

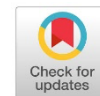


Review

Bioplastic: An accost towards sustainable development

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30 April 2021*Keywords:*Bioplastic, PHBs,
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In the current era, plastic has become a global environmental menace affecting terrestrial and aquatic ecosystems. Regular plastic resilient nature towards decomposition, and it pollutes the environment. Conventional plastic is widely used in various industrial setups with no alternate substitute available. The quest to find an alternate solution to the emerging problem development of bioplastic that is eco-friendlier and adds no pollution to the environment has been much focussed. Bioplastic is plastic synthesized from renewable biomass sources rather than petroleum origin. The development of bioplastic of microbial origin will be a promising innovation to keep our world plastic-free and promote sustainability. It can be degraded easily and gets broken down into carbon dioxide, biomass, and water rapidly. The present reviews highlight the sources of microbial-derived bioplastic, polyhydroxybutyrate (PHB), polyhydroxyalkanoates (PHAs), extraction methodologies, optimization strategies to improve yield, degradation, application areas, present challenges, and prospects in production. We have also provided a brief insight into gene and gene clusters responsible for bioplastic production. Overall, the article will provide a comprehensive update on bioplastic to help mitigate our current problem associated with conventional plastic usage.

Introduction

One of the 17 UN sustainable goals aims for a clean environment where bioplastic use could achieve sustainability to a great extent. Synthetic polymers are incredibly stable in the environment and do not degrade by biogeochemical cycles of the biosphere, which has to lead them to be a global pollutant and poses a threat to sustainable development in the world.¹ A primary environmental concern is a gradual accumulation of synthetic plastics, which are non-biodegradable, directly impacting global warming, eco-toxicity, ozone depletion, and soil-water pollution. Thus, it seeks to develop means to

reduce plastic use by practicing bioplastics in day-to-day use. One aspect of incorporating PHA bioplastic production within plants with the aid of genetic manipulation could significantly link agriculture with the material industry's demands. Sources of bioplastics range from a wide variety of renewable sources such as cellulose, starch, chitin, proteins, lipids, and majorly polymers from plant/microbial origin, i.e., PHAs and PHBs.² Polylactic acid has also shown great potential in bioplastic production apart from its other polymer like polycaprolactone, polyglycolic, PHB is used mainly in pharmaceutical applications.³ Compared

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to petroleum-based plastics, degradation of bioplastic supports its sustainable development approach, which quickly gets degraded into carbon dioxide, water, and biomass under aerobic conditions. Compositing under microbial action is useful in degrading polylactic acid, PHA, or other plant-derived bioplastics like bio-polyethylene terephthalate and 1,3-propanediol⁴, but PLA showed a slow degradation processing taking 11-month time for decomposition. Thus, to further enhance biodegradability, either the polymer's blending could be an effective solution or improve the composting methodology.

Classification of Plastics

Based on biodegradability, plastic can be classified into biodegradable and non-biodegradable. Chemical structure and degradation patterns have been explored mainly for various plastics. Fossil fuel-based plastics are conventional plastic, non-biodegradable due to their extensive repetition of monomer units and higher molecular weight.⁵ The domain of non-biodegradable plastic includes PVC, PP, PS, PET, and PUR. Due to their harsh nature and poor waste management practices, it has posed a severe threat of pollution to the earth. Polyolefins-based plastics are widely used in various domains of our day-to-day life; because of their highly stable nature in the environment, it is vital to look for proper waste management.⁶ Although to enhance its degradability, starch and prooxidants are preferred for better fragmentation of plastics. The diverse range of bioplastic is summarized in Figure 1.

While on the other hand, biodegradable bioplastics have a high biodegradability rate and get easily disintegrated by microbes' action, thus possess a lesser threat to the ecosystem. Factors like polymer molecular weight, functional group, and crystallinity affect plastic's degradation process.⁸ Exoenzymes released by microbe break down these complex molecules into simpler ones using aerobic and anaerobic processes.^{6,9} Fungi and bacteria degrade plastics finally into carbon dioxide, and water through various metabolic activities, e.g.,

strains like bacillus and Brevibacillus produce proteases with aid in the degradation of plastics.¹⁰ Fungi are natural decomposers that produce laccase enzymes for the degradation of plastics.¹¹ Under the anaerobic situation, bacteria use oxygen as an electron donor to synthesize smaller organic molecules. While under anaerobic conditions in the absence of oxygen, other molecules like nitrate, sulfate, and manganese are used as electron acceptors.¹²

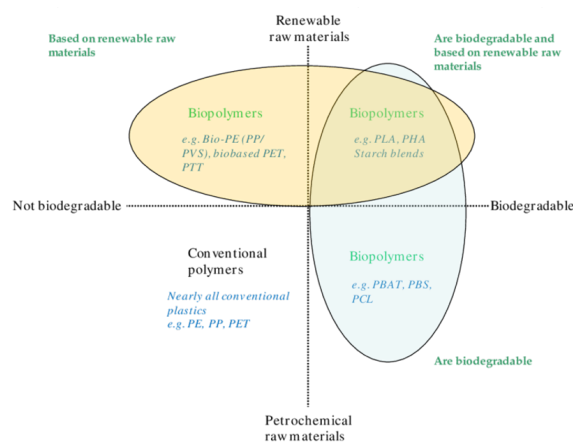


Figure 1: Types of bioplastics.⁷

Production of PHB and PHA from microbial sources

PHA are linear polyesters that comprise of molecular weight more than 60000 Daltons, which is commonly observed to get accumulated in a variety of microbes, including gram harmful bacteria, gram-positive bacteria, and some archaea as intracellular granules under unfavorable growth conditions.¹³⁻¹⁵

PHB is the principal subsidiary of PHA. Under limited nutrient conditions with surplus carbon, various bacteria such as *Alcaligene eutrophus*, *Azotobacter vinelandii*, *Bacillus*, *Streptomyces* accumulate PHA as stored food material.¹⁶⁻¹⁸ The optimized strain of *Rhizobium elti* E1 and *Pseudomonas stutzeri* E114 improves the yield of PHBs when given mannitol as carbon and ammonium sulfate as a nitrogen source at PH of 7. Optimum PHB production conditions were 48 h, temperature 30 oC under incubation period of 48 hrs during the highest yield.¹⁹

PHBs are biodegradable polyesters that have great potential to develop fully biodegradable plastics. The cheapest source for its production is agricultural products like cane molasses and steep corn liquor as carbon and nitrogen sources.²⁰ Fungi and bacteria utilize PHB, a thermoplastic polyester in intracellular carbon, and accumulate in the cell as an inclusion body.²¹ Obtaining a cheap carbon source is a significant hurdle in the production of PHA and PHBs. It is estimated that one-tonne polymer 3 tons of glucose are needed, which is pretty costly. PHB can be obtained using other cheap sources like agro-residues, dairy waste, molasses, corn steep liquor.^{22,23}

Microalgae have appeared as rich sources for bioplastic production.⁷ For bioplastic production, *Chlorella* and *Spirulina* species are the most promising microalgae.⁷ Biomass produced by microalgae is potentially high, and it is not dependent upon food sources as substrate and has potential for high lipid accumulation.^{24,25} Bioplastic from microalgae offers a more sustainable approach and contributes to the bioeconomy.^{26,27} Growing microalgae in a closed cultivation system involve much lower cost with higher production.²⁸

Strain improvement and optimization of culture

A gram-positive bacterium SRKP-3, which resembled *Bacillus megaterium*, was identified from brackish water showing an excellent capability to accumulate PHAs. Optimization using response surface methodology resulted in an enhanced yield of 6.37 g/L of PHB dry weight at pH 9.²² Optimization of *Alcaligenes sp.* in batch culture was performed for PHB production using cane molasses and urea as a nutrient source. Strain selection was made using Nile blue A staining; parameter optimization showed 34.5 °C temperature, 6.54 pH, 3.13 Hz agitation enhanced yield to result in PHB yield of 76.80% of dry cell mass.²⁹ Yang *et al.*, 2010 showed enhanced PHA production in *Ralstonia eutropha* H16 by using an optimal acetate, propionate, and butyrate dose. With increasing butyrate production, PHA production is enhanced by 1.5-fold, and further

optimizes phosphate buffer increased PHA production by fourfold.³⁰ Bacterium *Cupriavidus necator* is known to accumulate PHB in a large amount. Using the Plackett-Burman design, crucial factors affecting the accumulation of PHB granules were agitation speed, fructose, KH_2PO_4 , and initial pH, which yielded 7.46 g/L of PHB.³¹ Similarly, for *Botryococcus braunii* applying RSM, the optimal parameters were pH in the range of 4-11, temperature 30-50 °C, and sewage water as a substrate for maximum yield of PHB was studied.³² Likewise, various statistical optimization was employed for obtaining a maximum yield of PHB in *Bacillus subtilis* NG05, *Burkholderia sacchari*, *Bacillus sphaericus* NCIM 5149, with a variety of substrate.^{33,34}

Genetic approaches used in enhancing bioplastic production

Several archaea and eubacteria accumulate PHA within their cells to meet energy needs and carbon storage. The alteration in genetic and metabolic pathways for PHA metabolism has been achieved in these bacteria. However, finding a cost-effective production methodology remains a hurdle that could be sorted out using genetic engineering these microbes to produce PHAs in a high amount.³⁵ Earlier in 1990, *Pseudomonas saccharophila* and *Alcaligenes eutrophus* were engineered to uptake galactose and lactose, but they resulted in low PHB accumulation within the cell.³⁶ At the same time, incorporation of *lacZ*, *lacI*, and *lacO* genes from *E. coli* to *C. necator* allowed better lactose utilization resulting in higher accumulation.³⁷ Likewise, by introducing *Mannheimia succiniciproducens* MBEL55E *sacC* gene to *R. eutropha* for sucrose pathway utilization was engineered, which produced 83% of CDM in the defined medium.³⁸ Another recombinant that expressed *Pseudomonas sp.* 6-19 *phaC1437*, *Clostridium propionicum* *pct540*, was designed.³⁹ In the chemical mutagenesis approach, a mutant strain of *C. necator* was developed that produced 88% PHB using rapeseed oil.⁴⁰ The recombinant *R. eutropha* can utilize various plant oil as a substrate is well-

reviewed, which leads to a yield of PHB in the range of 72 to 87% of CDM.⁴¹ *A. caviae* based *phaJ* gene was inserted in the PHA biosynthesis operon that resulted in a higher PHA accumulation of 79% using soybean oil.⁴² By cloning *Pseudomonas* lipase genes which metabolize triacylglycerols (TAGs) were introduced to enhance PHA accumulation.⁴³ Biosynthetic pathway poly-beta-hydroxybutyrate from *Alcaligenes eutrophus* H16 was cloned and expressed in *E. coli* for PHA production.^{44,45} Balaji *et al.*, 2013⁴⁶ mentioned applying genetic modifications in a microorganism to produce PHB and its extraction process and have shown cyanobacteria's potential as a PHB source by using alternate substrates. Bacterial genes were incorporated into the tobacco plastome to enhance PHB production using an operon extension strategy.⁴⁷ Likewise, transgenic cotton was used for PHB production. Deciphering the methylperillate biosynthesis pathway from *Salvia dorisiana* trichomes is another novel route for the biosynthesis of bioplastics from plants. The engineered enzymes for metabolic engineering could be used in biobased chemicals.⁴⁸ Thus, apart from using conventional techniques, recombinant technology could provide a significant boost to PHA production.

Degradation of bioplastics

Current polymer degradation strategies rely on chemical, thermal, photo, and biological procedures. In proper waste management strategies coupled with industrially controlled biodegradation facilities, biodegradable plastics for some applications such as the packaging or health industry are promising and attractive options for economic, environmental, and health benefits.⁴⁹ Many studies have reported the striking ability of certain microorganisms, including bacteria and fungi, in fast degradation of both bio-based and fossil-based biodegradable polymers under stress conditions by producing exoenzymes and their products.⁵⁰⁻⁵³

Various microorganisms tend to degrade bioplastic and finally convert it to carbon dioxide and water

via microbial metabolic activity. On the contrary, petrochemical plastic is resistant to microbial degradation.⁵⁴ Degradation of bioplastic to a greater extent, depends on the resident microbial population and begins once the colonization process is completed, leading to a lag phase.⁵⁵ The extent of biodegradability of biopolymers depends on their physical and chemical properties and their located environment, which is vital in their biodegradation.⁵⁶ The molecular structure of PLA, material properties, and environmental factors such as humidity, temperature, pH, microbes, and enzymes' presence determine the degradation of bioplastics.^{57,58} Anaerobic conditions favor the degradation of polylactide, phosphinocarboxylic acid, and polyhydroxyalkanoates. Energy derived from mineralization is utilized by microbes and biomass.⁵⁹

Conclusions and Future Prospectives

The extensive studies on the degradation aspect of bioplastic have deepened our understanding regarding degrading microbes and their enzymes. The knowledge will further enhance our understanding of designing biodegradable polymers. Besides, the blending of a biopolymer with natural biomaterials could be one way to reduce the high cost. Our knowledge on the subject will open new avenues towards the development of cost-effective greener bioplastic. Novel approaches for enhanced production of bioplastic and their integration in daily use will offer a greener alternate to petrol-based plastic and help attain sustainable development goals. Consequently, looking out for newer sources will further strengthen its applicability and ensure environmental safety and sustainability.

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