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Production of Polyhydroxyalkanoates by Fermentation of Bacteria

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SUMMARY: Polyhydroxyalkanoates (PHAs) are carbon and energy reserve material accumulated by numerous microorganisms and have been drawing much attention as biodegradable substitutes for conventional nondegradable plastics and elastomers. There are a number of different PHAs having a variety of material properties based on the different monomer composition. Poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) are now efficiently produced by bacterial fermentation at reasonable production costs. Recent advances in the production of short-chain-length (SCL) PHAs by bacterial fermentation are reviewed. Current status of the production of medium-chain-length (MCL) PHAs and SCL-MCL-PHA copolymers is also reviewed.

Introduction

Polyhydroxyalkanoates (PHAs, Fig. 1) are thermoplastic or elastomeric polyester with biodegradable and biocompatible properties, and can be produced from various carbon substrates by numerous microorganisms[1-7]. These microbial PHAs have recently attracted much industrial attention to be used in a wide range of consumer products, agricultural, marine, and medical applications[3,4,8]. The physical and mechanical properties of PHA can vary with the number of main chain carbon atoms and types of alkyl-pendent groups (R groups) in the monomer units[8,9]. PHAs can be divided into two groups by the number of carbon atoms in the monomer; short-chain-length (SCL) PHA consisting of 3 – 5 carbon atoms and medium-chain-length (MCL) PHA consisting 6 –14 carbon atoms[9,10].

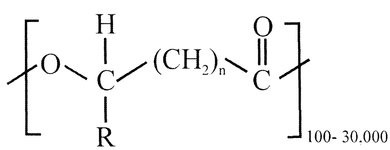

Fig. 1: General structure of polyhydroxyalkanoate.

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Although many different PHAs possessing useful properties have been discovered, they are currently too expensive to produce[4,11]. Commercial uses of PHAs have so far been limited to specialty applications where properties are more important than costs. Much current research focuses on the development of strategies for the efficient production of different types of PHAs[4,7]. In this paper, recent advances in the production of PHAs with high productivity and content are presented. Particularly, it was aimed to review the current status of producing SCL-PHAs, MCL-PHAs, and SCL-MCL-PHA copolymers.

Poly(3-hydroxybutyrate)

Poly(3-hydroxybutyrate) (PHB) is the best characterized member of PHAs. PHB is often compared with isotactic polypropylene because it is a enantiomerically pure polymer with a single methyl substituent adjacent to the methylene group placed regularly along its backbone[8]. PHB isolated from bacterial cells usually possesses very high number-average molecular weight (approximately 10^5 – 10^6) and high crystallinity (more than 50%)[8,12]. A wide variety of prokaryotic organisms accumulate PHB when growth is limited by the depletion of an essential nutritional component such as nitrogen, oxygen, phosphorous, sulfur, or magnesium in the presence of excess carbon source. However, only several bacteria such as *Ralstonia eutropha*, *Alcaligenes latus*, *Azotobacter vinelandii*, methylotrophs, and recombinant *Escherichia coli* can be employed for the efficient production of PHB mainly because high PHB content and high PHB concentration can be obtained with these bacteria[3,10].

A. latus grows fast, accumulates PHB during growth, and utilizes sucrose, and thus inexpensive raw sugar and beet/cane molasses[10,13]. However, the PHB content that could be obtained was typically less than 50% of cell dry weight[13]. To increase the PHB content, the strategy of applying nutrient limitation was examined during the cultivation of *A. latus*[14]. After nitrogen limitation was applied, the increase of cell concentration was only due to the increase of PHB concentration. By the fed-batch culture with nitrogen limitation strategy, PHB content could be increased to 88 wt% of dry cell weight. With this strategy, the PHB productivity of 4.94 g/L-h, which is the highest value reported to date, was obtained[14)].

Recombinant *E. coli* has been investigated for the production of PHB because it has several advantages as reviewed elsewhere[15,16)]. By the fed-batch culture of recombinant *E. coli* harboring the *R. eutropha* PHA biosynthesis genes and *E. coli ftsZ* gene, cell dry weight of 206 g/L, PHB concentration of 149.7 g/L, and PHB content of 73% were achieved in a chemically defined medium, resulting in the PHB productivity of 3.4 g/L-h[17)]. Recently, we constructed recombinant *E. coli* strains harboring the newly cloned PHA biosynthesis genes from *A. latus* and examined production of PHA by the recombinant *E. coli*[18)]. By the fed-batch culture of recombinant *E. coli* harboring the *A. latus* PHA biosynthesis genes, the final cell and PHB concentrations of 194.1 g/L and 141.6 g/L, respectively, were obtained in 30.6 h, resulting in a much higher productivity of 4.63 g PHB/L-h[18)].

From the economical evaluation of the processes for the production of PHB by various bacteria, it was found that these processes for the production of PHB by *A. latus* or by recombinant *E. coli* harboring the *A. latus* PHA biosynthesis genes were more economical compared with the processes by other bacteria[19,20)].

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)

PHB is a highly crystalline material mainly due to its stereoregularity, and its 3-substituted structure makes it thermally unstable at temperature immediately above the melting point[8)]. Both of these characteristics suggested potential difficulties of employing PHB in plastics and fibre application. The breakthrough came through the development of a family of copolyesters based on 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate, referred to as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(HB/V)][21)]. P(HB/V) had been produced commercially by fed-batch culture of *R. eutropha* from glucose and propionic acid by Imperial Chemical Industries under the trade name Biopol[21)].

There have been a series of papers on the production of P(HB/V) by the wild type producers[10]. Recently, a high level production of P(HB/V) by the fed-batch culture of recombinant *E. coli* was also reported[22]. For the production of P(HB/V) copolymer by recombinant *E. coli*, co-substrate such as propionic acid was also required to provide 3-hydroxyvalerate precursors[23]. Acetic acid and/or oleic acid induction resulted in the enhanced production of P(HB/V) in both flask and fed-batch cultures[24]. By the fed-batch culture of recombinant *E. coli*, P(HB/V) concentration, P(HB/V) content and P(HB/V) fraction of 158.8 g/L, 78.2 wt%, and 10.6 mol%, respectively, could be obtained in 55.1 h, resulting in a high P(HB/V) productivity of 2.88 g/L-h[22]. These results together with the simple purification method recently developed for recombinant *E. coli*[20,25] demonstrated the possibility of producing P(HB/V) at a cost much lower than previously thought. Also, the P(HB/V) fraction could be varied by varying the propionic acid concentration in the feeding solution during the culture[22].

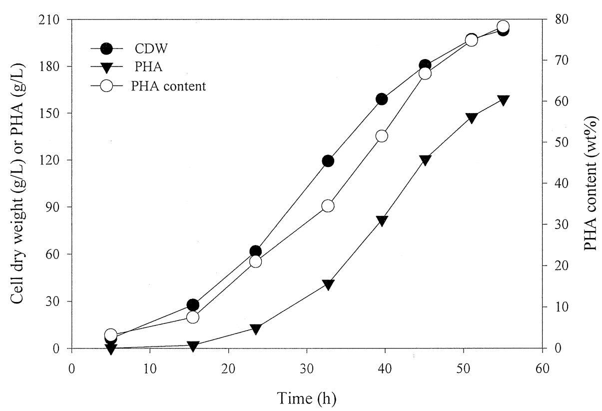

Fig. 2: Time profiles of cell dry weight, PHA concentration and PHA content (wt%) during the fed-batch culture of XL1-Blue (pJC4) with oleic acid supplementation after acetic acid induction. The feeding solution was added to increase the concentrations of glucose and propionic acid to 20 g/L and to 5 mM, respectively, after each feeding (reprinted from ref. 22 with the permission).

It has also been reported that several other SCL-PHA copolymers such as poly(3-

hydroxybutyrate-co-4-hydroxybutyrate) could be produced, even though at much lower efficiency[7)]. Because these copolymers possess superior polymer properties to PHB homopolymer, development of efficient fermentation process for the production of these copolymers will allow development of a wide range of applications.

Medium chain length-PHAs

Medium chain length (MCL-) PHAs are rubbery and flexible material with low crystallinity, and can be used in a wide range of applications which cannot be fulfilled by PHB and other SCL-PHAs[26)]. *Pseudomonas* strains have been employed for the production of MCL-PHAs from a number of organic compounds such as alkanes, alkenes or alkanoic acids[3,10)]. However, to date, the final MCL-PHA concentration and PHA content obtained have been at relatively low levels compared with those of SCL-PHAs, which hampered the development of their applications[27,28)]. By the fed-batch culture of *Pseudomonas oleovorans*, high cell concentration of 112 g/L was obtained, but the PHA content was less than 25 wt%[27)]. Recently, efficient production of MCL-PHA by *Pseudomonas putida* has been reported[29)]. By the application of phosphorus limitation and optimized feeding strategy, PHA concentration and PHA content obtained in 38 h were 72.6 g/L and 51.4 wt%, respectively, resulting in a high PHA productivity of 1.91 g/L-h[29)]. The molar fractions of 3-hydroxyhexanoate (3HHx), 3-hydroxyoctanoate, 3-hydroxydecanoate, 3-hydroxydodecanoate, and 3-hydroxy-5-cis-tetradecanoate in PHA were 10.1 mol%, 37 mol%, 34.7 mol%, 8.6 mol%, and 9.6 mol%, respectively[29)].

Several bacteria produce PHAs consisting of both SCL- and MCL-hydroxyalkanoate monomer units[30-32)]. Some *Pseudomonas* strains accumulate PHAs consisted of 3-hydroxyalkanoates (3HAs) of C_4 to C_{12}[31)], and *Aeromonas caviae* produces a copolymer composed of 3HB and 3HHx[32)]. These copolymers were found to possess useful material properties of their own[33)]. *Aeromonas hydrophila* isolated from raw sewage samples was found to produce a copolymer composed of 3-hydroxybutyrate and 3-hydroxyhexanoate[34]. By the fed-batch culture of *A. hydrophila*, PHA concentration and PHA content obtained in 43 h were 43.3 g/L and 45.2 wt%, respectively, resulting in a high PHA productivity of 1.01 g PHA/L-h[34]. The fraction of 3-hydroxyhexanoate in PHA was 32.1 mol% at maximum.

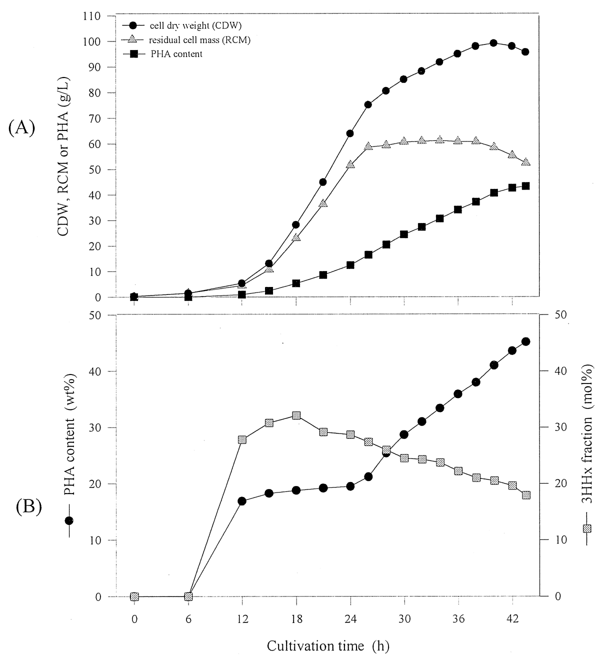

Fig. 3: Time profiles of (A) cell dry weight, residual cell mass, and PHA concentration and (B) PHA content, and 3HHx fraction during fed-batch cultivation of *A. hydrophila* (reprinted from ref. 34 with the permission).

Conlcusion

PHAs have been thought to be good candidates for completely degradable plastic and elastomeric materials. However, their high production cost has limited their development. To reduce the high production cost, much effort has been devoted to the isolation of better bacterial strains and development of efficient fermentation and recovery processes. PHB and P(HB/V) copolymer can now be produced efficiently with the production cost less than \$ 3.5 per kg. Reports on the production of other SCL PHAs, MCL PHAs, and SCL-MCL PHAs to high concentrations with high productivities are beginning to appear. With further development, various members of PHAs will be produced at reasonable costs, which will allow PHAs to become a leading biodegradable polymer.

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