

Overview on Polyhydroxyalkanoates: A Promising Biopol

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Abstract

Plastic pollution is creating the significant environmental and economic burdens since they consume the natural fuels (energy) and other natural resources. Beside this, they have long shelf time, debasing the environment in the numerous ways. The only way to trim down the hazards of plastic pollution is to decrease the use of plastic and thereby reducing its production. Degradations and recycling steps followed to diminish these plastic was of no use since it takes abundant stress (mechanical and chemical) to do so and also considered being costly. Biodegradable plastics became major approach to solve this issue and also became eminent since 1970s. From the inventory of biopolymers acting as bioplastics, PHA has gained major importance because of its analogous behavior to that of petro based plastics. PHAs are the linear polymers produced by the microbes to store energy and carbon. Present review discusses about the PHA production method, recent advances in producing, its degradation and applications of PHA which elucidates the gaining importance in today's industrial world.

Keywords: Polyhydroxyalkanoates; Bioplastics; Poly-Lactic Acid (PLA); BIOPOL; Polyhydroxybutyrate; PHA depolymerases.

Introduction

The substance widely used in our clothing, housing, automobiles, packaging, recreation items and medical implants are Plastics, which encompass wide varieties of organic and inorganic elements. Durability being an prime characteristic of them, plastics can be used in various means and thus gaining an importance in our everyday life.

Plastics are the combinations of high molecular weight compounds, called polymers which include polystyrene, polypropylene, polyethylene, polyvinyl chloride and PTFE. Many fossil based plastics are used in industries made of petroleum, nylon, elastomer (natural rubber) etc which releases dioxins when incinerated and thus disposal is a very big problem causing immense risks to the environment (direct or indirect impact on carbon footprint) [1,2] and mankind (accumulation in the food chains). Plastics don't have competence to degrade and thus recycling is done in some cases to reduce this disposal problem but this cannot be the good remedy. Although many new technologies are evolved to make plastic degradable like photodegradable plastics [3], scientists are seeking for better alternative to this so as to resolve this problem and make the substance degradable or have a controlled life time [4].

In response to problems associated with plastic waste and its effect on the environment, there has been considerable interest in the development and production of an alternative, biodegradable plastics or Bioplastics. In the current context of environmental problems, the swap of conventional plastics with bioplastics is a big confront. Also their development became very much essential due to the depletion of petroleum reserves, high oil prices and increased greenhouse gas emissions. Reports also suggested that burning bioplastic will also avoid the problems caused by them breaking down and producing methane, which is 25 times more potent as a greenhouse gas than CO₂ [5] and more over this produced methane can be further used as source of energy [6]. Bioplastics are a form of plastics derived from renewable biomass sources, such as vegetable fats and oils, corn starch, pea starch, microbiota and thus do not lead to the depletion of finite resources which shows a good balance between mechanical property and biodegradability [7].

Poly-Lactic Acid (PLA) is the early bioplastics, discovered at around 1890. From that day till now, many such different biopolymers have been introduced in the market. The pressures of diminishing resources and increasing waste have lead many professionals to try to re-discover natural polymers and put them to use as materials for manufacture and industry.

Biopolymers can be formed in two ways, those which accumulate and can be collected from the living organisms (microbes and plants) and those which need to be polymerized using renewable resources [8]. Figure 1 describes about the different biopolymer used in routine life coming from different sources [9-12].

Commercially available biopolymers considered as Bioplastics include

- Polyhydroxyalkanoates (PHA)
- Polylactic acid
- Thermoplastic starch
- Bio-polyamides (nylons)
- Bio-polyols
- Cellulosics

Although most of these are produced from different plant and other sources, many of them lack the durability property of the plastic (as packing material) which is a key characteristic considered [13]. Of the all PHA and PLA are having a high demand over other because of its availability and higher potentials to be used as Bioplastics [14].

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Production of bioplastics can be carried out mainly in two ways

1. Using fermentation techniques
2. By growing plastics in plants (using new genetic engineering techniques)

Fermentation procedures, even though costly are considered best for producing such polymers since they give good outcome within less time [15]. Fermentation carried out by the bacteria may or may not relay on separate polymerization step. This depends on type of substrate which has to be degraded, the microbe which is involving and the conditions which are provided during the process. Some microbes like *Ralstonia eutropha* [16], *Lactobacillus sp.* [17,18] have the ability to produce the polymerized substance directly while in case of production of PLA, lactic acid is the outcome of the fermentation which has to be polymerized separately using traditional polymerization process.

Present review gives a note on PHA as Bioplastics, their production process, recent advances involved with the production, PHA degradations, their applications, advantages and disadvantages in using these biopolymers.

Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHA), natural polyesters of bacteria stored as intracellular inclusions are attractive substitutes for petrochemical plastics because they have analogous material properties to thermoplastics and elastomers [19]. This property of PHA, acting as an ideal storage compound is due to its insolubility inside bacterial cytoplasm, which exerts slight increase in osmotic pressure. Besides serving as storage compounds of carbon and energy sources, PHA also acts as sink for reducing equivalents for some microorganisms. It was reported that the bacteria containing PHA storage materials would be able to survive during starvation period compared to those without PHA, as this energy-reserve material slows down the cell autolysis and its mortality rate [20].

PHAs are composed of R(-)-3-hydroxyalkanoic acid monomers

ranging from C3 to C14 carbon atoms with variety of saturated or unsaturated and straight or branched chain containing aliphatic or aromatic side groups. The molecular mass of PHA depends up on the type of growth conditions and microorganism, which may range between 2×10^5 to 3×10^6 daltons. Thus, this property of PHA production, to incorporate monomers of different length made it possible to be used in wide range field of applications. Intracellular depolymerases have the ability to degrade these PHA to carbon and energy source, as soon as the supply of the limiting nutrient is restored [21].

PHA production

PHA is produced by bacteria under unstable growth conditions. Reports predicted that, some bacteria are capable to produce PHA as much as 90% (w/w) of dry cells during depletion of essential nutrients such as nitrogen, phosphorus or magnesium in the medium [22,23]. Polyhydroxybutyrate (PHB), a short-chain length PHAs, was first discovered to be constituent of the bacterium *Bacillus megaterium* in 1926, by Maurice Lemoigne [14]. Various microbes such as *Ralstonia eutropha*, *Alcaligenes latus*, *Aeromonas hydrophila*, *Pseudomonas putida*, Recombinant *E.coli* (having higher success rates) [24] and *Bacillus spp.* were used for industrial production of PHA [25-28]. In addition to heterotrophic microbes acting as potent PHA producers, several cyanobacteria such as *Chlorogloea fritschii*, *Aphanothece sp.*, *Synechococcus sp.*, *Spirulina platensis* have the key enzyme producing PHA [29,30].

The key enzyme producing this PHA is PHA synthases. Figure 2 illustrates about the sequential steps followed by the bacteria in production of PHBs. Carbon derivatives break down to pyruvate through common glycolysis. This pyruvate further forms Acetyl Co-A up on action of pyruvate dehydrogenase, later sequentially forming PHB.

PHA is produced commercially nowadays to meet the requirement of the bioplastic using simple fermentation techniques. Commercial production of PHA as Bioplastics was first conducted by by Zeneca

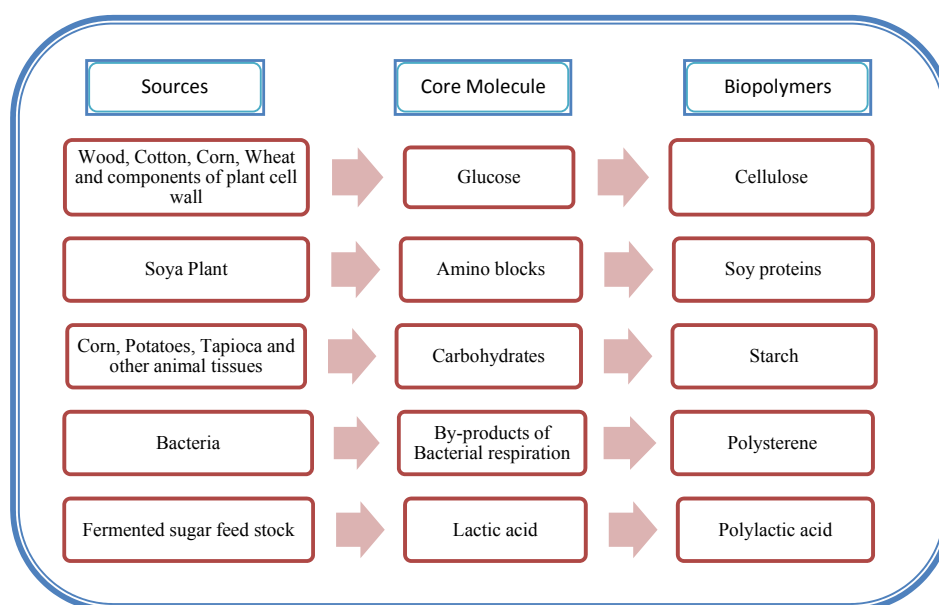


Figure 1: Different sources for the bioplastic production.

Bioproducts using *Aliccaligenes eutrophus* [31]. In the 1980s, a glucose-utilizing mutant of *Cupriavidus necator* was employed by Imperial Chemical Industries (UK) for the industrial production of PHA which was sold under the trade name of Biopol™ [32]. Industrial production procedures considered various factors [33] for the efficient production of PHA. The factors include culture conditions, type of media required (which usually include complex mixtures of amino acids, vitamins, proteins, lipids, carbohydrates, nucleotides, nucleosides and minerals) [9,34], suitable microbe to be used, type of fermentation process, downstream techniques to get the purified raw form of PHA Bioplastics. Figure 3 give the brief description of all the above factors followed for the industrial production of PHA [35].

Certain organism will only produce the compounds, only when they are starved or limited with certain nutrients like nitrogen in the media [36]. The first type of culture requirement discussed in the Figure 3, needs limitation of the nutrient source like nitrogen, phosphorous etc. and therefore, PHA accumulation takes place at stationary phase [37], which can be removed easily using activated carbon adsorption, solvent extraction methods [38,39]. This may require additional supply of carbon source [40] after depletion of other nutrients in the medium and thus fed batch operations are considered to be suitable [41]. In the other type, PHA being accumulated in log phase requires two-stage fermentation method [35].

By varying the producing strains, substrates and co-substrates, a number of different PHAs can be synthesized which differ in monomer composition. The only problem faced by the PHA production is its cost. Due to specific culture conditions requirements, the procedure still becomes costly and more over the carbon sources used is actually

an edible raw material like corn, wheat, potato etc. which goes waste later. Hence improvements and future developments in fermentation/separation technology will help in bringing the production costs slightly down [42].

New developments in this field helped in bringing down the difference between the petro based plastic and PHA Bioplastics which improved its competitiveness [43]. Scientists have shown immense progress in searching for new bacterial strains, creating new types of recombinant strains and tailoring myriad of PHA to reduce the cost of production. The simplest approach to reduce the cost of production is to choose renewable, inexpensive and most readily available carbon substrates that could support both the microbial growth and PHA production efficiently. Nowadays microbes are isolated from or employed over the effluent wastes, discharges etc which contain polluted and residual matter, so that they can be degraded in an eco-friendly way [44-51]. Reports illustrated that few microbes are capable of producing PHA using carbon sources from complex waste effluents, residual effluents, oil paints [52], alkanes [53], fatty acids etc. Various industrial and food wastes are used as carbon source like malt wastes from a beer brewery plant which is used by *Alcaligenes latus* [54]. *Ralstonia eutropha* uses fragmented organic wastes (conversion of organic wastes to short chain volatile fatty acids through hydrolysis and acidogenesis) for PHA production from the food and beverage industries for the PHA production [55].

It is greatness of the scientists and researchers to use the industrial effluents as carbon source for the production of PHA Bioplastics. This is proved by using *Pseudomonas putida* CA-3 strain against toxic pollutant like styrene, naphthalene used as the sole source of carbon and

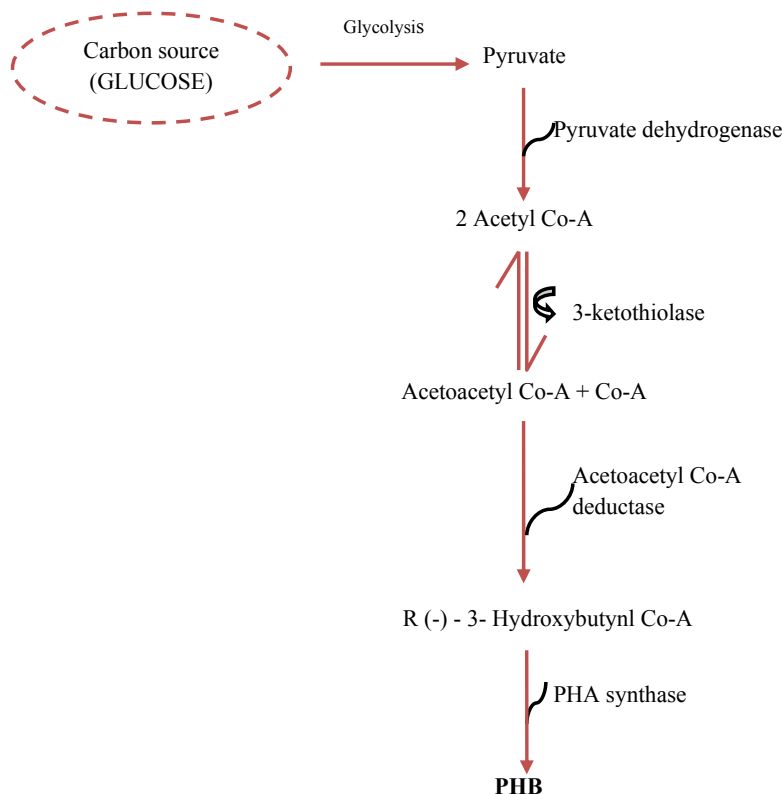
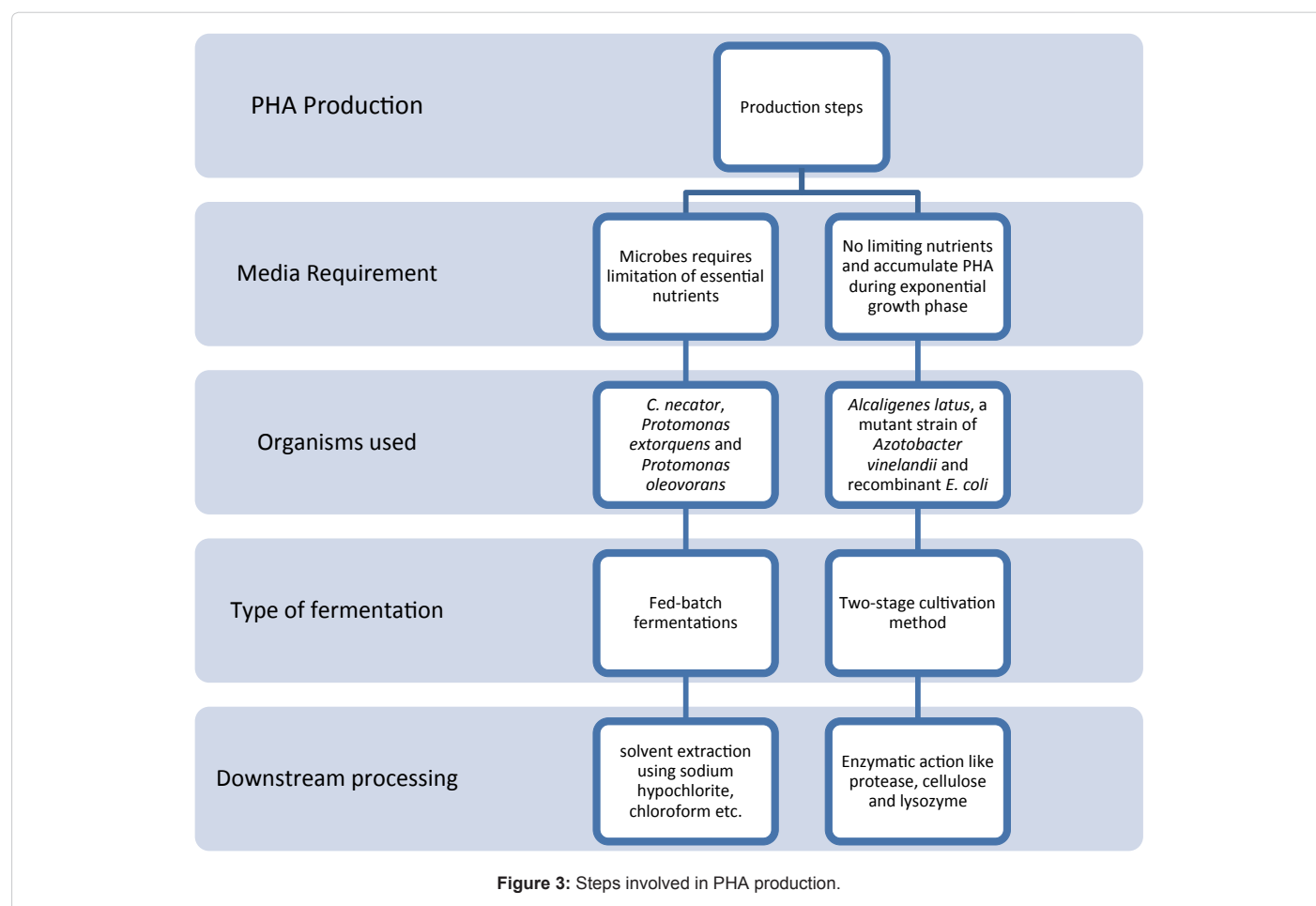


Figure 2: Sequential steps of production of PHB involving PHA synthases.



energy for accumulating medium-chain-length polyhydroxyalkanoates (MCL-PHAs) [56-58]. In few cases, mixed cultures (miniature of complex microorganisms) are required for complete polymerization process since the carbon source provided may be in complex form and cannot be degraded so easily [59-61]. To overcome this cost ineffective process, several studies have been made to isolate such microbes which utilize these complex compounds and degrade them giving bioplastics at last. One such example is *Saccharophagus degradans* (ATCC 43961) which can degrade insoluble cellulose [62] from the textile and dye effluents [63].

Industrial PHA production technology is currently based on bacteria cultivation using pure cultures grown in well-defined nutrient deficient synthetic media with single substrates [64]. Corn plants have high contents of carbon source and thus corn is directly used as substrate, thus the edible part of the plant is getting wasted. Researchers are trying to overcome this problem by incorporating bacterial gene in to the plant which helps in accumulating PHA, using the other parts of plants like roots, leaves, stem etc. People found genetically engineered plants also help to get through several environ problems [65-67]. Also transgenic plants are coming in to limelight in producing PHAs, MIT researchers being first to start it at 1989. It has been reported that PHA are produced in plastids of transgenic plants like *Arabidopsis thaliana* and *Brassica napus*. While in few, peroxisomes also serve as storage bags for PHAs [68]. *Nicotiana tabacum*, *Gossypium hirsutum*,

Medicago sativa, *Elaeis guineensis*, *Linum usitatissimum* are some of the other plants which produces PHAs by transgenic methods [69].

Recent advancements in production of PHAs

- Mixed microbial cultures are used for the synthesis of biofuels from the waste glycerols [70-73] also produces PHA as the by-product [74]. Jatropa biodiesel byproducts are used as carbon source by *Bacillus sonorensis* and *Halomonas hydrothermalis* for production of PHAs [75].
- Cupriavidus necator* organism is reported to produce PHAs from the waste frying oils [76], oil sludge [77] and organic acid wastes from kitchen [78].
- Contaminated oil residues containing polycyclic aromatic hydrocarbons are degraded using *Sphingobium scionense* sp. WP01, producing PHBs [79].
- Ogunjobi reported that *Pseudomonas putrefaciens*, an omnipotent species isolated from the poultry wastes when fed with corn cob as carbon source produced PHAs with an yield of 66.67% w/w of cell dry weight [80-82].
- Liquid bean curd waste is used as low cost carbon source by *Alcaligenes latus* for the production of PHAs [83]. *Alcaligenes latus* also have capability to degrade hydrocarbons in the same way [84].

- Transgenic sugarcane (*Saccharum sp.*) is used extensively nowadays for production of PHAs [85].
- A new type of PHA containing lactide as a co-monomer is synthesized which is believed to have higher durability [86].

PHA biodegradation

Biopolymers have the ability to degrade by itself taking sufficient shelf life period. BIOPOL was actually degraded in soil in 4 weeks. Microbial (enzymatic) action on high molecular weight PHA is the only way to degrade these bioplastics in an eco-friendly manner which is done by secreting the PHA depolymerases. Many such bacteria and fungi are widely distributed which have capability of producing these PHA depolymerases. Two different PHA depolymerases exist, extracellular and intracellular. Intracellular PHA depolymerases (i-PHA depolymerases) are released when the required nutrients are supplied back to the medium and actively degrade the endogenously stored native (amorphous) PHA. This is generally of no use, as this work is done by accumulating bacteria itself and that too internally. While extracellular PHA depolymerases (e-PHA depolymerases), are carboxyesterases have capability to hydrolyze the water insoluble PHA to water soluble monomers [87]. These e-PHA depolymerases are composed from the accumulating cells of microbes after death and cell lysis, hence can be separated easily, purified and used extensively for PHA degradations.

Various bacterial and fungal species have been reported which degrade PHA extracellularly such as aerobic and anaerobic PHA-degrading microorganisms isolated from various ecosystems such as soil (*Pseudomonas lemoignei*), compost, aerobic and anaerobic sewage sludge (*Alcaligenes faecalis*), fresh (*Comamonas testosteroni*) and marine water (*Pseudomonas stutzeri*), including deep sea, estuarine sediment, and air. These microbes are separated from the specific sites, treated to excrete e-PHA depolymerases, enzyme is purified and further used for bioplastic degradation. Such biodegradation of polymer essentially depends on the certain conditions like environment temperature, inorganic composition of nutrients etc. [88]. Recent reports have added *Gracilibacillus* and *Enterobacter* contributions to degrade PHA Bioplastics [89].

Applications of PHA bioplastics

Medical applications include development of cardiovascular products, for drug delivery (acting as microparticulate carriers), nerve repair processes, soft tissue repair (tissue engineering), cell implants etc. PHA Bioplastics are used in and as packing films, disposable items (utensils, hygiene products), cosmetic products, in agricultural applications like biodegradable plastic films for crop protection, seed encapsulation etc. [90]. Nowadays these Bioplastics are used as mobile phone casings, for E- devices, for CD etc.

Benefits and drawbacks of PHA bioplastics

PHA Bioplastics are derived using renewable resources through eco-friendly synthesis process and also can be biodegradable and showing sufficient transparency. Besides having such good collection of pros, they have certain disadvantages too like their compostable nature only under specific conditions due to its brittleness, some show dominant hydrophilic character, unsatisfactory mechanical properties particularly under wet environments.

Conclusion

Plastic usage in the daily routine life has increased its significance

and production which actually created problem in the long run. To replace these, biodegradable polymers came in to limelight which are considered as Bioplastics. PHA being one of them is naturally accumulated in the microbes which can be driven out using some simple downstream techniques. This phenomena lead researchers to plant their intellect and commercially produce these PHA bioplastics from different sources like natural residues, waste effluents etc using fermentation technology. The setback of plastic reuse and degradation is also solved by using these PHA bioplastics, since they can be biodegraded easily by the same microbe producing it or by the use of other microbe producing the key enzyme called PHA depolymerases. Although they have certain disadvantages like brittleness in moulding the plastic, because of their wide application range and analogous nature to that of petro based plastics, techniques are still more evolving to overcome these difficulties in handling these bioplastics, considering them to be a promising biopol.

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