Open access Journal International journal of Emerging Trends in Science and Technology

Bioprospects of PHB: A Review

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Abstract

Polyhydroxybutyrates (PHB) are bio-plastics that are produced by many microbial species under carbon rich and nutrient starvation conditions. Poly (ß-hydroxybutyrate) (PHB) belongs to a family of microbial energy/carbon storage compounds collectively known as poly hydroxyalkanoates. The organisms producing PHBs have been isolated, identified and the conditions of maximum production optimized. The cheaper raw material for the mass production of PHB are constantly being studied and suggested to lower the production cost. Most of the commercial productions are at present more expensive than synthetic polymer production. Since biopolymers offer the dual advantage of being formed from renewable resources and in addition to it they are also completely biodegradable, the structure, properties and regulation of synthesis and degradation of PHB should be reviewed and the microbial production of copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate, with properties varying according to copolymer composition, must be considered.

Keywords: *Polyhydroxybutyrate, Bioplastics, production, raw material, optimization*

1. Introduction

PHBs have been defined in various ways by several workers. PHB is an intracellular carbon and energy source synthesized by a wide range of microorganisms under nutrient-limiting conditions (Aderson et al., 1990). Poly-3-hydroxybutyrate (PHB) is an intracellular lipid reserve material accumulated by many bacteria under conditions of nutrient stress, normally when an external carbon source is available but when concentrations of nutrients such as nitrogen, phosphorous or oxygen are limiting growth (Bitar and Underhill, 1990). It is an alternative source of plastics which has similar physical properties like polypropylene and it can be easily biodegradable aerobically and anaerobically (Arun et al., 2006).

Bioplastics are plastics that are bio-based i.e. the composition of the bioplastic is biodegradable. Bio-based plastics are made of renewable resources which means their use can limit the depletion of petroleum reserves, the oil prices and the greenhouse gas emissions.Poly-3-hydroxybutyrate (PHB) is a linear polyester of D (-)-3-hydroxybutyric acid which was first discovered in bacteria by Lemoigne in 1925. It is accumulated in intracellular granules by a wide variety of Gram-positive and Gram-negative organisms under conditions of a nutrient limitation other than the carbon source (Dawes and Senior, 1973). Intensive research and industrial efforts have focused on the production of biopolymers as green alternatives to synthetic polymers. PHAs are degraded biologically to carbon dioxide and water in aerobic environments and to methane under anaerobic conditions.

2. PHB Production

The PHB production process is performed in two phases. In the first phase, all nutrients necessary for biomass growth (carbon source, NH_3, O_2) are provided so that the biomass concentration rises until a desired level. In the second phase, nutrient limiting conditions are established by stopping the nitrogen feeding so that only carbon source and O_2 are added to the fermentor which leads to a stop in biomass growth and redirection of the excess carbon source to PHB production. Its production has most commonly been studied on micro-organisms belonging to the genera *Alcaligenes*, *Azotobacter*, *Bacillus* and *Pseudomonas* (Aysel et al., 2002). The percent of PHB in the biomass was measured using a gas chromatography (GC) method proposed by Comeau et al. (1988). Characterizations of native PHB as well as blends have also been carried out by FTIR, DSC, ^{1}H- NMR and ^{13}C- NMR analysis (Raveendran et al. 2011). Lemos et al. (1998) showed that the types of carbon sources (acetate, propionate and butyrate) had an impact on biopolymer production with acetate giving the best polymer production of the three carbon used.

3. Applications of Bioplastics

Bioplastics can be used in numerous applications such as agricultural and construction materials, automotive interior materials, electrical devices, bottles and containers, sanitary goods, general packaging materials, etc. (Kaneka, 2009). However, due to their often inferior resistance and durability, they cannot replace all petroleum-based plastics (Kosior, 2006). PHB can also be extruded, moulded, spun into fibres, made into films and blended with synthetic polymers (in order to improve bioplastics physical properties) (Lee et al., 1997). PHB is also compatible with body tissues which makes their use in medical areas like surgical sutures, wound dressings and ocular devices possible (Patnaik, 2007). Because of its biodegradability and promising applications, PHB as an organic polymer has attracted interest in the medical, pharmaceutical and chemical industries (B.Senthil Kumar and G.Prabakaran, 2006) The extracted PHB is also used for the preparation of PHB polymer film and polymer blends. Bioplastics have a wide range of agricultural, marine and medical applications (Arun et al.2006 and Kitamara et al. 2004).

4. PHB producing organisms

Many bacterial species have been isolated and optimized for the production of PHB. A number of bacteria such as *Azotobacter*, *Bacillus*, Archaebacteria, Methylobacteria, *Pseudomonas* have been found to synthesize PHA to varying levels. *Ralstonia eutropha* (formerly *Alcaligenes eitrophus*) has been the subject of much published research work because it can accumulate PHAs up to 80 per cent dry weight (Lee, 1996). *Pseudomonas extorquens* grows on methanol as the carbon source and ammonium as the nitrogen source (Laurens Goormachtigh, 2013). The production of Polyhydroxybutyrate (PHB) has also been determined in species of Rhizobium japonicum, Rhizobium cicer and Bradyrhizobium japonicum (Edwin A. Dawes, 1988). *Cupriavidus necator* is the most well-studied PHB producing bacteria due to its capability to synthesize large amounts of PHB (ca. 80 % (w/w) of dry cell mass (Lee, 1996b)) from easy obtainable carbon sources such as acetic acid, glucose, oleic acid, beet molasses, CO_2 and H_2O_2, glycerol, soybean oil, sucrose and lactic acid. *Alcaligenes latus* is a fast growing growth associated PHA producer (Lee, 1996b) that is able to utilize sucrose as a carbon source. Another attractive bacterium for PHA production is *Azotobacter vinelandii* since it can accumulate PHB up to 75 % of the dry cell mass during exponential growth (Page & Knosp, 1989). Suzuki et al. (1986) demonstrated production of PHB by a fed-batch culture of *Protomonas extorquens* on methanol. Recombinant E.coli, is also able to produce PHA after cloning PHA biosynthesis genes (mostly from A. eutrophus) (Adwitiya Pal.et al, 2009).

5. Degradation of PHB

PHB completely degrades into carbon dioxide and water under aerobic conditions. The ability to degrade PHA is widely distributed among bacteria and fungi and depends on the secretion of specific extracellular PHA depolymerases (e-PHA depolymerases), which are carboxyesterases (EC 3.1.1.75 and EC 3.1.1.76), and on the physical state of the polymer (amorphous or crystalline)(Dieter Jendrossek and René Handrick, 2002).

6. Conclusion

Considering future technical improvements and economies of scale, PHAs are a more sustainable and environmentally friendly alternative for petrochemical plastics provided that the production cost be reduced considerably by using cheaper substrates such as industrial or agricultural wastes or by optimization of the production process appropriately as the productivity of PHA fermentations is mostly quite low due to imprecise modelling and optimization. Venkateswar Reddy et al. (2012) obtained PHA accumulation of 39.6% using aerobic mixed culture and fermented food waste as a substrate. Coats et al. (2011) used fermented municipal wastewater solids to accumulate approximately 28% of poly-hydroxyalkonates (PHA) in the biomass. In their studies it appeared that municipal wastewater treatment can be made more sustainable by producing PHBs. Studies on process analysis and economic evaluation by Choi and Lee (1997) shown that PHB productivity, PHB content, PHB yield, and the cost of carbon substrate considerably affect the final price of PHB (Sindhu Raveendran et al.,2011). Development of an efficient recovery method is also important to lower the price of PHB. Since the cost of carbon source accounts for 70 to 80% of total raw material cost, the price of PHB can be significantly lowered if cheap carbon substrate can be used (Choi and Lee, 1997). The search for promising strains of PHA producers is a continuous process and development of efficient polyhydroxyalkanoate producing bacteria is the need of the hour (Nandini Phanse et al, 2011).

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