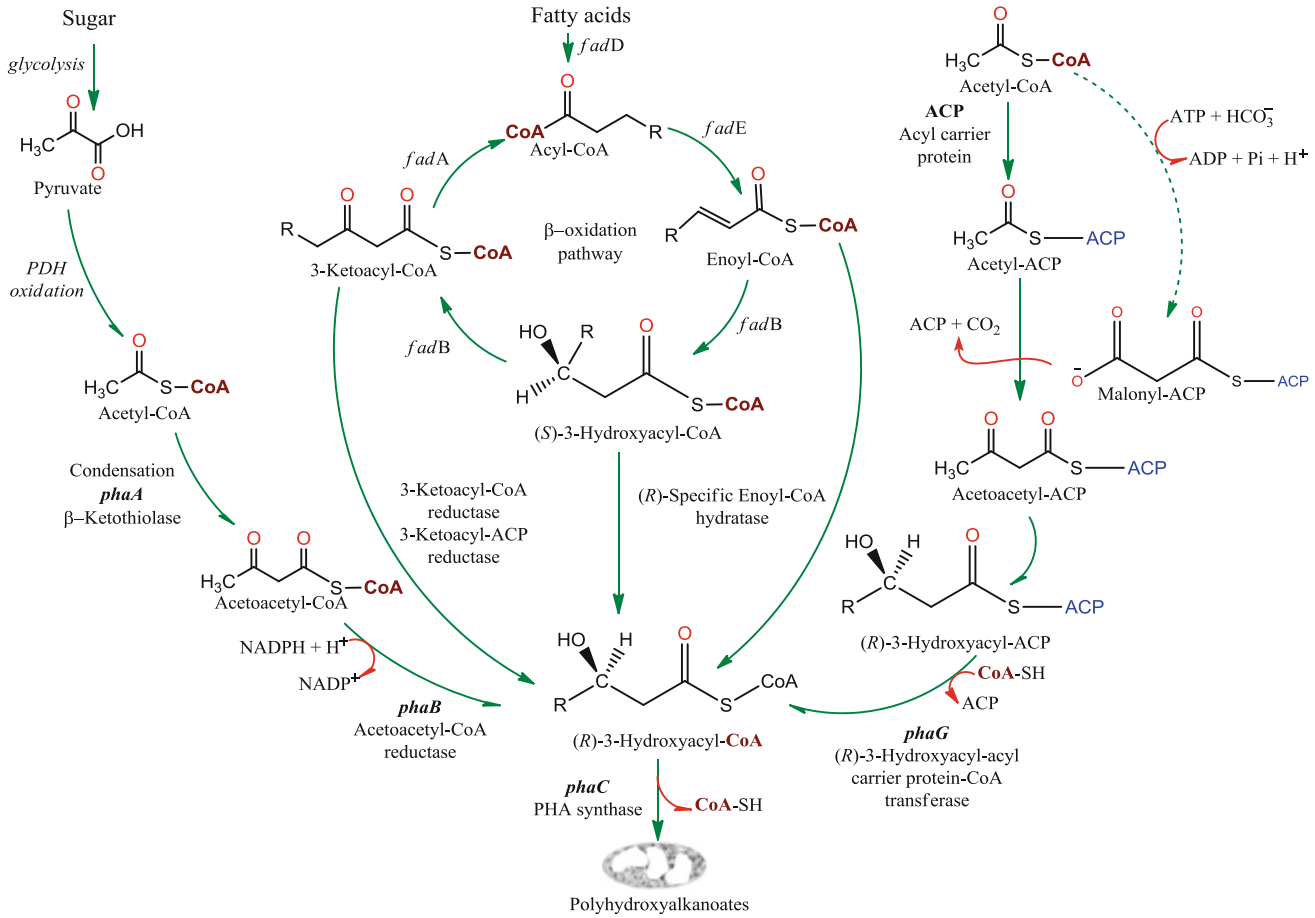



**Fig. 1** General scheme of PHA biosynthesis from sugar catabolism, fatty acid *β*-oxidation and intermediary pathways

polymer biosynthesis. Recently, Jian et al. [52] reported a 2-fold increase in production of biomass and 3.5-fold increase in production of PHA in an *E. coli* mutant that had been engineered to reduce the excretion of acetate, lactate, ethanol and formate. The excretion of acetate by the mutant was 90 % lower compared to the parent strain [52]. Simultaneous production of succinate and PHA in a metabolically engineered *Escherichia coli* has also been reported [53, 54]. This was achieved by deleting *pts*G, *sdh*A and *pta* genes and overexpressing the *pha*C1 gene of *Pseudomonas aeruginosa*. The engineered *E. coli* produced nearly 21 g $L^{-1}$ succinate and 0.54 g $L^{-1}$ PHA, equivalent to a polymer content of nearly 6 % in the biomass. The feed used was a mixture of glycerol and fatty acids. The PHA produced consisted of 3-hydroxyoctanoate (58.7 % by mol) and 3-hydroxydecanoate (41.3 % by mol).

Liu et al. [55] reported the use of $\beta$-oxidation inhibition to produce mcl-PHA homopolymer in a mutant *Pseudomonas putida* (KTQQ20) fed with the relevant fatty acid. Six genes of the $\beta$-oxidation pathway were knocked out to significantly reduce the fatty acid $\beta$-oxidation activity. Feeding dodecanoic acid to the mutant strain resulted in mcl-PHA accumulation at a 10 % (w/w) level in the biomass. The PHA homo-copolymer contained 3-hydroxydecanoate monomer (16 % by mol) and 3-hydroxydodecanoate monomer (84 % by mol). Changing the feed carbon source to decanoic acid resulted in a total PHA accumulation of about 5 % (w/w) in the biomass. The accumulated PHA was a pure homopolymer of 3-hydroxydecanoate. If the feed was changed to tetradecanoic acid, the PHA content of the biomass was higher at 78 % (w/w) and the product was a pure homopolymer of 3-hydroxytetradecanoate. The polymers produced in the $\beta$-oxidation inhibited mutant had improved mechanical properties compared to the polymers produced in the native bacterium [55].

Similar observations have been reported in relation to the effect of inhibition of $\beta$-oxidation on production of PHA (Fig. 1) in a mutant *Pseudomonas entomophila* [56]. The $\beta$-oxidation inhibited mutant produced by knocking out some of the genes of the relevant pathway, accumulated PHA at the level of >90 % (w/w) in the biomass [56]. The product consisted of mainly (99 % by mol) 3-hydroxydodecanoates. The number averaged molecular weight of the polymer formed was as high as 39,000 Da and it had a polydispersity index of 2.1.

In the biosynthesis of PHA copolymers when the focus is on increasing the mole fraction of a particular monomer in the copolymer, the use of a high concentration of the precursor of the preferentially desired monomer is required in the culture medium. However, a high concentration of certain precursors can be quite toxic to cells. One possible way of overcoming this toxicity is to use a PHA synthase gene that has a high affinity for polymerizing the toxic co-monomer [57]. For example, if a copolymer with a high content of 3HV is wanted, the culture medium would need to be rich in a 3HV precursor such as valeric acid which may be toxic. The producing microorganism may be engineered to contain the PHA synthase gene such as *pha*C of *Chromobacterium* sp. USM2, which is reported to have a high affinity towards valeric acid. This strategy was used to accumulate poly(3HB-*co*-3HV-*co*-3HHx) (HHx = hydroxyhexanoate) to the level of 86 % (w/w) in the biomass of an engineered *Cupriavidus necator* [57]. The 3HV monomer content in the terpolymer was nearly 91 % (by mol) and the polymer had mechanical properties comparable to those of the common low density polyethylene [57].

An *Aeromonas hydrophila* mutant with two genes of the acetic acid metabolic pathway deleted, accumulated poly (3HB-*co*-3HHx) at the level of 47 % (w/w) in the dry biomass, corresponding to a polymer concentration of around 3 g $L^{-1}$ in the broth [55]. This level of production was nearly 45 % greater compared to the native strain [55]. Further transformation of the already engineered bacterium to harbor genes relating to fatty acids biosynthesis increased production of PHBHHx by 63 % compared to the strain with only the genes of the acetic acid metabolism deleted.

Yeasts generally have a larger cell size than bacteria and consequently are comparatively easier to recover from the fermentation broth by processes such as centrifugation and filtration. Compared to bacteria, yeast cells are also easier to break for recovering intracellular products [58]. For these reasons, there is an interest in using yeasts to produce PHAs. PHA production by different metabolically engineered yeasts has been reported [7, 8, 59, 60]. For example, Buelhamd et al. [59] produced PHAs in a transgenic *Saccharomyces pombe*. The yeast was engineered to produce PHB by transfection with the plasmid pBHR68 harboring the PHB synthesis genes encoding $\beta$-ketothiolase (*phb*$A_{Re}$), acetoacetyl-CoA reductase (*phb*$B_{Re}$) and PHB synthase (*phb*$C_{Re}$) of *Ralstonia eutropha*. Under optimized conditions, the yeast accumulated PHB at the level of nearly 9 % (w/w) in the biomass.

Properties of the PHA synthesized in engineered *Saccharomyces pombe* and *Saccharomyces cerevisiae* have been discussed in detail [59]. PHA produced in these yeasts was found to have a melting temperature in the range of 153–171 °C. The degree of crystallinity of the product ranged from 27 to 32 % during heating and 36 to 50 % during cooling [7].

A metabolically engineered yeast of the genus *Kloeckera* accumulated PHA within the cells and secreted a water-soluble bioflocculant polymer in the extracellular medium [7]. The mutant accumulated PHA to the level of about 7 % (w/w) in the biomass. This yeast had been made

by transfection with *R. eutropha*'s *pha*ABC operon harboring genes encoding *pha*A, *pha*B and *pha*C [59].

PHAs accumulate as intracellular inclusions; therefore, their recovery from the cells is inherently expensive. The overall cost of producing PHAs would reduce greatly if the cells could be coaxed into secreting the polymer into the extracellular broth. Extracellular production may be made possible through genetic engineering of the producing cells. This approach is attracting attention. Extracellular secretion of PHA in an *Alcanivorax borkumensis* mutant specifically engineered for this purpose was observed when the microorganism was fed on either pyruvate or octadecane as the sole carbon source [61].

### *Recovery of PHAs*

Scalable processes are needed for inexpensively recovering the intracellular PHAs from microorganisms. Examples of the recently published recovery methods are provided in Table 2. Although other recovery methods have been published [62], extraction of the polymer with organic solvents appears to be a commonly used approach and has been reported to have an undoubted advantages over the other PHA extraction methods [63]. Its simplicity and rapidity is reported to incur its frequent use in laboratory scale PHA extractions. Solvents extract the polymer without degrading it by improving the cellular membrane permeability and subsequent solubilization of the PHA [63]. Solvent extraction process was also reported to effectively prevent Gram-negative bacterial endotoxin contamination of the polymer, therefore improving the polymeric quality for biomedical applications [64].

In some cases, the biomass recovered by centrifugation is first washed with an organic solvent to remove fatty acids and oils left from the culture medium. Subsequently, the biomass may be freeze-dried prior to extracting the polymer with a solvent such as chloroform [4]. Cold methanol is then generally used to precipitate the polymer from chloroform [65]. Alternatively, a freshly harvested biomass paste may be washed with acetone and dried under vacuum at ambient temperature prior to solvent extraction of the polymer [66]. PHB-*co*-PHV has been recovered from *Halomonas campisalis* cells using a similar process [65] (Table 2). P3HB-*co*-4HB has been similarly extracted [66] and a similar extraction has been reported for poly-3HB-*co*-3HV from *Pseudomonas oleovorans* cells [67]. Variations of solvent extraction methods have been used by others [27, 44, 68] (Table 2). In some processes dichloromethane has been used for extraction instead of chloroform [69].

Some studies have used mechanical disruption by ultrasonication of cells in combination with solvent extraction [70]. Use of other mechanical disruption methods has been reported [62]. Use of chemicals to digest the

**Table 2** PHA recovery processes

| Recovery method | Advantages | Recovery agent | Microorganism | Yield[a] (purity) (%) | References |
|---|---|---|---|---|---|
| Solvent extraction | High purity; endotoxin removal; limited polymer degradation | Chloroform, methanol | *Halomonas campisalis* MCM B-1027 | 36.82 | [64] |
| | | Chloroform, hexane | *Cupriavidus necator* A-04 | 78 | [27] |
| | | Chloroform, hexane | *Wautersia eutropha* ATCC 17699 | 90 | [67] |
| | | Dichloromethane, hexane | *Pseudomonas oleovorans* | 38 | [68] |
| | | Chloroform | Mixed microbial culture | 77 | [43, 44] |
| Mechanical disruption | Less use of chemicals; reduced polymer degradation | Sonication, chloroform | *Alcaligenes lata* DSM1123 | 95 | [70] |
| Chemical digestion | No polymer degradation; high purity; applicable to large volumes and high cell densities | NaClO, chloroform/ethanol | *Escherichia cloacae* SU-1 | 94 | [71] |
| | | SDS, LAS-99, ES702, AOS-04, Brij®58, NaOH | *Ralstonia eutropha*, *Escherichia coli* | 99(90) | [72] |
| Enzymatic digestion (with or without mechanical treatment) | Good polymer recovery; high purity; reduced use of chemicals other than enzymes | Alcalase, SDS, EDTA | *Pseudomonas putida* | 90(92.6) | [75] |
| | | Benzonase, Alcalase, lysozyme, flavourzyme; microfluidizer | *P. putida* PGA1 | (99.2) | [76] |
| Supercritical fluids | Low toxicity; low cost; high polymer purity | $CO_2$ | Bacterial cells | 90(99) | [79] |

[a] Yield is given in terms of PHA content (% of cell dry weight)

cell envelope to facilitate solvent extraction has been reported for PHA recovery from *Enterobacter cloacae* cells [71] as well as from other microorganisms.

A variety of digestive detergents have been evaluated for PHA recovery [72] with varying efficacy. Presumably, the effectiveness of a particular detergent in dissolving the cell envelope depends on the microbial species, but there is no information on this. Detergents such as sodium dodecylsulfate (SDS) have been used to rupture cell membranes to recover granules of the crude PHA polymers [62]. Incubation of *R. eutropha* and *E. coli* cells with 5 % (w/v) SDS for 3 to 6 h has allowed recovery of 95 % of the intracellular PHA [72], but the purity of the recovered polymer was improved by extending the detergent treatment to longer than 6 h. Digestion of the cells was enhanced by increasing the incubation temperature. Digestion of cells with alkali (NaOH) has been used for PHA recovery [62, 72] and there is evidence that overzealous treatment with alkali damages the polymer. A treatment regimen for PHA recovery from *E. coli* may be the use of 1 M NaOH at 50 °C with gentle mixing for 10 min [73].

A comparison of chemical digestion and solvent extraction suggest the latter to afford a greater purity of the PHA product, but if the cells contain a high level of PHA (e.g. >80 % (w/w) of cell mass) digestion with chemicals may give as high a purity as solvent extraction [72]. A case-based evaluation is always necessary in selecting a preferred recovery method.

Enzymatic digestion of *Pseudomonas putida* cells to recover mcl-PHA with a purity of nearly 93 % was reported by Kathiraser et al. [74]. Solvent extraction gave a product of a somewhat higher purity (~96 % pure). The recovery of mcl-PHA from *P. putida* cells by combined enzymatic-chemical digestion has been reported [75]. Alcalase and lysozyme enzymes were effective in digesting the cellular material. Chemicals such as sodium dodecylsulfate (SDS) and ethylenediamine tetra-acetic acid (EDTA) helped in solubilizing the non-PHA materials. This combined treatment allowed nearly 90 % of the PHA to be recovered with a purity of nearly 93 % [75]. Enzymes in general may be too expensive for use in a large-scale extraction process. Some enzymes may digest the PHA polymer [74]. Also, cell wall digesting enzymes tend to the microorganism specific [58]; therefore, a case-based evaluation is necessary.

Combinations of enzymatic and mechanical cell disruption treatments have also been used for recovering the intracellular PHAs [76]. Bacteria contain a significant proportion of their biomass as DNA, a jelly-like polymer, and disruption of the cells releases this DNA in the cell homogenate. Therefore, the homogenate can be quite viscous and difficult to process. Thus, enzymes such as benzonase (a commercial nuclease) may need to be added, for example at a concentration of 10 μL/L cell broth at pH 10, to reduce viscosity by digesting DNA and ease processing through certain mechanical cell disruption devices [76]. In some cases, the PHA pellet recovered from the digested cells has been further treated with ozone or peroxide to remove contaminants [76].

Supercritical fluids have attracted attention for PHA recovery [64, 77–79] (Table 2). Of particular interest is supercritical $CO_2$ as it is inexpensive, readily available, does not leave behind a toxic residue, has a low reactivity, is nonflammable and has a moderate critical temperature (31 °C) and pressure (7.29 MPa). Supercritical fluids have been used to extract nearly 90 % of the PHA in the biomass at purities ranging from 86 to 99 % [64, 79] (Table 2). A variety of other relatively less used methods of recovering PHA exist [64].

## PHA Production in Genetically Modified Plants

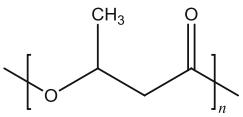
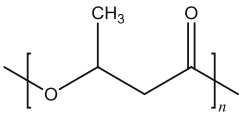
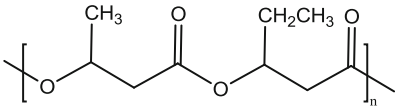
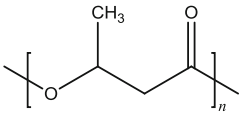
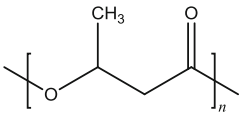
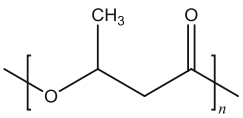
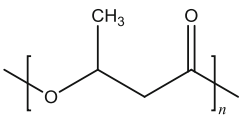
Cost of the carbon source is a substantial contributor to the cost of producing PHAs by microbial processes [80]. Furthermore, recovering the microbial biomass from the fermentation broth and further processing to extract the PHA are expensive. A potentially cheaper production option is to use atmospheric carbon dioxide and sunlight to produce PHAs in genetically modified plants [9, 81, 82]. Plants are easily harvested and a large amount of water does not need to be removed from plant biomass for extracting PHAs. Plant platforms for producing PHA have been extensively reviewed [80, 83, 84]. Some recent studies on PHA biosynthesis in plants are summarized in Table 3.

Synthesis of PHB in genetically modified tobacco plant (*Nicotiana tabacum*) has been reported [85]. The plant had been transformed with a plasmid construct containing genes from *Acinetobacter* sp. and *Bacillus megaterium* to code the enzymes required for PHA synthesis. The modified tobacco produced between 17 and 19 % (w/w) PHB in leaf tissue and nearly 9 % in the total plant biomass.

Matsumoto et al. [86] used the codon optimization method to improve expression of PHA in tobacco inserted with the PHA synthesis genes of *R. eutropha*. The codon-optimization of *pha*B gene resulted in a two-fold increase in PHB content of the plant tissue compared to the case for the non-optimized gene (Table 3) [86]. In contrast, the codon-optimization of *pha*C gene had no significant effect on PHB accumulation [86]. This led to the conclusion that *pha*B gene product had a rate determining influence on PHB production in tobacco leaves [86].

Ariffin et al. [81] reported the transfection of immature palm oil seedlings with *Agrobacterium tumefaciens* carrying pRMIN and pLMIN plasmids harboring the various PHA

**Table 3** PHA biosynthesis in plants

| Plant | PHA genes | Product | Yield | References |
|---|---|---|---|---|
| *Nicotiana tabacum* | *Acinetobacter* sp. thiolase (*pha*A), synthase (*pha*C) |  poly(3-hydroxybutyrate) | 17.3–18.8 % DW in leaf tissues | [9] |
| *Nicotiana tabacum* | *Bacillus megaterium* reductase (*pha*B) |  poly(3-hydroxybutyrate) | 8.8 % DW in total plant biomass | [85] |
| *Elaeis guineensis* | *bkt*B, *pha*B, *pha*C and *tdc*B |  poly(*3*-hydroxybutyrate-*co*-*3*-hydroxyvelarate) | About 91.2 % PHB GUS positive test transformation | [81] |
| *Panicum virgatum* L | *pha*A, phaB of *R. eutropha* hybrid phaC<br>*P. oleovorans/Zoogloea ramigera* |  poly(3-hydroxybutyrate) | 3.72 % DW in leaves and 1.23 % DW from stalk and sprouts | [87] |
| *Arabidopsis thaliana* | *phaA, phaB, phaC* |  poly(3-hydroxybutyrate) | 14.3 % (w/w) PHB in younger leaves; 7 % (w/w) PHB in older ones | [88] |
| *Nicotiana tabacum* | *R. eutropa phaA, phaB, phaC* |  poly(3-hydroxybutyrate) | 2 mg $g^{-1}$ DW | [86] |
| *A. thaliana*, *Saccarum* sp. | *R. eutropa phaA, phaB, phaC* |  poly(3-hydroxybutyrate) | 1.6–1.8 % in the leaves | [11] |

*DW* dry weight

synthesis genes. Nearly 90 % of the transfected calli were successfully transformed (Table 3). A PHB yield of 3.7 % of leaf dry weight and 1.2 % of stalk dry weight has been reported for production in genetically engineered switchgrass *Panicum virgatum* L harboring genes of *Ralstonia eutropha* and a hybrid gene construct from *Pseudomonas oleovorans/Zoogloea ramigera* [87] (Table 3).

A high level of PHB expression in plants causes chlorosis, a condition characterized by a reduced chlorophyll production, consequently reduced production of carbohydrate and reduced plant growth [80]. This problem may be alleviated by delaying the synthesis of PHB until the photosynthetic tissues of the plant are well developed. This may be achieved, for example, by using a chemically induced gene-switch ([88]. The gene switching approach has been demonstrated in *Arabidopsis thaliana* [88]. With this approach, a PHB level of around 14 % (w/w) was obtained in younger leaves and 7 % in older ones [89] (Table 3).

Gene switching is not the only strategy for reducing or overcoming chlorosis. An alternative approach is to produce PHA within the plant peroxisomes [11]. Peroxisomal production of PHA has been reported in *A. thaliana* and *Saccarum* sp. (sugarcane) using *R. eutropha* genes [11]. PHB yields of 1.6 % (w/w) and 1.8 % (w/w) based on dry biomass were obtained in sugarcane leaves and *A. thaliana* seedlings, respectively (Table 3). Although, peroxisomes were the targeted production sites, in sugarcane PHB accumulated throughout most of the leaf cell including in the peroxisomes and the vacuoles.

## Enzymatic Synthesis of PHA In Vitro

Enzyme catalyzed synthesis of PHAs in vitro without involving any microorganisms is an alternative production method [21]. Enzymatic syntheses are generally highly stereoselective, chemoselectvie, regioselective and enantioselective. This ensures a well-defined structure of the synthesized polymer. Furthermore, enzyme catalyzed reactions typically occur under ambient reaction conditions and the separation of the polymer from the reaction mixture is straightforward.

PHA polymers can be produced in vitro from a wide range of substrates including cyclic lactones and carbonates (Table 4). PHAs such as poly-3-hydroxypropionate, poly-4-hydroxybutyrate and poly-6-hydroxyhexanoate have been produced this way. Enzymes used in PHA synthesis include cutinases [20], oxidoreductases [90–92] and hydrolases, especially the serine hydrolases such as lipase (EC 3.1.1.3) [20, 93]. Lipases function as hydrolases in aqueous media to hydrolyze ester bonds [94]; however, in microaqeuous media, lipases can catalyze the formation of ester bonds (Fig. 2). Immobilized lipase B of *Candida antarctica* has proved to be especially effective in production of PHAs via ring opening polymerization of cyclic lactones [91, 95, 96].

Cutinase of the soft-rot fungus *Humicola insolens* has been used to catalyze polycondensations and ring-opening polymerizations of lactones [97]. The optimal activity temperature for this enzyme was 70 °C. The enzyme, immobilized on beads of an ion exchange resin, catalyzed the ring-opening polymerization of both ε-caprolactone (PCL) and ω-pentadecalactone (PDL) in toluene to poly (ε-caprolactone) and poly(ω-pentadecalactone), respectively [97]. Polymerization did not occur if the monomers were changed to (*R*,*S*)-β-butyrolactone and *L*-lactide. In ring-opening polymerization of ε-caprolactone at 70 °C, a monomer conversion of nearly 99 % was achieved (Table 4). The number averaged molecular weight and the polydispersity index of the product depended on whether the polymerization occurred in toluene or in the bulk substrate as the solvent [97].

Using ω-pentadecalactone monomer in toluene, a polymer with a high molecular weight of 44,600 Da could be produced [97]. Lactones with 7- and 16-carbon rings were found to be good monomers for ring-opening polymerization with the immobilized cutinase of *H. insolens* [97]. The enzyme also catalyzed polycondensation of diols and diacids with good activity depending on the chain length of the substrate [97].

Feder and Gross [98] explored the chain length selectivity of *H. insolens* cutinase immobilized on Amberzyme oxirane resin, in polycondensation of ω-hydroxyalkanoic acids having 6, 10, 12 and 16 carbons. The catalytic activity increased as the chain length increased from C12 to C16. No polymerization occurred with C6 and C10 substrates. The C16 substrate could be polymerized to a product with a number averaged molecular weight of 40,400 Da. Using the C16 substrate and immobilized *C. antarctica* lipase B (Novozym 435) as the catalyst, the polymer produced had a number averaged molecular weight of only 25,500 Da [98]. The lipase catalyzed the polymerization of the C12 substrate as effectively as it did that of the C16 substrate, but had no polymerization activity with the C6 substrate [98].

Advances in enzyme catalysis have made possible the production of functionalized PHAs without the need for protection/deprotection of the functional group during the polymerization reaction. Takwa et al. [99] reported the synthesis of ω-functionalized polypentadecalctone containing dithiol, thiol acrylate, diacrylate or dimethacrylate end groups. A single-step solvent-free lipase catalyzed polymerization process was used (Table 4). Two approaches were explored for the synthesis. In the first approach, a difuntionalized polymer with dithiol or thiolacrylate end groups was obtained by mixing the lipase, the lactone and an equimolar mixture of the functional initiator (6-mercapto-1-hexanol) and terminator (11-mercapto-1-undecanoic acid or vinyl acrylate). This way, about 96 % of the polymer was functionalized with dithiol or thiolmethacrylate end groups. In the second approach, a functional diester (ethylene glycol diacrylate or ethylene glycol dimethacrylate) was mixed with lactone and non-dehydrated lipase, allowing the enzymatic water content to serve as initiator. After 2 h of reaction, the pressure was reduced to evaporate the accumulated water so that the reaction could be driven further towards polymerization. This way, more than 96 % of the polymer product could be functionalized with diacrylate or dimethacrylate end groups.

Lipase-catalyzed synthesis of functionalized poly-ω-pentadecalactone-co-butylene-co-carbonate has been reported [19]. A two-stage synthesis was used (Table 4). In the first stage, oligomerization was carried out under a low vacuum (600 mmHg, 18 h, 80 °C). This was followed by the second stage in which polymerization occurred under a high vacuum (2.4 mmHg, 48 h, 80 °C). The copolymer yield from the monomers was nearly 92 % and the product had a high molecular weight of nearly 33,000 Da. The composition of the copolymer could be influence by varying the ratio of the monomers in the feed [19].

Lipase catalyzed synthesis of a copolymer made of 3-hydroxybutyric acid (3HA) and D-glucono-δ-lactone monomers, has been reported [100] (Table 4). Of the several lipases tested for this reaction, the Novozym 435 lipase B from *Candida antarctica* proved to be the best. The reaction was carried out at 80 °C and did not require a

**Table 4** Enzyme-catalyzed polymerization

| Enzyme | Reaction conditions | Monomer | Solvent | Yield | References |
|---|---|---|---|---|---|
| *Hemicola insolens* cutinase | 70 °C, 24 h | Both ε-caprolactone (PCL), ω-pentadecalactone (PDL), (R,S)-β-butyrolactone L-lactide | Toluene, bulk substrate | ~99 % | [97] |
| *Hemicola insolens* cutinase | 70 °C, 8 h | ω-Hydroxyalkanoic acids | Diphenyl ether | ~83 % | [98] |
| *Fervidobacterium nodosum* lipase | 90 °C, 72 h | ε-Caprolactone | Dioxane, acetone, THF, dichloromethane, chloroform, toluene, cyclohexane, *n*-hexane, bulk substrate | 45–94 % | [38] |
| *Candida antarctica* lipase *B* | 60 °C, 48 h | D,D-lactide 1-phenylethanol | $D_8$-Toluene | 11 % monomer conversion; 71 % monomer conversion; 89 % monomer conversion | [112] |
| *C. antartica* lipase *B mutant Q157A* | | | | | |
| *C. antartica* lipase *B mutant Q157A, I189A, L278A* | | | | | |
| *Candida antarctica* lipase B 435 | 80 °C, 24 h | 3-Hydroxybutyric acid D-glucono-δ-lactone | *tert*-butanol/dimethylsulfoxide [Bmim]$PF_6$, bulk substrate | 99 % 3HA monomer conversion | [100] |
| *Candida antarctica* lipase B 435 | 80 °C, 66 h | ω-Pentadecalactone (PDL), diethyl carbonate 1,4-butanediol | Diphenyl ether | 92 % | [19] |
| *Candida antarctica* lipase B 435 | 65 °C, 168 h | ε-Caprolactone, δ-velarolactone | Supercritical $CO_2$ ($scCO_2$) and 1,1,1,2-tetrafluoroethane (R-134a), bulk substrate | 70.5–89.4 % | [114] |

prior derivatization of the monomers. The composition of the product was strongly influenced by the nature of the reaction medium: using a blended *tert*-butanol/dimethylsulfoxide solvent or a medium of the ionic liquid [Bmim]$PF_6$, around 99 % of the 3HA monomer was converted to product. However, the same reaction carried out in the bulk substrate (i.e., without any solvent) resulted in an almost 1.5 fold reduced conversion of the 3HA monomer [100]. The product had a low molecular weight ($\leq$470 Da) and this was attributed to a poor specificity of the lipase towards 3-hydroxybutyric acid [100].

Controlling the concentration of the initiator in the reaction medium in an enzyme catalyzed polymerization can provide some control over the molecular weight of the polymer formed [101]. For example, in the absence of water (an initiator), the polymer formed tends to have a high molecular weight, but the molecular weight is reduced as the concentration of water in the solvent increases. The use of specific initiators may allow an improved tailoring of the polymerization with a better defined outcome in molecular weight and improved fidelity in the terminating end.

Lipases and cutinases are not the only enzymes capable of polymerizing substrates in vitro. Purified PHA synthase has been shown to polymerize substrates in vitro [102]. Using class II PHA synthase ($PhaC1_{Pp}$) from *Pseudomonas putida* and class III PHA synthase ($PhaEC_{Av}$) from *Allochromatium vinosum*, polyhydroxyalkanoates could be synthesized on a hydrophobic support of highly oriented pyrolytic graphite (HOPG) [102]. A poly-3-hydroxyoctanoate film of a few nanometers thickness was formed on the HOPG support when $PhaC1_{Pp}$ and 3-hydroxyoctanoyl-CoA were used. Using the synthase $PhaEC_{Av}$ and 3-hydroxybutyryl-CoA, a homogenous poly-3-hydroxybutyrate was formed on the support. This technology provides a method of forming an ultra-thin PHA film on a hydrophobic support and may have other industrial applications in surface-coatings.

Although enzyme-mediated in vitro polymerization has important advantages, problems remain. Hazardous organic solvents are generally required for achieving high activity with enzymes such as lipases [18, 103]. Also, as polymerization progresses, the concentration of the dissolved polymer increases and so does the viscosity of medium. This imposes diffusion limitations and leads to a polymer with a characteristically low molecular weight [15]. In vitro polymerization with enzymes may be improved by some of the following approaches: (1) microwave irradiation of the reaction mixture [23, 104, 105]; (2) ultrasonic irradiation [96]; (3) replacement of conventional organic solvents with supercritical fluids [106–108] and ionic liquids [104, 109, 110]; (4) use of co-solvent blends [18]; and (5) the use of continuous flow microreactors [111]. In addition, enzyme catalysts themselves may be improved, for example, through molecular

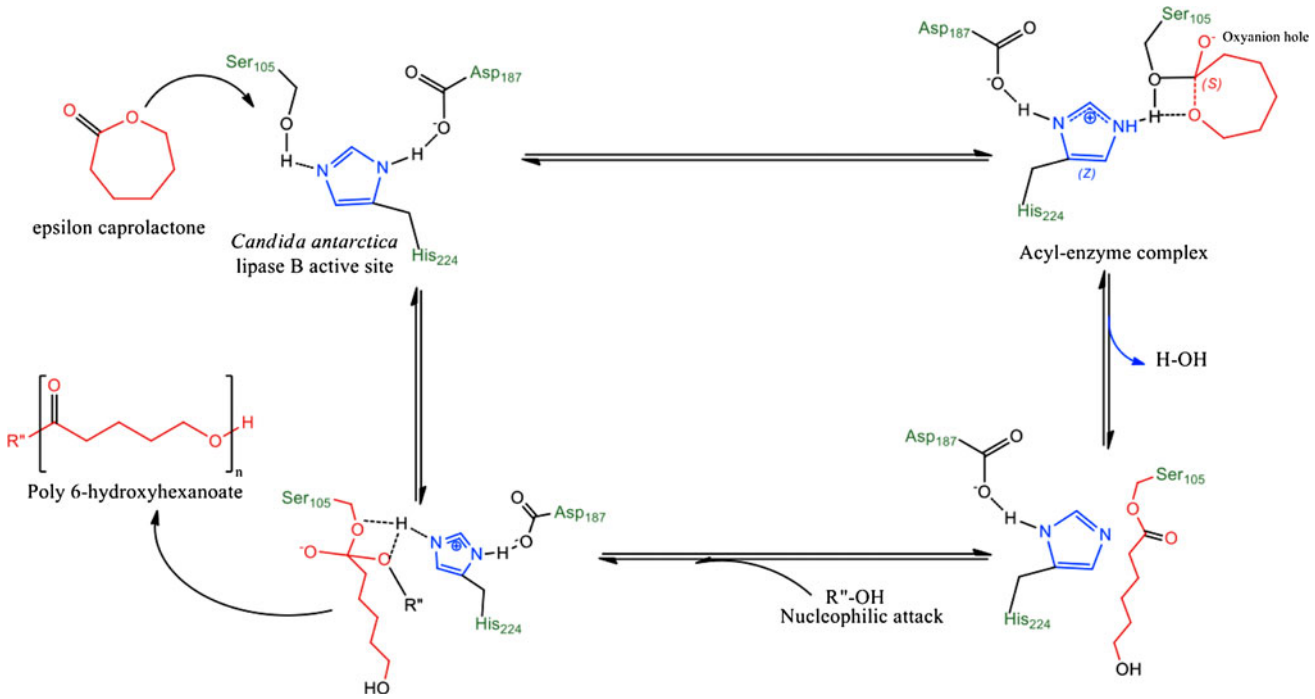



**Fig. 2** Substrate–enzyme interaction at the active site during enzyme-catalyzed ring opening polymerization

engineering [112, 113], modification of immobilization methods [114] and co-lyophilization with non-buffer salts.

Functionalized linear hyperbranched polymers have been produced in supercritical fluids from lactones using lipase-catalyzed synthesis [115]. Supercritical $CO_2$ ($scCO_2$), the bulk substrates (i.e., lactones) and 1,1,1,2-tetrafluoroethane (R-134a) were compared as media for this reaction. Polymerization occurred in both solvents, but was faster in supercritical carbon dioxide. The maximum yield in $scCO_2$ was 89.4 % at 120 h compared to 71.2 % in R-134a and 70.4 % in the bulk substrate. Beyond 120 h of reaction, the yield began to decline in both solvents, but particularly strongly in $scCO_2$ [115]. This was attributed to polymer degradation presumably through hydrolysis.

Enzyme-catalyzed polymerization in a continuous flow microreactor has been reported [111]. The reactor was configured to provide a high catalyst surface area compared to the reactor volume, to improve contact of the substrate with the immobilized enzyme and mass transfer was enhanced through flow. The reaction medium was toluene and the catalyst was Novozym 435 lipase. The reaction occurred at 70 °C. A monomer conversion of >90 % was achieved in less than 5 min in the microreactor whereas a batch process took 30 min to attain a monomer conversion of about 70 % [111].

Molecular modeling techniques have been used to design a mutant lipase of *Candida antarctica* with a 90-fold increased activity relative to the wild-type enzyme during ring-opening polymerization of D,D-lactide [113]. Simulations of molecular dynamics were used to identify steric hindrances preventing effective catalysis at the active site of the native enzyme. Site-specific mutagenesis was then used to delete three amino acids at the entrance of the enzyme's active site. This modification caused the aforementioned increase in enzyme activity relative to the wild-type enzyme and improved monomer conversion. Similar approaches have been used to improve the performance of *Rhizomucor miehei* lipase [116]

A thermophilic lipase from *Fervidobacterium nodosum* has been reported to catalyze the ring opening polymerization of ε-caprolactone [38]. This enzyme had optimal activity at 90 °C compared to an optimum temperature of 60 °C for most other lipases. The thermophilic enzyme achieved a near 100 % conversion of the monomer and yielded a product with a number averaged molecular weight of 2,340 Da. The *F. nodosum* lipase (FNL) had a higher affinity towards ε-caprolactone monomer compared to the commonly used *Candida antarctica* lipase B (CALB). However, in ring-opening polymerization of ε-caprolactone, the specificity and selectivity of FNL were far below those of CALB [38].

A naturally occurring alkaline lipase isolated from *Acinetobacter* sp. has been reported to be stable in a variety of solvents (ethanol, methanol, isopropyl alcohol, dimethylformamide, dimethylsulfoxide, *n*-hexane, acetone), retaining 80 % of its initial activity after 90 min at pH 10 and 50 °C [112].

The selectivity of Novozym 435 in ring-opening polymerization of lactones has been found to depend on the conformation of the substrate: in *cisoid* lactones, the

enzyme shows *S*-selectivity whereas in *transoid* lactones it has a pronounced *R*-selectivity. Iterative tandem catalysis has been used to polymerize 6-methyl-ε-caprolactone [117]. In iterative tandem catalysis, two different catalysts work together to accomplish polymer propagation. For example, combining Novozym 435 with a racemization catalyst results in turning the unreactive terminal alcohols with an *S*-configuration into reactive ones with an *R*-configuration that can be propagated. Using this approach, a racemic monomer could be quantitatively converted into a homochiral polymer [117]. In vitro production of PHAs has been further reviewed [21].

## Chemo-Biosynthesis

Chemical, morphological and physical properties of polymers can be usefully modified by functionalization with different structural and chemical motifs. Synthesis of novel functionalized polymers has been shown to be possible by using a combination of chemical and enzymatic processes [91, 118].

Synthesis of symmetric quintuplet CBABC-type pentablock copolymers has been achieved with a combination of lipase catalysis and atom transfer radical polymerization (ATRP) [118]. The lipase Novozym 435 and ε-caprolactone were used in a first step to produce a tri-block copolymer of di-hydroxyl terminated polycaprolactone block polyethylene oxide (PCL-*b*-PEO-*b*-PCL) using the terminal hydroxyl of di-hydroxyl-capped polyethylene oxide (PEO) as initiator (Fig. 3). Further chemical esterification of this tri-block copolymer with α-bromopropionyl bromide in the presence of dichloromethane gave bromine ended tri-block microinitiator that accepted ATRP of styrene in the presence of copper (I) chloride and 2, 2′-bipyridine, forming quintuplate pentablock copolymer (PSt-*b*-PCL-*b*-PEO-*b*-PCL-*b*-PSt). The number averaged molecular weight of this product was around 38,900 Da. The polymer was capable of assuming different self assembled aggregate morphologies in aqueous media. Similar synthetic processes have been reported for H-shaped block copolymers (Fig. 4) [119] and a Y-shaped $ABA_2$-type tri-block copolymer (Fig. 5) [120]. Chemo-enzymatic synthesis of biodegradable PHA copolymers with an excellent-shape memory has been reported [121].

Most of the copolymerization processes require two consecutive steps. After the formation of the first block, an intermediate transformation step is used to convert the end-groups of the block into active micro-initiators for the next block. An alternative one-pot cascade synthesis has been reported for making block copolymers using a bifunctional initiator to allow consecutive polymerization without the need for intermediate transformation steps [122]. However, the one-pot cascade approach does present major challenges in production of copolymers with a high molecular weight [120, 122].

Tajima et al. [123] reported a chemo-enzymatic synthesis of poly-lactate-co-3-hydroxybutyrate in a water–organic solvent two-phase reaction system (Fig. 6). Chemically synthesized thiophenyl (*R*)-lactate [(*R*)-LATP] and thiophenyl (*R*)-3-hydroxylbutyrate [(*R*)-3HBTP] were used as substrate precursors to first produce hydroxyalkanoylCoA (HACoA) by the ester exchange reaction between the thiophenyl alkanoate and CoA. Then an engineered lactate-polymerizing PHA synthase was used to polymerize the hydroxyalkanoyl-CoA (HACoA) to PHA. This resulted in a copolymer with a number averaged molecular weight of 11,000 Da and a polydispersity index of 1.4. The ratio of the monomers in the copolymer could be controlled by varying the molar ratio of (*R*)-LATP and (*R*)-3HBTP fed to the process. Other similar schemes involving a two-phase reaction system have been reported [124].

## Functionalized Biopolymers

Biodegradable polymers are continuously finding applications in numerous fields especially biomedical. However, most of the synthesized biopolymers lack biological stimulus found in either intra or extra cellular matrix, thus specialized biopolymers are needed to be applied for this purpose. Recent advances in biopolymer engineering resulted in significant efforts towards the synthesis of biopolymers with specific functional groups capable of coupling bioactive ligands (Table 5). For example, stimuli responsive biopolymers having an ability to mimic the cellular response process were reported [125–127]. It is not surprising that these biopolymers have attracted much attention recently due to their ability to respond to specific changes in basic environmental stimuli such as temperature [128], pH [129–131], photo [132] and eletro [133] stimuli while others were reported to respond to multiple stimuli [134]. Recently, a thermo-sensitive triblock of PLA-*b*-PNIPAAm-*b*-PLA having low critical solution temperature (LSCT) of 31.15–32.62 °C has been reported [128]. It has been reported that the thermal stimuli of these polymers to have arisen as a result of the hydrophobic interactions among PNIPAAm molecular chains, the intermolecular hydrogen bonding between the PNIPAAm chains, water molecules, and the intramolecular hydrogen bonding between the $–CONH_2$ groups [125]. Poly-3-hydroxybutyrate macro initiator was used in ATRP to initiate the synthesis of a novel thermo sensitive amphiphilic triblock hydrophobic PHB flanks by hydrophilic PNIPAAm (Table 5) [135]. Zhu et al. [136] reported the synthesis of polycaprolactone based temperature sensitive

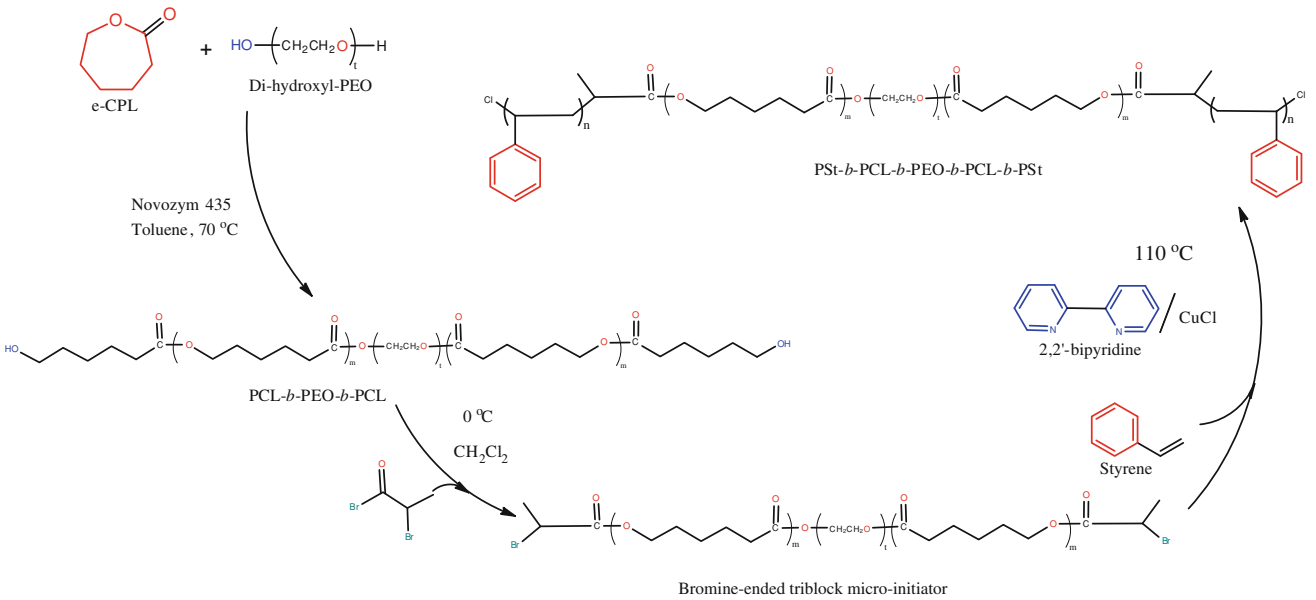



**Fig. 3** Chemo-enzymatic synthesis of a symmetric quintuplet CBABC-type pentablock copolymer. Adapted from [118]

polymer (PNIPAM-b-(HEMA-PCL)) using PNIPAAm as the macroinitiator in RAFT polymerization process. Differences in cellular pH have been utilized in designing novel drug delivery devices (Table 5). For instance, Bawa et al. [130] reported the general blood and tissue pH to be about 7.4 while in carcinogenic cells the pH was found to be about 1.0. Yin et al. [137] recently observed the physico-chemical characteristics of pH sensitive PHF-*b*-PEG micelles (Table 5). The researchers reported that the p$K_a$ value of the copolymer can generally be controlled by changing the ratio of the amino acid residue to that of the lactide and ethylene glycol. Taking these biopolymers as drug delivery devices for example, differences in stomach acidic pH to that of intestinal basic pH could determine their target site for delivering the drug. Thus, serve as pH dependent specific delivery devices. Despite their poor biodegradability, poor polymer-cellular interaction and low solubility in most organic solvents; electro conductive polymers were reported to be used as scaffold in nerve regeneration culture and other biomedical fields [138]. Wei et al. [139] reported the synthesis of specialty polymer with enhanced PC-12 cellular attachment and differentiation using a film of PANi funtionalized with bioactive laminin-derived adhesion peptide. Plant bioactive coumarin is reported to be used as photoinduced cross linker in the synthesis of photosensitive polymers [140]. Coumarin encapped PCL-co-TMC were use as photocurable precursors in biomedical devices fabrication and drug encapsulation devices [125, 141]. Polyethyleneglycole functionalized gadolinium ion ($Gd^{3+}$) has been reported to be used as in vivo paramagnetic probe for magnetic resonance imaging [142].

Sugars such as galactose and mannose were reported to be specific ligands to the ASGPR receptor, which is overexpressed in hepatocellular carcinoma [143]. Jiang et al. [144] observed the solution behavior of PCL functionalized hydroxyethyl cellulose (HEC). Previously, Lu et al. [145] reported the synthesis of poly(1′-O-vinyladipoyl-sucrose) in chemo-enzymatic process resulting in polymer with molecular weight as high as 53 kDa having improved solvation properties that can be explored as promising biomaterial. In general, polymer functionalization has resulted in recent increasing demand of biodegrabable polymers in diverse industrial applications. Functionalization of polymer has opened a new avenue for the production of novel polymers with specific application that were not possible earlier.

## Applications of Polyhydroxyalkanoates

### Biomedical Applications

PHAs have attracted much attention as materials for biodegradable implants in biomedical and tissue engineering applications. Specifically, PHB has been reported to be biocompatible with various kinds of cells [146] (Table 6).

Use of PHAs as drug delivery systems (Table 6) for prolonged release of therapeutics into systemic circulation is receiving attention [147]. Polyhydroxyesters such as block copolymer of PEG-*b*-PCL in the form of micelles and nanoparticles have been used for parenteral delivery of taxanes [148] (Table 6). A matrix of nanoparticles of poly-3-hydroxybutyrate-*co*-3-hydroxyhexanoate (PHBHHx) has

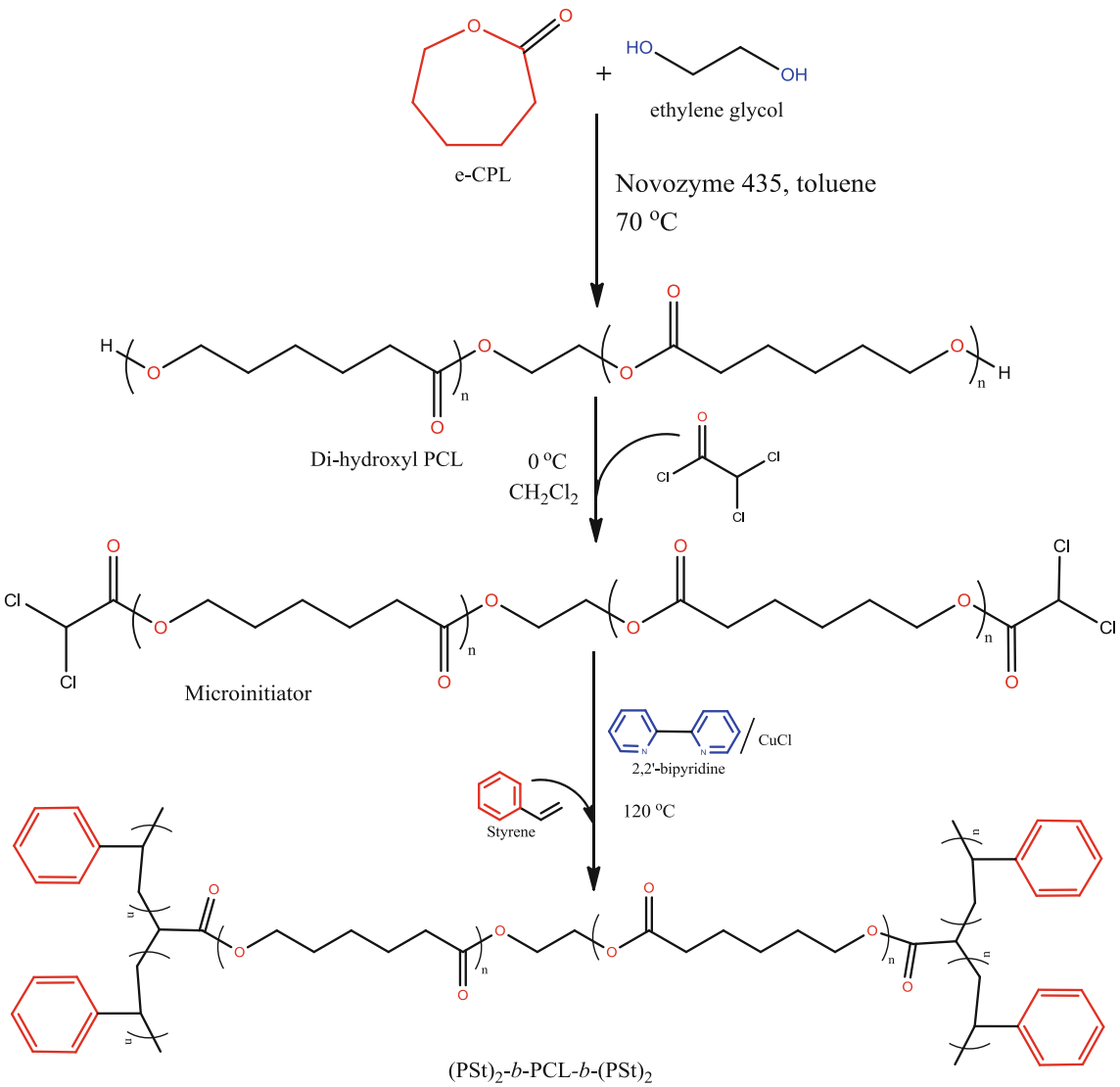



**Fig. 4** Chemo-enzymatic synthesis of H-shaped block copolymer. Adapted from [119]

been used to deliver antineoplastic agents to cancer cells [149] (Table 6). PHB nanoparticles functionalized with a tumor-specific ligand have been examined for specifically targeting certain breast cancer cells [150]. The non-steroidal anti-inflammatory drug ibuprofen has been conjugated to nontoxic oligo(3-hydroxybutyrate), in attempts to improve drug delivery but this novel formulation remains to be thoroughly assessed [151].

Poly-3-hydroxybutyrate microsphere have been tested in vitro for releasing the antibiotics gentamycin and tetracycline [152]. Multifunctional PHB/45S5Bioglass composite system has been discussed as drug delivery agents and for use in certain bone tissue engineering applications [152].

Polycaprolactone (PCL) tends to be highly permeable and this is an attractive feature in some drug delivery applications. Use of PCL in certain drug delivery applications has been approved by the US Food and Drug Administration [146]. PCL degrades slowly (2–4 years) in vivo and is therefore useful for developing drug release implants for long-term use [153].

Silver nanoparticles have attracted much attention because of their antibacterial properties [154–156], but slurries of such particles tend to be unstable. Use of polyhydroxyalkanoates in prolonging stability of such slurries has been reported [157].

Poly-3-hydroxybutyrate-*co*-3-hydroxyhexanoate scaffolds have been evaluated for use in eyelid reconstruction in experimental animals [158] (Table 6). Although the scaffold performed satisfactorily, it produced some inflammation that took about 2 weeks to clear. Poly-3-hydroxybutyrate-*co*-3-hydroxyhexanoate was found to induce cartilage development from mouse mesenchymal stem cells and preserve the chondrocytic phenotype of the cells [159] (Table 6).

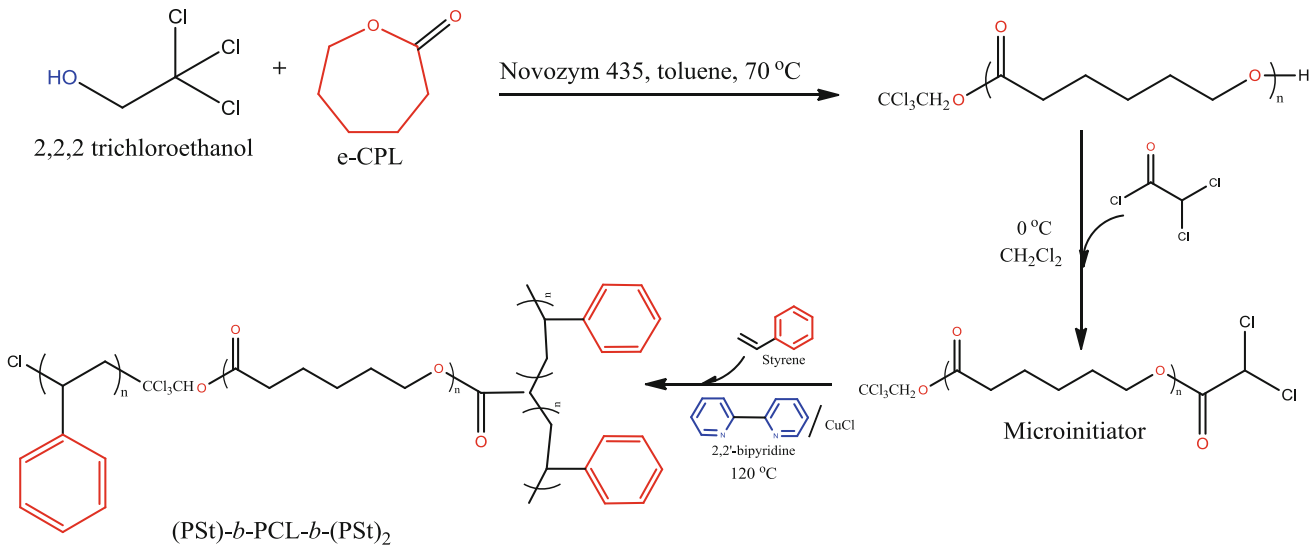



**Fig. 5** Chemo-enzymatic synthesis of Y-shaped block copolymer. Adapted from [120]

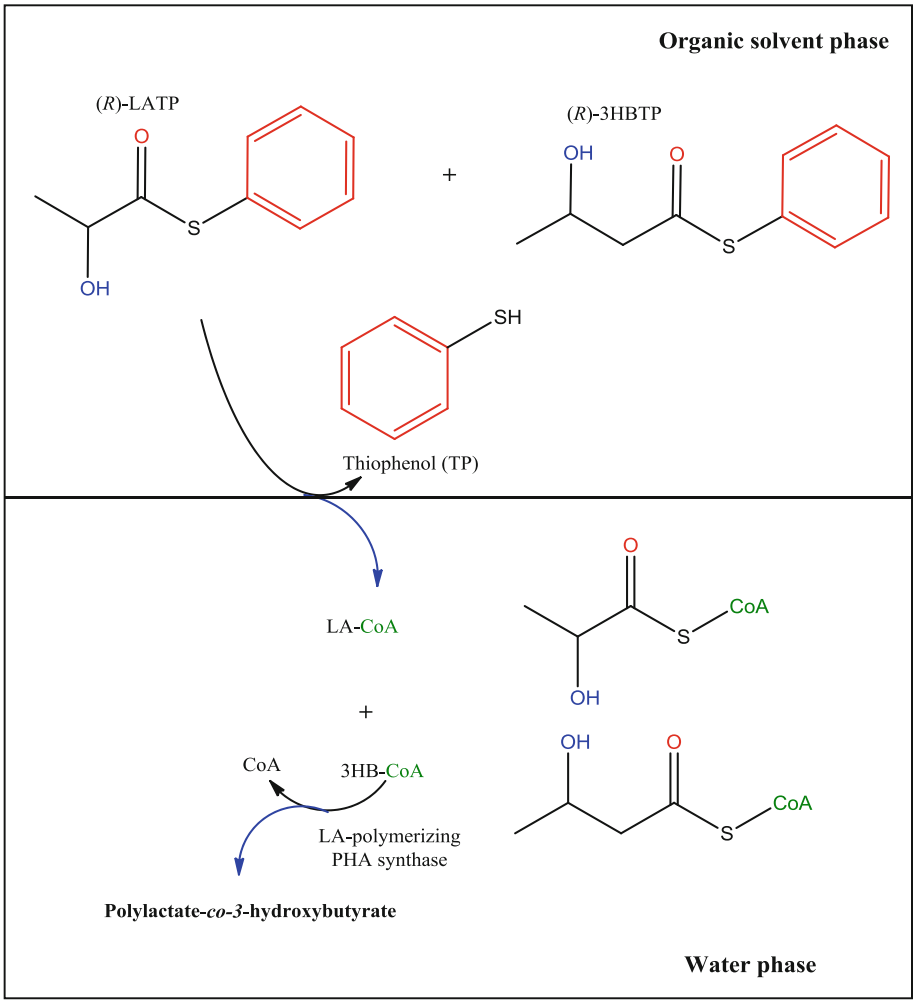
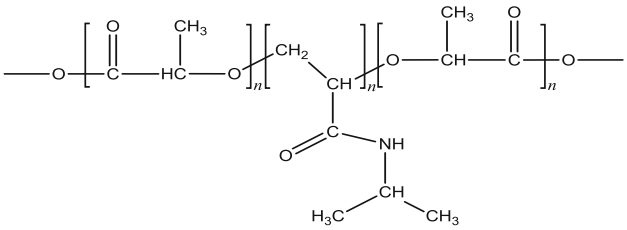
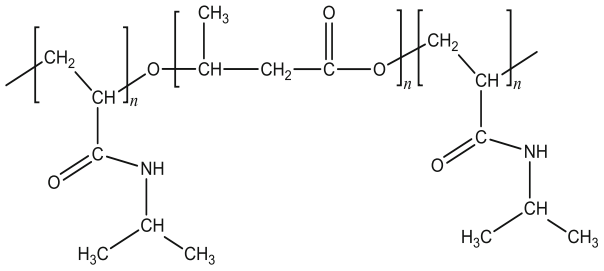
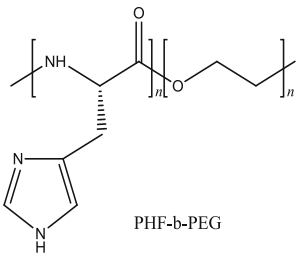
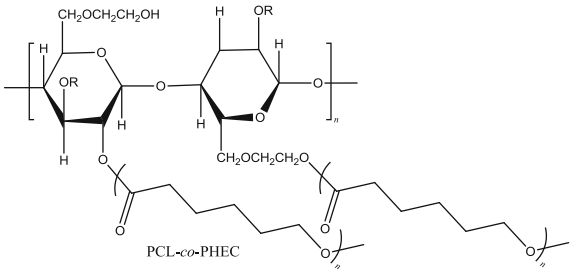



**Fig. 6** Chemo-enzymatic synthesis of polylactate-co-3-hydroxybutyrate in a two-phase reaction system. Adapted from [123]

**Table 5** Functionalized biopolymers

| Functionalized character | Polymer | Application | References |
|---|---|---|---|
| Temperature responsive |  PLA-*b*-PNIPAAm-*b*-PLA | Drug delivery device | [128] |
| |  PNIPAAm-*b*-PHB-*b*-PNIPAAm | Drug delivery device | [135] |
| pH responsive |  PHF-b-PEG | Drug delivery device | [137] |
| Specific binding |  PCL-*co*-PHEC | Drug delivery device | [144] |

The use of a terpolyester of 3-hydroxybutyrate-*b*-3-hydroxyvalerate-*b*-3-hydroxyhexanoate (PHBVHHx) as scaffold for promoting differentiation of human bone marrow mesenchymal stem cell into nerve cells (Table 6) has been reported [160]. PHBVHHx scaffolds with a pore size of 30–60 μm were found to be best.

PCL reinforced with phosphate glass fibers has been used to make fixation pins for intramedullary fractures, craniofacial repairs and general bone repair [146]. PCL can be modified in various ways to improve mechanical strength and alter properties such as degradability, compatibility, hydrophilicity and crystallinity. For example, poly-ε-caprolactone functionalized polyethylene glycol copolymer has been reported to have a strong amphiphilicity, a controlled biodegradability and excellent biocompatability.

PCL blended with poly-glycerol sebacate has been used as a fibrous scaffold for aortic valve regeneration [161]. Use of both PCL and PHA has been reported as substrates for cardiovascular tissue engineering [162] (Table 6). The diverse biomedical applications of PCL have been further reviewed by others [163, 164]. Among the various other biomedical applications, PHA is being used in tablet formulations [165, 166], surgical sutures [39], wound dressings [167, 168], surgical implants to join tubular body parts [169, 170], controlled release contraceptive devices [171–173], lubricating powders, blood vessels, tissue scaffolds, and bone fracture fixation plates [39, 174–177]. Of these

**Table 6** Applications of polyhydroxyalkanoates

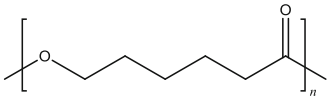
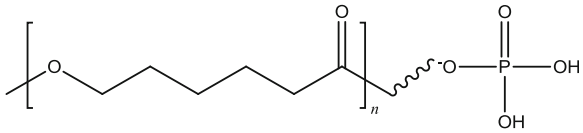
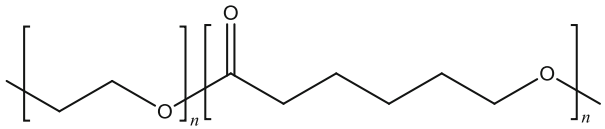
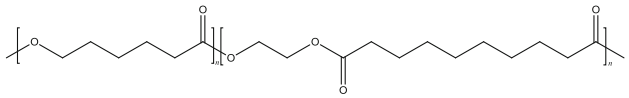
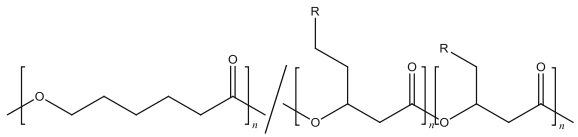
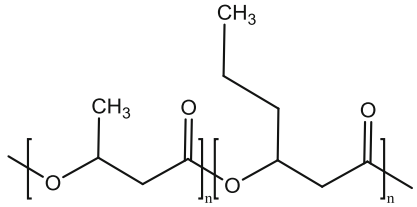
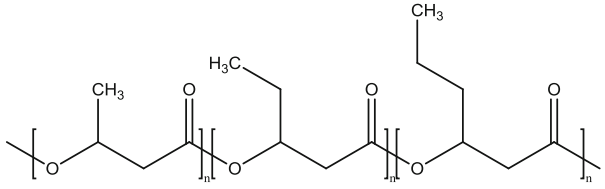
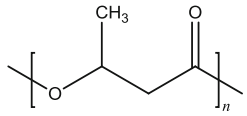
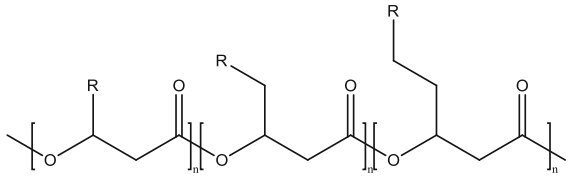
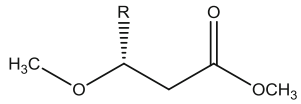
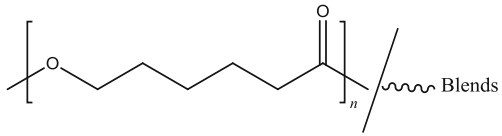
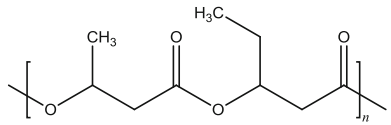
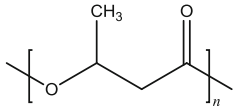
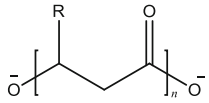
| Polymer | Chemical structure | Polymer form used | Applications | References |
|---|---|---|---|---|
| Biomedical applications | | | | |
| PCL |  | Drug delivery devices | Chemopreventive curcumin delivery | [147] |
| PCL/phosphate glass fibers |  | Fibers | Fracture fixation pins, craniofacial repair and general bone regeneration | [146] |
| PEG-b-PCL |  | Fibrous scaffolds | Antineoplastic taxanes delivery | [148] |
| PCL/PEG sebacate blends |  | Fibrous scaffolds | Aortic valve regeneration | [161] |
| PCL/PHA blends |  | Scaffolds | Cardiovascular tissue engineering | [162] |
| PHBHHx |  | Nanoparticles matrix | Antineoplastics agents carrier | [149] |
| | | Scaffolds | Eye lid tissue regeneration | [158] |
| | | | Induce chondrogenesis of mesenchymal stem cells | [159] |
| PHBHVHHx |  | Scaffold | Differentiation of human bone marrow mesenchymal stem cells (hBMSC) into nerve cells | [160] |

**Table 6** continued

| Polymer | Chemical structure | Polymer form used | Applications | References |
|---|---|---|---|---|
| PHB/(RGD4C) |  poly(3-hydroxybutyrate) | Functionalized nanoparticles | Breast cancer therapy | [150] |
| PHB/45S5® bioglass composite | | Microsphere | Tissue engineering | [152] |
| PHA |  | Colloids | Stabilizes silver nanoparticles | [157] |
| Industrial applications | | | | |
| PHA methyl esters |  | 3-Hydroxy alkanoate methyl esters | Biofuel and fuel additives | [53, 179, 180] |
| PCL blends |  | Nanocomposites | Packaging | [183] |
| PHBHV |  | Copolymer film | Packaging | [182] |
| PHB |  | Granules in-feed | Pathogenic bacterial growth inhibitor in aquaculture | [183–185] |
| | | Micro and nanoparticles | Herbicides controlled release carrier | [186] |
| | | Composite | Biomimetic absorbent | [187] |
| PHA |  | Granules | Paper sizing | [181] |

applications, surgical sutures constitute perhaps the largest use category with a 2010 market value exceeding US$1.3 billion annually [178].

### Industrial Applications

In addition to their biomedical applications, PHAs can potentially replace petrochemicals-based plastics in diverse other applications (Table 6).

A recently suggested application is the use of PHAs as precursors of biofuels. Like bioethanol from sugars, PHAs can be made into renewable biofuels. Hydrolysis of PHAs followed by methyl esterification provides 3-hydroxyalkanoates methyl esters with an energy content that is comparable to that of bioethanol [53, 179, 180]. Whether hydroxyalkanoate esters would be as cheap as bioethanol in fuel blends is debatable. This is because in making a PHA-based fuel, a carbon source first needs to be polymerized, the polymer then needs to be hydrolyzed and a subsequent methylation step is required. In contrast, glucose and other sugars can be directly fermented to bioethanol.

Polyhydroxyalkanoate latexes have been used in the paper industry for surface coating of paper and as sizing agents [181] (Table 6). PHB has had limited applications in packaging because of its high glass transition temperature which results in brittleness under typical use conditions. PHB's utility in packaging has been improved by copolymerizing with various levels (5–20 %) of valerate to produce PHBV polymers with glass transition temperatures of 4 °C at 5 % and 1 °C at 20 % valerate content [182] (Table 6). Novel polymeric materials for food packaging consisting of PCL blends have been developed [183]. They have been reported to have an excellent durability and a remarkable tensile strength [183] (Table 6). Uses of PHAs in the food industry have been discussed elsewhere [184, 185].

PHAs appear to be potentially useful in controlling bacterial pathogens in certain aquaculture applications [186–188]. For example, administration in the feed of 1,000 mg $L^{-1}$ of PHB particles of an average diameter of 30 μm, or addition of inactivated cells ($10^7$ cells $mL^{-1}$) of PHB-containing *Brachymonas* bacteria (equivalent to ~10 mg $L^{-1}$ PHB) to the culture water of brine shrimp (*Artemia nauplii*) larvae, conferred a complete protection from a virulent strain of the intestinal pathogen *Vibrio campbellii* [187]. Other similar reports have claimed an inhibitory effect of PHB on certain gut microflora of the giant freshwater prawn (*Macrobrachium rosenbergii*) larvae [188]. Administration of PHB (5 g $L^{-1}$) in the feed significantly increased the survival of the prawn larvae and improved their development. The total bacterial counts and *Vibrio* spp. counts were significantly reduced in PHB-fed larvae compared to the control larvae.

Polyhydroxyalkanoates have been used as controlled release agents for herbicides in agricultural. Controlled release potentially reduces the impact of the herbicides on nontarget species and reduces the need for repeated applications [189]. Micro- and nanoparticles of PHB and PHBV were used in a controlled release formulation of the herbicide ametryn [189] (Table 6). PHB has been successfully tested for removing lipid-soluble organic pollutants from water by adsorption [190] (Table 6).

## Concluding Remarks

PHAs are versatile biopolymers with diverse applications. PHAs are biodegradable and they may be produced in a sustainable way using renewable feedstocks. PHAs can be produced in vitro using enzymes without involving microbial cells. Alternatively, they may be produced by microbial fermentation processes and using recombinant plants. Production of PHAs using microorganisms and enzymes remains relatively expensive compared to plastics derived from petroleum. Production in plants is likely to establish itself as the least expensive option for certain PHAs, but does not currently offer the molecular versatility of the products that can be made using microbial fermentations and enzymes. In view of their relatively high cost, PHAs are likely to be used first in high-value niche applications, particularly in biomedicine, but their broader industrial use will increase as the cost of production declines.

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## References

1. Bauwens T (2011) First estimates suggest around 4% increase in plastics global production from 2010. Plastics Europe. http://www.plasticseurope.org/information-centre/press-room-1351/press-releases-2012/first-estimates-suggest-around-4-increase-in-plastics-global-production-from-2010.aspx. Accessed 18 July 2012
2. Piet LPJ (2010) World-wide production of crude steel and plastics 1950–2010. Eindhoven University of Technology, Netherlands
3. Prieto MA (2007) From oil to bioplastics, a dream come true? J Bacteriol 189(2):289
4. Ko-Sin N, Wong Y-M, Tsuge T, Sudesh K (2011) Biosynthesis and characterization of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) copolymers using jatropha oil as the main carbon source. Process Biochem 46(8):1572–1578. doi:10.1016/j.procbio.2011.04.012
5. Ni Y–Y, Kim DY, Chung MG, Lee SH, Park H-Y, Rhee YH (2010) Biosynthesis of medium-chain-length poly(3-hydroxyalkanoates) by volatile aromatic hydrocarbons-degrading *Pseudomonas fulva* TY16. Bioresour Technol 101(21):8485–8488. doi:10.1016/j.biortech.2010.06.033

6. Hofer P, Vermette P, Groleau D (2011) Production and characterization of polyhydroxyalkanoates by recombinant *Methylobacterium extorquens*: combining desirable thermal properties with functionality. Biochem Eng J 54(1):26–33
7. Abu-Elreesh G, Zaki S, Farag S, Elkady MF, Abd-El-Haleem D (2011) Exobiopolymer from polyhydroxyalkanoate-producing transgenic yeast. Afr J Biotechnol 10(34):6558–6563
8. Sabirova J, Golyshin P, Ferrer M, Lunsdorf H, Abraham W, Timmis K (2011) Extracellular polyhydroxyalkanoates produced by genetically engineered microorganisms. US Patent 20,110,183,388
9. Bohmert-Tatarev K, McAvoy S, Daughtry S, Peoples OP, Snell KD (2011) High levels of bioplastic are produced in fertile transplastomic tobacco plants engineered with a synthetic operon for the production of polyhydroxybutyrate. Plant Physiol 155(4):1690
10. Brumbley S, Petrasovits LA, McQualter RA, Zhou L, Nielsen LK (2008) Sugarcane: an industrial crop for the production of polyhydroxyalkanoates. In: ComBio2008, Canberra, Australia, 21–25 Sept 2008
11. Tilbrook K, Gebbie L, Schenk PM, Poirier Y, Brumbley SM (2011) Peroxisomal polyhydroxyalkanoate biosynthesis is a promising strategy for bioplastic production in high biomass crops. Plant Biotechnol J 9(9):958–969
12. Van Beilen JB, Poirier Y (2008) Production of renewable polymers from crop plants. Plant J 54(4):684–701
13. Otari S, Ghosh J (2009) Production and characterization of the polymer polyhydroxy butyrate-co-polyhydroxy valerate by *Bacillus megaterium* NCIM 2475. Curr Res J Biol Sci 1(2): 23–26
14. Bhubalan K, Chuah JA, Shozui F, Brigham CJ, Taguchi S, Sinskey AJ, Rha CK, Sudesh K (2011) Characterization of the highly active polyhydroxyalkanoate synthase of *Chromobacterium* sp. strain USM2. Appl Environ Microbiol 77(9):2926
15. Kobayashi S (2010) Lipase-catalyzed polyester synthesis—a green polymer chemistry. Proc Jpn Acad Ser B 86(4):338–365
16. Schultz A (2011) Consumers push plastics industry to find bio-based solutions. CNBC News. http://www.cnbc.com/id/42194558/Consumers_Push_Plastics_Industry_to_Find_Bio_Based_Solutions. Accessed 4 Aug 2011
17. Mohan AM (2010) Biodegradable polymers market packaging world report. http://www.packworld.com/news-29339
18. Gumel AM, Annuar MSM, Heidelberg T, Chisti Y (2011) Thermo-kinetics of lipase-catalyzed synthesis of 6-O-glucosyldecanoate. Bioresour Technol 102(19):8727–8732
19. Jiang Z (2011) Lipase-catalyzed copolymerization of dialkyl carbonate with 1, 4-butanediol and $\omega$-pentadecalactone: synthesis of poly ($\omega$-pentadecalactone-co-butylene-co-carbonate). Biomacromolecules 12:1912–1919
20. Miletic N, Loos K, Gross RA (2011) Enzymatic polymerization of polyester. In: Katja L (ed) Biocatalysis in polymer chemistry. Wiley-VCH, pp 84–128
21. Thomson N, Roy I, Summers D, Sivaniah E (2010) In vitro production of polyhydroxyalkanoates: achievements and applications. J Chem Technol Biotechnol 85(6):760–767
22. Yao D, Li G, Kuila T, Li P, Kim NH, Kim SI, Lee JH (2011) Lipase catalyzed synthesis and characterization of biodegradable polyester containing L-malic acid unit in solvent system. J Appl Polym Sci 120(2):1114–1120
23. Matos TD, King N, Simmons L, Walker C, McClain AR, Mahapatro A, Rispoli FJ, McDonnell KT, Shah V (2011) Microwave assisted lipase catalyzed solvent-free poly-$\varepsilon$-caprolactone synthesis. Green Chem Lett Rev 4(1):73–79
24. Akaraonye E, Keshavarz T, Roy I (2010) Production of polyhydroxyalkanoates: the future green materials of choice. J Chem Technol Biotechnol 85(6):732–743
25. Suriyamongkol P, Weselake R, Narine S, Moloney M, Shah S (2007) Biotechnological approaches for the production of polyhydroxyalkanoates in microorganisms and plants—a review. Biotechnol Adv 25(2):148–175
26. Annuar MSM, Tan IKP, Ramachandran KB (2008) Evaluation of nitrogen sources for growth and production of medium-chain-length poly-(3-hydroxyalkanoates) from palm kernel oil by *Pseudomonas putida* PGA 1. Asia Pac J Mol Biol Biotechnol 16(1):11–15
27. Chanprateep S, Buasri K, Muangwong A, Utiswannakul P (2010) Biosynthesis and biocompatibility of biodegradable poly(3-hydroxybutyrate-co-4-hydroxybutyrate). Polym Degrad Stab 95(10):2003–2012. doi:10.1016/j.polymdegradstab.2010.07.014
28. Franz A, Song HS, Ramkrishna D, Kienle A (2011) Experimental and theoretical analysis of poly ([beta]-hydroxybutyrate) formation and consumption in *Ralstonia eutropha*. Biochem Eng J 55(1):49–58
29. Grothe E, Chisti Y (2000) Poly (ß-hydroxybutyric acid) thermoplastic production by *Alcaligenes latus*: behavior of fed-batch cultures. Bioproc Biosys Eng 22(5):441–449
30. Grothe E, Moo-Young M, Chisti Y (1999) Fermentation optimization for the production of poly ([beta]-hydroxybutyric acid) microbial thermoplastic. Enzyme Microb Technol 25(1–2): 132–141
31. Salim YS, Abdullah AA-A, Nasri CSSM, Ibrahim MNM (2011) Biosynthesis of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and characterisation of its blend with oil palm empty fruit bunch fibers. Bioresour Technol 102(3):3626–3628. doi:10.1016/j.biortech.2010.11.020
32. Tian P, Shang L, Ren H, Mi Y, Fan D, Jiang M (2010) Biosynthesis of polyhydroxyalkanoates: current research and development. Afr J Biotechnol 8(5):709–714
33. Quillaguamán J, Guzmán H, Van-Thuoc D, Hatti-Kaul R (2010) Synthesis and production of polyhydroxyalkanoates by halophiles: current potential and future prospects. Appl Microbiol Biotechnol 85(6):1687–1696
34. Sudesh K, Bhubalan K, Chuah JA, Kek YK, Kamilah H, Sridewi N, Lee YF (2011) Synthesis of polyhydroxyalkanoate from palm oil and some new applications. Appl Microbiol Biotechnol 89(5):1373–1386
35. Jensen TE, Sicko LM (1971) Fine structure of poly-{beta}-hydroxybutyric acid granules in a blue-green alga, *Chlorogloea fritschii*. J Bacteriol 106(2):683
36. Yan Q, Zhao M, Miao H, Ruan W, Song R (2010) Coupling of the hydrogen and polyhydroxyalkanoates (PHA) production through anaerobic digestion from Taihu blue algae. Bioresour Technol 101(12):4508–4512. doi:10.1016/j.biortech.2010.01.073
37. Gumel AM, Annuar MSM, Heidelberg T (2012) Effects of carbon substrates on biodegradable polymer composition and stability produced by *Delftia tsuruhatensis* Bet002 isolated from palm oil mill effluent. Polym Degrad Stab 97(8):1227–1231. doi:10.1016/j.polymdegradstab.2012.05.041
38. Li Q, Li G, Yu S, Zhang Z, Ma F, Feng Y (2010) Ring-opening polymerization of $\varepsilon$-caprolactone catalyzed by a novel thermophilic lipase from *Fervidobacterium nodosum*. Process Biochem 46(1):253–257
39. Rai R, Keshavarz T, Roether J, Boccaccini A, Roy I (2011) Medium chain length polyhydroxyalkanoates, promising new biomedical materials for the future. Mater Sci Eng R Reports 72(3):29–47
40. Ramalingam S, Vikram M, Vigneshbabu M, Sivasankari M (2011) Flux balance analysis for maximizing polyhydroxyalkanoate production in *Pseudomonas putida*. Indian J Biotechnol 10(1):70–74
41. Hori K, Ichinohe R, Unno H, Marsudi S (2011) Simultaneous syntheses of polyhydroxyalkanoates and rhamnolipids by

*Pseudomonas aeruginosa* IFO3924 at various temperatures and from various fatty acids. Biochem Eng J 53:196–202
42. de Almeida A, Giordano AM, Nikel PI, Pettinari MJ (2010) Effects of aeration on the synthesis of poly (3-hydroxybutyrate) from glycerol and glucose in recombinant *Escherichia coli*. Appl Environ Microbiol 76(6):2036–2040
43. Albuquerque MGE, Concas S, Bengtsson S, Reis MAM (2010) Mixed culture polyhydroxyalkanoates production from sugar molasses: the use of a 2-stage CSTR system for culture selection. Bioresour Technol 101(18):7112–7122. doi:10.1016/j.biortech.2010.04.019
44. Albuquerque MGE, Martino V, Pollet E, Avérous L, Reis MAM (2011) Mixed culture polyhydroxyalkanoate (PHA) production from volatile fatty acid (VFA)-rich streams: effect of substrate composition and feeding regime on PHA productivity, composition and properties. J Biotechnol 151(1):66–76. doi:10.1016/j.jbiotec.2010.10.070
45. Matsumoto K, Kobayashi H, Ikeda K, Komanoya T, Fukuoka A, Taguchi S (2011) Chemo-microbial conversion of cellulose into polyhydroxybutyrate through ruthenium-catalyzed hydrolysis of cellulose into glucose. Bioresour Technol 102:3564–3567
46. Zúñiga C, Morales M, Le Borgne S, Revah S (2011) Production of poly-[beta]-hydroxybutyrate (PHB) by *Methylobacterium organophilum* isolated from a methanotrophic consortium in a two-phase partition bioreactor. J Hazard Mater 190(1–3):876–882
47. Xu Z, Chen H, Wu H, Li L (2010) 7 mT static magnetic exposure enhanced synthesis of poly-3-hydroxybutyrate by activated sludge at low temperature and high acetate concentration. Process Saf Environ Prot 88(4):292–296
48. Shrivastav A, Mishra SK, Mishra S (2010) Polyhydroxyalkanoate (PHA) synthesis by *Spirulina subsalsa* from Gujarat coast of India. Int J Biol Macromol 46(2):255–260
49. Filippou PS, Koini EN, Calogeropoulou T, Kalliakmani P, Panagiotidis CA, Kyriakidis DA (2011) Regulation of the *E. coli* AtoSC two component system by synthetic biologically active 5, 7, 8-trimethyl-1, 4-benzoxazine analogues. Bioorg Med Chem 19(16):5061–5070
50. Theodorou EC, Theodorou MC, Kyriakidis DA (2011) AtoSC two-component system is involved in cPHB biosynthesis through fatty acid metabolism in *E. coli*. BBA Gen Subj 1810(5):561–568
51. Theodorou EC, Theodorou MC, Samali MN, Kyriakidis DA (2011) Activation of the AtoSC two-component system in the absence of the AtoC N-terminal receiver domain in *E. coli*. Amino Acids 40(2):1–10
52. Jian J, Zhang SQ, Shi ZY, Wang W, Chen GQ, Wu Q (2010) Production of polyhydroxyalkanoates by *Escherichia coli* mutants with defected mixed acid fermentation pathways. Appl Microbiol Biotechnol 87(6):1–10
53. Gao X, Chen JC, Wu Q, Chen GQ (2011) Polyhydroxyalkanoates as a source of chemicals, polymers, and biofuels. Curr Opin Biotechnol 22(6):768–774
54. Kang Z, Du L, Kang J, Wang Y, Wang Q, Liang Q, Qi Q (2011) Production of succinate and polyhydroxyalkanoate from substrate mixture by metabolically engineered *Escherichia coli*. Biores Technol 102(11):6600–6604
55. Liu Q, Luo G, Zhou XR, Chen GQ (2011) Biosynthesis of poly(3-hydroxydecanoate) and 3-hydroxydodecanoate dominating polyhydroxyalkanoates by $\beta$-oxidation pathway inhibited *Pseudomonas putida*. Metab Eng 13:11–17
56. Chung AL, Jin HL, Huang LJ, Ye HM, Chen JC, Wu Q, Chen GQ (2011) Biosynthesis and characterization of Poly (3-hydroxydodecanoate) by $\beta$-oxidation inhibited mutant of *Pseudomonas entomophila* L48. Biomacromolecules 12(10):3559–3566
57. Bhubalan K, Rathi D-N, Abe H, Iwata T, Sudesh K (2010) Improved synthesis of P(3HB-co-3HV-co-3HHx) terpolymers by mutant *Cupriavidus necator* using the PHA synthase gene of *Chromobacterium* sp. USM2 with high affinity towards 3HV. Polym Degrad Stab 95(8):1436–1442. doi:10.1016/j.polymdegradstab.2009.12.018
58. Chisti Y, Moo-Young M (1986) Disruption of microbial cells for intracellular products. Enzyme Microb Technol 8(4):194–204
59. Buelhamd A, Abd-El-Haleem D, Zaki S, Amara A, GMS A (2007) Genetic engineering of *Schizosaccharomyces pombe* to produce bacterial polyhydroxyalkanotes. J Appl Sci Environ Manag 11(2):83–90
60. Desuoky A, El-Haleem A, Zaki S, Abuelhamd A, Amara A, Aboelreesh G (2007) Biosynthesis of polyhydroxyalkanotes in wildtype yeasts. J Appl Sci Environ Manag 11(3):1119–8362
61. Sabirova JS, Haddouche R, Van Bogaert I, Mulaa F, Verstraete W, Timmis K, Schmidt Dannert C, Nicaud J, Soetaert W (2011) The 'LipoYeasts' project: using the oleaginous yeast *Yarrowia lipolytica* in combination with specific bacterial genes for the bioconversion of lipids, fats and oils into high value products. Microb Biotechnol 4(1):47–54
62. Tamer IM, Moo-Young M, Chisti Y (1998) Disruption of Alcaligenes latus for recovery of poly ($\beta$-hydroxybutyric acid): comparison of high-pressure homogenization, bead milling, and chemically induced lysis. Ind Eng Chem Res 37(5):1807–1814
63. Kunasundari B, Sudesh K (2011) Isolation and recovery of microbial polyhydroxyalkanoates. eXPRESS Pol Lett 5(7):620–634. doi:10.3144/expresspolymlett.2011.60
64. Jacquel N, Lo C-W, Wei Y-H, Wu H-S, Wang SS (2008) Isolation and purification of bacterial poly(3-hydroxyalkanoates). Biochem Eng J 39(1):15–27. doi:10.1016/j.bej.2007.11.029
65. Kulkarni SO, Kanekar PP, Jog JP, Patil PA, Nilegaonkar SS, Sarnaik SS, Kshirsagar PR (2011) Characterisation of copolymer, poly (hydroxybutyrate-co-hydroxyvalerate) (PHB-co-PHV) produced by *Halomonas campisalis* (MCM B-1027), its biodegradability and potential application. Bioresour Technol 102(11):6625–6628. doi:10.1016/j.biortech.2011.03.054
66. Rao U, Sridhar R, Sehgal PK (2010) Biosynthesis and biocompatibility of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) produced by *Cupriavidus necator* from spent palm oil. Biochem Eng J 49(1):13–20. doi:10.1016/j.bej.2009.11.005
67. Allen AD, Anderson WA, Ayorinde FO, Eribo BE (2010) Biosynthesis and characterization of copolymer poly (3HB-co-3HV) from saponified *Jatropha curcas* oil by *Pseudomonas oleovorans*. J Ind Microbiol Biot 37(8):849–856
68. López-Cuellar M, Alba-Flores J, Rodríguez J, Pérez-Guevara F (2011) Production of polyhydroxyalkanoates (PHAs) with canola oil as carbon source. Int J Biol Macromol 48(1):74–80
69. Chardron S, Bruzaud S, Lignot B, Elain A, Sire O (2010) Characterization of bionanocomposites based on medium chain length polyhydroxyalkanoates synthesized by *Pseudomonas oleovorans*. Polym Test 29(8):966–971. doi:10.1016/j.polymertesting.2010.08.009
70. Penloglou G, Chatzidoukas C, Kiparissides C (2012) Microbial production of polyhydroxybutyrate with tailor-made properties: an integrated modelling approach and experimental validation. Biotechnol Adv 30(1):329–337. doi:10.1016/j.biotechadv.2011.06.021
71. Samrot A, Avinesh R, Sukeetha S, Senthilkumar P (2011) Accumulation of poly[(*R*)-3-hydroxyalkanoates] in *Enterobacter cloacae* SU-1 during growth with two different carbon sources in batch culture. Appl Biochem Biotechnol 163(1):195–203. doi:10.1007/s12010-010-9028-7
72. Yang YH, Brigham C, Willis L, Rha CK, Sinskey A (2011) Improved detergent-based recovery of polyhydroxyalkanoates (PHAs). Biotechnol Lett 1–6
73. Lo C-W, Wu H-S, Wei Y-H (2011) High throughput study of separation of poly(3-hydroxybutyrate) from recombinant

*Escherichia coli* XL1 blue. J Taiwan Inst Chem E 42(2):240–246. doi:10.1016/j.jtice.2010.08.001
74. Kathiraser Y, Aroua MK, Ramachandran KB, Tan IKP (2007) Chemical characterization of medium chain length polyhydroxyalkanoates (PHAs) recovered by enzymatic treatment and ultrafiltration. J Chem Technol Biot 82(9):847–855
75. Yasotha K, Aroua MK, Ramachandran KB, Tan IKP (2006) Recovery of medium-chain-length polyhydroxyalkanoates (PHAs) through enzymatic digestion treatments and ultrafiltration. Biochem Eng J 30(3):260–268. doi:10.1016/j.bej.2006.05.008
76. Horowitz DM, Brennan EM (2010) Methods for the separation and purification of biopolymers. European Patents 1(070):135
77. Darani KK, Reza Mozafari M (2010) Supercritical fluids technology in bioprocess industries: a review. J Biochem Technol 2(1):144–152
78. Khosravi-Darani K (2010) Research activities on supercritical fluid science in food biotechnology. Crit Rev Food Sci Nutr 50(6):479–488
79. Posada JA, Naranjo JM, López JA, Higuita JC, Cardona CA (2011) Design and analysis of poly-3-hydroxybutyrate production processes from crude glycerol. Process Biochem 46(1):310–317. doi:10.1016/j.procbio.2010.09.003
80. Reemmer J (2009) Advances in the synthesis and extraction of biodegradable polyhydroxyalkanoates in plant systems—a review. MMG 445 Basic Biotechnol eJ 5(1):44–49
81. Ariffin N, Abdullah R, Rashdan Muad M, Lourdes J, Emran NA, Ismail MR, Ismail I, Fadzil MFM, Ling KL, Siddiqui Y, Amir AA, Berahim Z, Husni Omar M (2011) Constructions of expression vectors of polyhydroxybutyrate-co-hydroxyvalerate (PHBV) and transient expression of transgenes in immature oil palm embryos. Plasmid 66(3):136–143. doi:10.1016/j.plasmid.2011.07.002
82. Snell KD (2010) Multi-gene epression construct containing modified intein. European Patents 1(255):846
83. Börnke F, Broer I (2010) Tailoring plant metabolism for the production of novel polymers and platform chemicals. Curr Opin Plant Biol 13(3):353–361
84. Poirier Y, Brumbley S (2010) Metabolic engineering of plants for the synthesis of polyhydroxyalkanaotes plastics from bacteria. In: Chen GG-Q (ed) vol 14. Microbiology Monographs. Springer Berlin / Heidelberg, pp 187–211. doi:10.1007/978-3-642-03287-5_8
85. Bohmert-Tatarev K, McAvoy S, Daughtry S, Peoples OP, Snell KD (2011) Focus issue on plastid biology: high levels of bioplastic are produced in fertile transplastomic tobacco plants engineered with a synthetic operon for the production of polyhydroxybutyrate. Plant Physiol 155(4):1690
86. Matsumoto K, Morimoto K, Gohda A, Shimada H, Taguchi S (2010) Improved polyhydroxybutyrate (PHB) production in transgenic tobacco by enhancing translation efficiency of bacterial PHB biosynthetic genes. J Biosci Bioeng 111(4):485–488
87. Somleva MN, Snell KD, Beaulieu JJ, Peoples OP, Garrison BR, Patterson NA (2008) Production of polyhydroxybutyrate in switchgrass, a value added co product in an important lignocellulosic biomass crop. Plant Biotechnol J 6(7):663–678
88. Kourtz L, Peoples OP, Snell KD (2010) Chemically inducible expression of biosynthetic pathways. US Patent App. 20,100/196,974
89. Kourtz L, Dillon K, Daughtry S, Peoples OP, Snell KD (2007) Chemically inducible expression of the PHB biosynthetic pathway in *Arabidopsis*. Transgenic Res 16(6):759–769
90. Gogoi P, Hazarika S, Dutta N (2010) Kinetics and mechanism on laccase catalyzed synthesis of poly (allylamine) catechin conjugate. Chem Eng J 163(12):86–92
91. Kadokawa J, Kobayashi S (2010) Polymer synthesis by enzymatic catalysis. Curr Opin Chem Biol 14(2):145–153
92. Kim S, Silva C, Evtuguin DV, Gamelas JAF, Cavaco-Paulo A (2011) Polyoxometalate/laccase-mediated oxidative polymerization of catechol for textile dyeing. Appl Microb Biot 89(4):981–987
93. Liu W, Chen B, Wang F, Tan T, Deng L (2011) Lipase-catalyzed synthesis of aliphatic polyesters and properties characterization. Process Biochem 46(10):1993–2000. doi:10.1016/j.procbio.2011.07.008
94. Sharma R, Chisti Y, Banerjee UC (2001) Production, purification, characterization, and applications of lipases. Biotechnol Adv 19(8):627–662
95. Arumugasamy SK, Ahmad Z (2011) *Candida antarctica* as catalyst for polycaprolactone synthesis: effect of temperature and solvents. Asia Pac J Chem Eng 6:398–405. doi:10.1002/apj.583
96. Gumel AM, Annuar MSM, Chisti Y, Heidelberg T (2012) Ultrasound assisted lipase catalyzed synthesis of poly-6-hydroxyhexanoate. Ultrason Sonochem 19(3):659–667
97. Hunsen M, Azim A, Mang H, Wallner SR, Ronkvist A, WENCHUN X, Gross RA (2008) Cutinase: a powerful biocatalyst for polyester synthesis by polycondensation of diols and diacids and ROP of lactones. In: Polymer biocatalysis and biomaterials II, vol 999. ACS symposium series, vol 999. American Chemical Society, pp 263–274
98. Feder D, Gross RA (2010) Exploring chain length selectivity in HIC-catalyzed polycondensation reactions. Biomacromolecules 11(3):690–697
99. Takwa M, Hult K, Martinelle M (2008) Single-step, solvent-free enzymatic route to $\omega$-functionalized polypentadecalactone macromonomers. Macromolecules 41(14):5230–5236
100. Kakasi-Zsurka S, Todea A, But A, Paul C, Boeriu CG, Davidescu C, Nagy L, Kuki Á, Kéki S, Péter F (2011) Biocatalytic synthesis of new copolymers from 3-hydroxybutyric acid and a carbohydrate lactone. J Mol Catal B Enzym 71:22–28
101. Veld M, Palmans A (2011) Hydrolases part I: enzyme mechanism, selectivity and control in the synthesis of well-defined polymers. In: Palmans ARA, Heise A (eds) Enzymatic polymerisation. Adv Polym Sci 237:55–78. doi:10.1007/12_2010_86
102. Sato S, Minato M, Kikkawa Y, Abe H, Tsuge T (2010) In vitro synthesis of polyhydroxyalkanoate catalyzed by class II and III PHA synthases: a useful technique for surface coatings of a hydrophobic support with PHA. J Chem Technol Biot 85(6):779–782
103. Gumel AM, Annuar MSM, Heidelberg T, Chisti Y (2011) Lipase mediated synthesis of sugar fatty acid esters. Process Biochem 46:2079–2090
104. Mallakpour S, Rafiee Z (2011) New developments in polymer science and technology using combination of ionic liquids and microwave irradiation. Prog Polym Sci 36(12):1754–1765
105. Sosnik A, Gotelli G, Abraham GA (2011) Microwave-assisted polymer synthesis (MAPS) as a tool in biomaterials science: how new and how powerful. Prog Polym Sci 36:1050–1078
106. García-Arrazola R, López-Guerrero DA, Gimeno M, Bárzana E (2009) Lipase-catalyzed synthesis of poly-l-lactide using supercritical carbon dioxide. J Supercrit Fluid 51(2):197–201
107. Matsuda T (2011) Asymmetric catalytic synthesis in supercritical fluids. Catal Meth Asym Synth 373–390
108. Thurecht KJ, Villarroya S (2010) Biocatalytic polymerization in exotic solvents. In: Loos K (ed) Biocatalysis in polymer chemistry. Wiley-VCH Verlag GmbH & Co. KGaA, pp 323–348. doi:10.1002/9783527632534.ch13
109. Gorke J, Srienc F, Kazlauskas R, Flickinger MC (2010) Enzyme-catalyzed reactions in ionic liquids. In: Encyclopedia of

industrial biotechnology: bioprocess, bioseparation and cell technology. Wiley, New York. doi:10.1002/9780470054581.eib271
110. Mallakpour S, Dinari M (2011) High performance polymers in ionic liquid: a review on prospects for green polymer chemistry. Part II: polyimides and polyesters. Iranian Polym J 20(4): 259–279
111. Kundu S, Bhangale AS, Wallace WE, Flynn KM, Guttman CM, Gross RA, Beers KL (2011) Continuous flow enzyme-catalyzed polymerization in a microreactor. J Am Chem Soc 133(15): 6006–6011
112. Ahmed EH, Raghavendra T, Madamwar D (2010) An alkaline lipase from organic solvent tolerant *Acinetobacter* sp. EH28: application for ethyl caprylate synthesis. Bioresour Technol 101(10):3628–3634
113. Takwa M, Larsen MW, Hult K, Martinelle M (2011) Rational redesign of *Candida antarctica* lipase B for the ring opening polymerization of d, d-lactide. Chem Comm 47:7392–7394
114. Karagoz B, Bayramoglu G, Altintas B, Bicak N, Arica MY (2010) Poly (glycidyl methacrylate)-polystyrene diblocks copolymer grafted nanocomposite microspheres from surface-initiated atom transfer radical polymerization for lipase immobilization: application in flavor ester synthesis. Ind Eng Chem Res 49(20):9655–9665
115. López-Luna A, Gallegos JL, Gimeno M, Vivaldo-Lima E, Bárzana E (2010) Lipase-catalyzed syntheses of linear and hyperbranched polyesters using compressed fluids as solvent media. J Mol Catal B Enzym 67(1–2):143–149. doi:10.1016/j.molcatb.2010.07.020
116. Han S-Y, Zhang J-H, Han Z-l, Zheng S-P, Lin Y (2011) Combination of site-directed mutagenesis and yeast surface display enhances *Rhizomucor miehei* lipase esterification activity in organic solvent. Biotechnol Lett 33(12):2431–2438. doi:10.1007/s10529-011-0705-6
117. Palmans ARA, van As BAC, van Buijtenen J, Meijer E (2008) Ring-opening of $\omega$-substituted lactones by Novozym 435: selectivity issues and application to iterative tandem catalysis. In: Polymer biocatalysis and biomaterials II, vol 999. American Chemical Society Publications, pp 230–244
118. Sha K, Li D, Li Y, Zhang B, Wang J (2008) The chemoenzymatic synthesis of a novel CBABC-type pentablock copolymer and its self-assembled "crew-cut" aggregation. Macromolecules 41(2):361–371
119. Zhang B, Li Y, Xu Y, Wang S, Ma L, Wang J (2008) Chemozymatic synthesis and characterization of H-shaped triblock copolymer. Polym Bull 60(6):733–740. doi:10.1007/s00289-008-0906-x
120. Zhang B, Li Y, Wang W, Wang J, Chen X (2011) ABA 2-type triblock copolymer composed of PCL and PSt: synthesis and characterization. Polym Bull 67(8):1507–1518
121. Xue L, Dai S, Li Z (2010) Biodegradable shape-memory block co-polymers for fast self-expandable stents. Biomaterials 31(32):8132–8140
122. de Geus M, Palmans Anja RA, Duxbury Christopher J, Villarroya S, Howdle Steven M, Heise A (2008) Chemoenzymatic synthesis of block copolymers. In: Polymer biocatalysis and biomaterials II, vol 999. ACS symposium series, vol 999. American Chemical Society, pp 216–229. doi:10.1021/bk-2008-0999.ch014
123. Tajima K, Satoh Y, Satoh T, Itoh R, Han X, Taguchi S, Kakuchi T, Munekata M (2009) Chemo-enzymatic synthesis of poly (lactate-co-(3-hydroxybutyrate)) by a lactate-polymerizing enzyme. Macromolecules 42(6):1985–1989
124. Han X, Satoh Y, Tajima K, Matsushima T, Munekata M (2009) Chemo-enzymatic synthesis of polyhydroxyalkanoate by an improved two-phase reaction system (TPRS). J Biosci Bioeng 108(6):517–523
125. Tian H, Tang Z, Zhuang X, Chen X, Jing X (2012) Biodegradable synthetic polymers: preparation, functionalization and biomedical application. Prog Polym Sci 37(2):237–280. doi:10.1016/j.progpolymsci.2011.06.004
126. Motornov M, Roiter Y, Tokarev I, Minko S (2010) Stimuli-responsive nanoparticles, nanogels and capsules for integrated multifunctional intelligent systems. Prog Polym Sci 35(1–2): 174–211
127. Jocić D, Tourrette A, Lavrić PK (2010) Biopolymer-based stimuli-responsive polymeric systems for functional finishing of textiles. In: Elnashar MM (ed) Biopolymers. Sciyo, Croatia, pp 37–40. doi:10.5772/286
128. Xu F, Yan TT, Luo YL (2011) Synthesis and micellization of thermosensitive PNIPAAm-b-PLA amphiphilic block copolymers based on a bifunctional initiator. Macromol Res 19(12): 1287–1295
129. Meng F, Zhong Z, Feijen J (2009) Stimuli-responsive polymersomes for programmed drug delivery. Biomacromolecules 10(2):197–209
130. Bawa P, Pillay V, Choonara YE, du Toit LC (2009) Stimuli-responsive polymers and their applications in drug delivery. Biomed Mater 4:022001
131. Stuart MAC, Huck WTS, Genzer J, Müller M, Ober C, Stamm M, Sukhorukov GB, Szleifer I, Tsukruk VV, Urban M (2010) Emerging applications of stimuli-responsive polymer materials. Nature Mater 9(2):101–113
132. Cui W, Qi M, Li X, Huang S, Zhou S, Weng J (2008) Electrospun fibers of acid-labile biodegradable polymers with acetal groups as potential drug carriers. Int J Pharm 361(1–2):47–55
133. Barinov V, Dabrowski R, Levon K (2006) Methods and apparatus for modifying gel adhesion strength. WO Patent WO/2006/050,340
134. Costa RR, Custódio CA, Arias FJ, Rodríguez-Cabello JC, Mano JF (2011) Layer-by-layer assembly of chitosan and recombinant biopolymers into biomimetic coatings with multiple stimuli-responsive properties. Small 7(18):2640–2649
135. Loh XJ, Cheong WCD, Li J, Ito Y (2009) Novel poly (N-isopropylacrylamide)-poly [(R)-3-hydroxybutyrate]-poly (N-isopropylacrylamide) triblock copolymer surface as a culture substrate for human mesenchymal stem cells. Soft Matter 5(15):2937–2946
136. Zhu JL, Zhang XZ, Cheng H, Li YY, Cheng SX, Zhuo RX (2007) Synthesis and characterization of well-defined, amphiphilic poly (N-isopropylacrylamide)-b-[2-hydroxyethyl methacrylate-poly ($\epsilon$-caprolactone)]n graft copolymers by RAFT polymerization and macromonomer method. J Polym Sci, Part A: Polym Chem 45(22):5354–5364
137. Yin H, Lee ES, Kim D, Lee KH, Oh KT, Bae YH (2008) Physicochemical characteristics of pH-sensitive poly (L-histidine)-b-poly (ethylene glycol)/poly (L-lactide)-b-poly (ethylene glycol) mixed micelles. J Control Release 126(2):130–138
138. Guo Y, Li M, Mylonakis A, Han J, MacDiarmid AG, Chen X, Lelkes PI, Wei Y (2007) Electroactive oligoaniline-containing self-assembled monolayers for tissue engineering applications. Biomacromolecules 8(10):3025–3034
139. Wei Y, Lelkes PI, MacDiarmid AG, Guterman E, Cheng S, Palouian K, Bidez P (2004) Electroactive polymers and nanostructured materials for neural tissue engineering. In: Qifeng Z, Cheng SZD (eds) Contemporary topics in advanced polymer science and technology. Peking University Press, Beijing, China, pp 430–436
140. Yamamoto H, Kitsuki T, Nishida A, Asada K, Ohkawa K (1999) Photoresponsive peptide and polypeptide systems. 13. Photoinduced cross-linked gel and biodegradation properties of copoly-

(L-lysine) containing ε-7-Coumaryloxyacetyl-L-lysine residues. Macromolecules 32(4):1055–1061
141. Kwon IK, Matsuda T (2005) Photo-polymerized microarchitectural constructs prepared by microstereolithography (μSL) using liquid acrylate-end-capped trimethylene carbonate-based prepolymers. Biomaterials 26(14):1675–1684
142. Gupta H, Wilkinson RA, Bogdanov AA Jr, Callahan RJ, Weissleder R (1995) Inflammation: imaging with methoxy poly(ethylene glycol)-poly-L-lysine-DTPA, a long-circulating graft copolymer. Radiology 197(3):665–669
143. Ross JF, Chaudhuri PK, Ratnam M (1994) Differential regulation of folate receptor isoforms in normal and malignant tissues in vivo and in established cell lines. Physiologic and clinical implications. Cancer 73(9):2432–2443
144. Jiang C, Wang X, Sun P, Yang C (2011) Synthesis and solution behavior of poly (ε-caprolactone) grafted hydroxyethyl cellulose copolymers. Int J Biol Macromol 48(1):210–214
145. Lu D, Wu Q, Lin X (2002) Chemoenzymatic synthesis of biodegradable poly (1′-O-vinyladipoyl-sucrose). Chinese J Polym Sci 20(6):579–584
146. Puppi D, Chiellini F, Piras AM, Chiellini E (2010) Polymeric materials for bone and cartilage repair. Prog Polym Sci 35(4):403–440. doi:10.1016/j.progpolymsci.2010.01.006
147. Bansal SS, Goel M, Aqil F, Vadhanam MV, Gupta RC (2011) Advanced drug-delivery systems of curcumin for cancer chemoprevention. Cancer Prev Res 4:1158
148. Gaucher G, Marchessault RH, Leroux JC (2010) Polyester-based micelles and nanoparticles for the parenteral delivery of taxanes. J Control Release 143(1):2–12
149. Kiliçay E, Demirbilek M, Türk M, Güven E, Hazer B, Denkbas EB (2011) Preparation and characterization of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (Phbhhx) based nanoparticles for targeted cancer therapy. Eur J Pharm Sci 44(3):310–320. doi:10.1016/j.ejps.2011.08.013
150. Lee J, Jung S-G, Park C-S, Kim H-Y, Batt CA, Kim Y-R (2011) Tumor-specific hybrid polyhydroxybutyrate nanoparticle: surface modification of nanoparticle by enzymatically synthesized functional block copolymer. Bioorg Med Chem Lett 21(10): 2941–2944. doi:10.1016/j.bmcl.2011.03.058
151. Zawidlak-Wegrzynska B, Kawalec M, Bosek I, Luczyk-Juzwa M, Adamus G, Rusin A, Filipczak P, Glowala-Kosinska M, Wolanska K, Krawczyk Z (2010) Synthesis and antiproliferative properties of ibuprofen-oligo (3-hydroxybutyrate) conjugates. Eur J Med Chem 45(5):1833–1842
152. Francis L (2011) Biosynthesis of polyhydroxyalkanoates and their medical applications. University of Westminster, Westminster
153. Seyednejad H, Ghassemi AH, van Nostrum CF, Vermonden T, Hennink WE (2011) Functional aliphatic polyesters for biomedical and pharmaceutical applications. J Control Release 152: 168–172
154. Li W-R, Xie X-B, Shi Q-S, Duan S-S, Ouyang Y-S, Chen Y-B (2011) Antibacterial effect of silver nanoparticles on *Staphylococcus aureus*. Biometals 24(1):135–141. doi:10.1007/s10534-010-9381-6
155. Li WR, Xie XB, Shi QS, Zeng HY, Ou-Yang YS, Chen YB (2010) Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*. Appl Microbiol Biotechnol 85(4): 1115–1122
156. Mirzajani F, Ghassempour A, Aliahmadi A, Esmaeili MA (2011) Antibacterial effect of silver nanoparticles on *Staphylococcus aureus*. Res Microbiol 162(5):542–549
157. Phukon P, Saikia JP, Konwar BK (2011) Enhancing the stability of colloidal silver nanoparticles using polyhydroxyalkanoates (PHA) from *Bacillus circulans* (MTCC 8167) isolated from crude oil contaminated soil. Colloids Surf B Biointerfaces 86(2):314–318. doi:10.1016/j.colsurfb.2011.04.014
158. Zhou J, Peng S-W, Wang Y–Y, Zheng S-B, Wang Y, Chen G-Q (2010) The use of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) scaffolds for tarsal repair in eyelid reconstruction in the rat. Biomaterials 31(29):7512–7518. doi:10.1016/j.biomaterials.2010.06.044
159. Yan C, Wang Y, Shen X-Y, Yang G, Jian J, Wang H-S, Chen G-Q, Wu Q (2011) MicroRNA regulation associated chondrogenesis of mouse MSCs grown on polyhydroxyalkanoates. Biomaterials 32(27):6435–6444. doi:10.1016/j.biomaterials.2011.05.031
160. Wang L, Wang Z-H, Shen C-Y, You M-L, Xiao J-F, Chen G-Q (2010) Differentiation of human bone marrow mesenchymal stem cells grown in terpolyesters of 3-hydroxyalkanoates scaffolds into nerve cells. Biomaterials 31(7):1691–1698. doi:10.1016/j.biomaterials.2009.11.053
161. Butcher JT, Mahler GJ, Hockaday LA (2011) Aortic valve disease and treatment: the need for naturally engineered solutions. Adv Drug Del Rev 63:242–268
162. Bouten C, Dankers P, Driessen-Mol A, Pedron S, Brizard A, Baaijens F (2011) Substrates for cardiovascular tissue engineering. Adv Drug Del Rev 63:221–241
163. Wei XW, Gong CY, Gou ML, Fu SZ, Guo QF, Shi S, Luo F, Guo G, Qiu LY, Qian ZY (2009) Biodegradable poly (ε-caprolactone)-poly (ethylene glycol) copolymers as drug delivery system. Int J Pharm 381(1):1–18
164. Woodruff MA, Hutmacher DW (2010) The return of a forgotten polymer-polycaprolactone in the 21st century. Prog Polym Sci 35(10):1217–1256
165. Patel A, Velikov K (2011) Colloidal delivery systems in foods: a general comparison with oral drug delivery. LWT Food Sci Technol 44(9):1958–1964
166. Riekes MK, Barboza FM, Vecchia DD, Bohatch M Jr, Farago PV, Fernandes D, Silva MAS, Stulzer HK (2011) Evaluation of oral carvedilol microparticles prepared by simple emulsion technique using poly (3-hydroxybutyrate-co-3-hydroxyvalerate) and polycaprolactone as polymers. Mater Sci Eng C 31(5): 962–968
167. Öztürk F, Ermertcan AT (2011) Wound healing: a new approach to the topical wound care. Cutan Ocul Toxicol 30(2):92–99
168. Uppal R, Ramaswamy GN, Arnold C, Goodband R, Wang Y (2011) Hyaluronic acid nanofiber wound dressing—production, characterization, and in vivo behavior. J Biomed Mater Res Part B Appl Biomater 97B(1):20–29
169. Bodmeier R (2011) Implants particles. US Patents
170. Howe J (2011) Suture anchor inserter. US Patents
171. Bansal SS, Vadhanam MV, Gupta RC (2011) Development and in vitro-in vivo evaluation of polymeric implants for continuous systemic delivery of curcumin. Pharm Res 28(5):1121–1130
172. Waknis V, Jonnalagadda S (2011) Novel poly-DL-lactide-polycaprolactone copolymer based flexible drug delivery system for sustained release of ciprofloxacin. Drug Deliv 18(4):236–245
173. Wong VG, Wood LL (2011) Conveniently implantable sustained release drug compositions. US Patents
174. Thuaksuban N, Nuntanaranont T, Pattanachot W, Suttapreyasri S, Cheung LK (2011) Biodegradable polycaprolactone-chitosan three-dimensional scaffolds fabricated by melt stretching and multilayer deposition for bone tissue engineering: assessment of the physical properties and cellular response. Biomed Mater 6:015009
175. Williams SF, Martin DP, Gerngross T, Horowitz DM (2011) Polyhydroxyalkanoates for in vivo applications. US Patents
176. Williams SF, Martin DP, Gerngross T, Horowitz DM (2011) Medical device comprising polyhydroxyalkanoate having pyrogen removed. US Patents
177. Woodruff MA, Hutmacher DW (2010) Resorbable composite scaffolds for bone tissue engineering. In: Tissue and cell engineering society (TCES), Manchester, 28–30 July 2010. TCES

178. Pillai CKS, Sharma CP (2010) Absorbable polymeric surgical sutures: chemistry, production, properties, biodegradability, and performance. J Biomater Appl 25(4):291–366. doi:10.1177/0885328210384890
179. Wang SY, Wang Z, Liu MM, Xu Y, Zhang XJ, Chen GQ (2010) Properties of a new gasoline oxygenate blend component: 3-hydroxybutyrate methyl ester produced from bacterial poly-3-hydroxybutyrate. Biomass Bioenerg 34(8):1216–1222
180. Zhang X, Luo R, Wang Z, Deng Y, Chen GQ (2009) Application of (R)-3-hydroxyalkanoate methyl esters derived from microbial polyhydroxyalkanoates as novel biofuels. Biomacromolecules 10(4):707–711
181. Bourbonnais R, Marchessault RH (2010) Application of polyhydroxyalkanoate granules for sizing of paper. Biomacromolecules 11(4):989–993. doi:10.1021/bm9014667
182. Modi S, Koelling K, Vodovotz Y (2011) Assessment of PHB with varying hydroxyvalerate content for potential packaging applications. Eur Polym J 47:179–186
183. Johansson C (2011) Bio-nanocomposites for food packaging applications. Nanocomposites with biodegradable polymers: synthesis, properties, and future perspectives. Oxford University Press, UK
184. Del Nobile MA, Conte A, Buonocore GG, Incoronato AL, Massaro A, Panza O (2009) Active packaging by extrusion processing of recyclable and biodegradable polymers. J Food Eng 93(1):1–6
185. Siracusa V, Rocculi P, Romani S, Rosa MD (2008) Biodegradable polymers for food packaging: a review. Trends Food Sci Technol 19(12):634–643
186. De Schryver P, Dierckens K, Bahn Thi QQ, Amalia R, Marzorati M, Bossier P, Boon N, Verstraete W (2011) Convergent dynamics of the juvenile European sea bass gut microbiota induced by polyhydroxybutyrate. Environ Microbiol 13(4):1042–1051
187. Defoirdt T, Sorgeloos P, Bossier P (2011) Alternatives to antibiotics for the control of bacterial disease in aquaculture. Curr Opin Microbiol 14(3):251–258
188. Nhan DT, Wille M, De Schryver P, Defoirdt T, Bossier P, Sorgeloos P (2010) The effect of poly [beta]-hydroxybutyrate on larviculture of the giant freshwater prawn *Macrobrachium rosenbergii*. Aquaculture 302(1–2):76–81. doi:10.1016/j.aquaculture.2010.02.011
189. Grillo R, AdES Pereira, de Melo NFS, Porto RM, Feitosa LO, Tonello PS, Filho NLD, Rosa AH, Lima R, Fraceto LF (2011) Controlled release system for ametryn using polymer microspheres: preparation, characterization and release kinetics in water. J Hazard Mater 186(2–3):1645–1651. doi:10.1016/j.jhazmat.2010.12.044
190. Zhang X, Wei C, He Q, Ren Y (2010) Enrichment of chlorobenzene and o-nitrochlorobenzene on biomimetic adsorbent prepared by poly-3-hydroxybutyrate (PHB). J Hazard Mater 177(1–3):508–515