

## Polyhydroxy Alkanoates - A Sustainable Alternative to Petro-Based Plastics

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### Abstract

Industrial growth, urbanization and wrong agricultural practices are responsible for pollution and loss of environmental quality. Petro plastics, produced from mineral resources, are one of the most important materials, but the production process destructs the environment. Bio-based and biodegradable plastics can form the basis for environmentally preferable and sustainable alternative to current materials based exclusively on petroleum feed stocks. Bioplastic helps us to overcome the problem of pollution caused by synthetic plastics as they originate from renewable resources and are a new generation of plastics able to significantly reduce the environmental impact in terms of energy consumption and greenhouse effect. Polyhydroxyalkanoate (PHA) emerges as a potential candidate in a way to be used as a biopolymer material which not only possesses the characteristic similar to the traditional plastic, but it is also biodegradable in nature without any toxic production. A way to reduce the production cost is the use of alternative substrates, such as the agro industrial wastes. This review gives an overview of economical strategies to reduce production costs of PHA as well as its applications in various fields.

**Keywords:** Polyhydroxyalkanoates; Bioplastics; Environment

### Introduction

Plastic can be regarded as one of the greatest inventions and an indispensable commodity of human's life ever since it has been developed into a major industry [1]. It contributes to sustainable development and brings quality life to citizens. As example plastics makes many goods in our daily life more affordable and reduce the wastage of many valuable resources.

Plastics have eased the everyday life; its usage is increasing and annual production has increased substantially over the last 60 years, it has hiked by 3.8 % to around 280 million tons in 2011 over the world. The statistics of European market clearly reveal the annual turnover of 300 billion Euros [2]. The main source for the production of plastics are petrochemicals which are non renewable in nature. A recent estimation done on the earth's mineral resources showed an alarming rate of depletion of these valuable natural assets. This has created a renewed impetus to search for various other sustainable alternatives. The increasing cost and the awareness of consumers of the negative environmental impact of the fluid mineral fuels and related products like recalcitrance to biodegradation [3], toxicity after incineration and massive waste accumulation into the landfills as well as growing water and land pollution problems have led to concern about plastics.

### Biopolymer

With the excessive use of plastics, rising pressure is getting placed to meet the ever increasing demand of petrochemicals coupled with the search for a safe plastic waste disposal process. This awareness of the waste problem and its impact on the environment has awakened new interest in the area of economic and efficient biodegradable polymers sources for production of plastic or popularly known as the "bioplastic". Table 1 shows the comparative analysis between biopolymer and peto based plastics.

Numerous types of biodegradable polymers are under development that popularly includes Polylactides, Polyglycolic acids, Polyhydroxyalkanoates (PHAs), aliphatic polyesters, polysaccharides. On the other hand, natural renewable polymers include porous sponges (from cellulose wood fibres), fibres (made from natural fibres),

hydrogels, starch, cellulose, chitin, chitosan, lignin and proteins. Among these numerous aforementioned biodegradable polymers, PHAs is being considered as the most potential renewable substitute to petrochemical plastics because of its resemblance to commercially available plastic in context to physical and chemical properties [4,5].

Polyhydroxyalkanoates (PHAs) are polymers synthesized entirely by a biological process that involves conversion of carbon sources directly into PHAs through microbial fermentation [6,7]. In contrary, most of the other biopolymers like polybutylene succinate (PBS), Polytrimethylene Terephthalate (PTT) and Polylactic Acid (PLA) are chemically synthesized using fermentation-derived monomers. For example, PLA is prepared by Ring Opening Polymerization (ROP) of lactide, a cyclic dimer of dehydrated lactic acid that is produced by fermentation [8-11].

### Classification of PHAs

PHAs are lipoidic material [12] accumulated intracellularly as insoluble inclusions by a wide variety of microorganisms in the presence of an abundant carbon source. The assimilated carbon sources are biochemically processed into hydroxyalkanoate units under stressed conditions, polymerised and stored in the form of water insoluble inclusions in the cell cytoplasm. The ability of cells to carry out the polymerization process rests on the availability of a key enzyme known as PHA synthase. The product of this enzyme is high Molecular Weight (MW) optically active crystalline polyester [13]. The molecular weight of PHAs depends upon the type of growth

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Properties	Biopolymer	Petro-based plastics
Renewability	Yes or partially	No
Sustainability	Yes	No
Degradability	Biodegradable and/or compostable	Usually non-degradable (Some are degradable through polymer oxidation)
Polymer range	Biopolymers (lipids, proteins, Starch etc.), Bacterial polymers (Polyhydroxyalkanoates, polyhydroxybutyrate)	Extensive
Green house gas emissions	Low emission	High emission
Utilization of fossil fuels	Limited utilization	High utilization
Agriculture land utilization	Expected to increase	No utilization

Table 1: Comparison between Biopolymer and Petro-based plastics.

conditions and microorganism, which range between  $2 \times 10^5$  to  $3 \times 10^6$  daltons [6, 14, 15].

Among these Polyhydroxybutyrate, poly(3-hydroxybutyrate) [P(3HB)] was the first and most common type of PHA to be identified by the French microbiologist Maurice Lemoigne [16]. P(3HB) is an optically active biological linear polyester which is insoluble in water and exhibit a high degree of polymerization that ranges from  $10^5$  to approximately  $10^7$ . The biosynthesized P(3HB) is thus perfectly isotactic and upon extraction from the microorganisms has a crystallinity of about 55-80% with a melting point at around 180°C [17-20].

Structurally, these polymers are classified on the basis of the number of carbon atoms that ranges from 3-5, 6-14, mostly and some are more than 14 [7,20,21] and the type of monomeric units, producing homopolymers or heteropolymers. PHAs with 3-5 carbon atoms are considered as short chain length PHA's (scl-PHAs). Examples of this class include poly(3-hydroxybutyrate) [P(3HB)] and poly(4-hydroxybutyrate) [P(4HB)]. Medium chain length PHAs (mcl-PHAs) contains 6-14 carbon atoms which includes homopolymers poly(3-hydroxyhexanoate) [P(3HHx)], poly(3-hydroxyoctanoate) [P(3HO)] and heteropolymers such as P(3HHx-co-3HO) [22]. PHAs with more than 14 carbon atoms are considered as long chain length PHAs (lcl-PHAs) and they are very uncommon and least studied. For example P(3HB-3HV-3HHD-3HOD) copolymer in *Pseudomonas aeruginosa* [21].

## Characteristics of Polyhydroxyalkanoates

### Physical characteristics

Although aliphatic polyesters have been studied extensively but their remarkable properties such as sufficiently high molecular mass coupled with polymerization characteristics were realized only recently which can be exploited in order to replace conventional plastics such as polypropylene. Naturally occurring PHA's are optically active linear polyesters with each repeating unit in the stereochemical R-configuration. Monomeric compositions, chemical structure as well as the molecular weight are the key factors influencing the physical properties of the polymer. The molecular mass of PHAs varies per PHA producer but is generally in the order of 50,000 to 1,000,000 Dalton.

Scl-PHAs such as P(3HB) are crystalline polymers which are quite brittle and rigid, with high melting points and low glass transition temperatures [23]. These unique characteristics of this biologically synthesized P(3HB) arises due to its exceptional purity. The brittleness is due to the formation of large crystalline domains in the form of spherulites. On the other hand, P(4HB), P(3HB-co-4HB) P(HB-co-HV), scl-PHAs are strong and malleable thermoplastic polyesters [24], especially P(HB-co-HV). It is created by incorporating PV into PHB which is less stiff and brittle than PHB, as a result it can be used

to prepare films with excellent water and gas barrier properties similar to polypropylene. It can also be processed at lower temperature along with preserving most of mechanical properties of PHB [25]. Moreover, Mcl-PHAs are the thermoplastic elastomers with low crystallinity and tensile strength but high elasticity. They also have a lower melting point and glass transition temperatures in contrast to scl-PHAs and polypropylene.

However, for the replacement of petroleum-based plastics, PHA copolymers consisting of both SCL and MCL monomers are considered better choices than PHB due to the presence of certain enhanced properties, such as flexibility and ease of processing [26]. Table 2 gives an outline of comparison of physical properties of different PHAs with conventional polymers [6,27]

### Biological characteristics

The diversity of numerous hydroxy acid monomers that can be incorporated into PHAs, in conjunction with a biological polymerization system capable of generating high-molecular weight materials, has resulted in a manifold rise in the availability of wide range of new polymers. Important biological attributes of PHA includes:

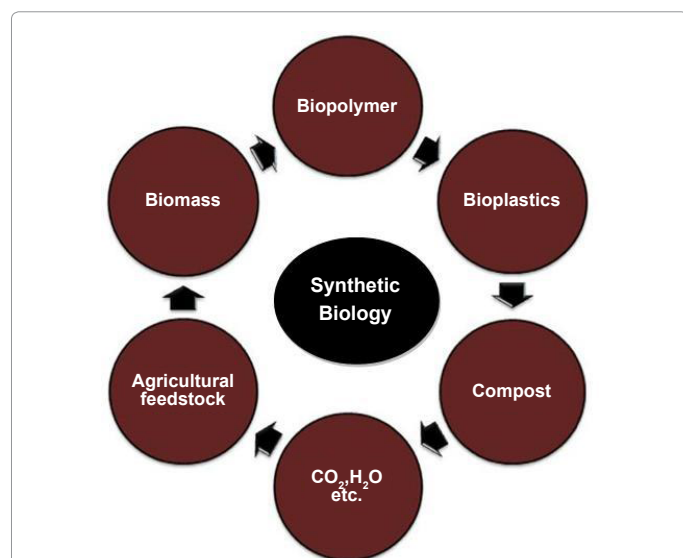
**Biodegradability:** Besides the typical polymeric properties described above, biodegradability is conceivably the most versatile property of PHAs. These biological polyesters are inert, insoluble in water, not affected by moisture and indefinitely stable in air [28].

These polymers are biologically processed by microbial enzymes into inorganic molecules such as carbon dioxide and water under aerobic conditions or methane and water under anaerobic conditions [29]. Such a processing is a part of the natural carbon recycling cycle that exists on the earth shown in (Figure 1). The ability to degrade PHA is broadly carried out by bacteria and fungi, which depends on secretion of specific extracellular PHA depolymerases into the environment or by the intracellular mobilization of PHA in the accumulating strain itself. Both Intracellular mobilization of PHA and extracellular degradation differ from each other due to difference in the biophysical conformations of extracellular (denatured) PHA from those of intracellular (native) PHA. Intracellular PHA depolymerases hydrolyze an endogenous carbon reservoir which are in amorphous state, the native PHA granules, consisting of the polymer with a surface layer of proteins and phospholipids which is sensitive to physical or chemical stress. Extracellular PHA depolymerases degrade denatured extracellular granules which are partially crystalline and are lacking a surface layer [30,31].

Intracellular depolymerases degrades poly[D(-)-3-hydroxybutyrate] (PHB) within the granules into 3-hydroxybutyric acid. It acts on the latter and oxidises it to acetylacetate and a  $\beta$ -ketothiolase acts on acetylacetate to break it down to acetyl-CoA. Under aerobic

POLYMER	MELTING TEMPERATURE (°C)	YOUNG'S MODULUS (GPA)	TENSILE STRENGTH (MPa)	ELASTICITY (%)
P(3HB)	175-180	3.5-4	40	3-8
P(4HB)	53	149	104	N.A.
P(3HB-co-3HV) (3 MOL% HV)	170	2.9	38	N.A.
P(3HB-co-3HV) (20 MOL% HV)	145	1.2	32	50-100
P(3HB-co-4HV) (3 MOL% HV)	166	N.A.	28	45
P(3HB-co-4HV) (10 MOL% HV)	159	N.A.	24	242
P(3HO)	61	N.A.	6-10	300-450
POLYSTYRENE (PS)	80-110	3.0-3.1	50	3-4
POLYPROPYLENE (iPP)	176	1.7	34.5	400
NYLON-6,6	265	2.8	83	60

**Table 2:** A comparison between the physical properties of different PHA's and commonly used conventional polymers.



**Figure 1: Synthetic biology, Bioplastics and Environment:** Bioplastics derived from synthetic biology approaches using renewable feed stock, are good candidates for sustainable development and eco-friendly environment, due to their biodegradability and compostability.

conditions, the acetyl-CoA enters the citric acid cycle and is oxidized to CO<sub>2</sub> [32]. Very little is known about the intracellular depolymerases since they are always found to be intimately connected to the P(3HB) granules and the overall process is very complex [31,33]. Extracellular depolymerases degrade polyhydroxyalkanoates present in the environment [31]. The environmental microbes attack the polymers on the surface [7] and secrete extracellular enzymes that solubilise the polymer and these soluble products are then absorbed through their cell walls and utilized. The PHA depolymerase enzymes act on the polymer mainly by hydrophobic interactions. Degradation by these depolymerases initially produces oligomers. Some microbes produce an additional dimer hydrolase, which further breaks down the oligomers into the corresponding monomer [34]. These extracellular enzymes are quite well understood. The activities of these enzymes may vary depending on the composition of the polymer, its physical

form (amorphous or crystalline), dimensions and most importantly, the environmental conditions.

**Biocompatibility:** According to Williams DF the biocompatibility may be defined as the ability of a material to perform with an appropriate host response in a specific application [35] without any toxic or injurious after effects on biological functions. PHA is highly biocompatible in nature as no toxic compound is generated during polymer degradation. The biocompatibility of PHA particularly P(3HB), is no surprise, as 3HB is a normal blood constituent and is found in the cell envelope of eukaryotes [36]. The ease of crystallization of PHB during precipitation makes entrapment of the drug difficult [37]. Hence, copolymers with HV have been used for drug formulation more than the pure PHB. Copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV), P(4HB), copolymers of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx) have been tested in animals. These tests revealed that all these polymers were biocompatible in various host systems [38]. PHAs have great contribution in medicine, it has been used in different medical applications, such as in wound management (sutures, skin substitutes), vascular system devices (heart valves, vascular grafts), orthopedic (scaffolds for cartilage engineering, screws, bone graft substitutes), controlled drug delivery (through macro and nanocarriers), urological stents etc [39]. Microcapsules from PHB and P(HB-co-HV) has been prepared by various techniques and investigated for the release of bovine serum albumin [40] P(3HB-3HV) capsules can be used as a suitable biodegradable matrix for drug delivery in veterinary medicine that can remain in the rumen of cattle [41,42]. PHB and P(HB-co-HV) are both crystalline materials [42] but the actual degree of crystallinity varies considerably depending on crystallization conditions and HV content [43]. This will, in turn, affect the drug release characteristics. Drug release rates have been reported to increase with increasing HV content [43-45]. For instance, microspheres from P(HB-co-HV) containing tetracycline for the treatment of periodontal diseases showed decreasing encapsulation efficiencies and increasing delivery rates with a higher HV content. Sulbactam-cefoperazone antibiotic was integrated into rods made of poly(3-hydroxybutyrate-co-22 mol%-3-hydroxyvalerate) and studies were carried out to use these rods as antibiotic-loaded carriers to treat implant related and chronic osteomyelitis. The rods were implanted into a rabbit tibia that was artificially infected by *Staphylococcus aureus*. After 15 days, the infection subsided and within 30 days there was complete healing. The high melting points of P(3HB) and P(HB-co-HV) is sometimes a disadvantage and the use of medium chain PHAs with lower melting points could provide a considerable advancement in drug delivery [46]. P(4HB) as biocompatible material has been used as a cardiovascular patching material with great success. It has also been used to make heart valves and in vascular grafting. Sutures, ligaments, surgical meshes and pericardial substitutes made with P(4HB) are now being used [47]. PHA matrices have also been tested for hemocompatibility by inspecting the response of mammalian blood when incubated with polymer films. It was shown that PHB or P(HB-co-HV), when in contact with blood, did not affect platelet responses, nor did the polymer activate complement system.

**Renewable nature:** The production of PHA is solely based on the usage of renewable resources. Fermentative production of PHAs is based on carbon and energy sources obtained from agricultural products such as sugars and fatty acids. These agricultural feed stocks are derived from CO<sub>2</sub> and water, and after their conversion to biodegradable PHA, the breakdown products are again CO<sub>2</sub> and water. Thus, apart from being biodegradable and biocompatible, PHAs receive



general attention because they are based on renewable compounds instead of diminishing fossil fuel stockpiles [48].

It is the consequence of PHAs inherent physical and biological properties along with the striking similarity of physical and chemical profile with conventional polymers such as polypropylene, it has attracted commercial interest and has been extensively studied in the last two decades [49,50].

## Sustainable PHAs: Using Low Cost Production Technology

The global interest of many companies in large scale production of PHA polymers, has been established [51]. The biopolymer production cost is the major stumbling block in the commercialization of PHA. The high production cost of biopolymer is mainly depends upon the methods adopted for its synthesis and the composition of media which limit the widespread use of PHA [52].

By utilizing using inexpensive renewable carbon source and bacterial strains through a fermentation process, it is plausible to produce all sorts of related biopolymers having properties ranging from stiff and brittle polymers to rubbery polymers [22].

## Microorganisms

Application of genetic engineering is essentially paving the way for the development of super PHA production strains that are able to grow to a high cell density within a short period of time on low cost substrates under less demanding fermentation conditions, such as micro-aerobic conditions [6,53]. This approach has significantly reduced the production costs. Engineering approaches including incorporation of engineered PHA synthases and engineered precursor supplying enzymes into the pathways have been applied to achieve enhanced production.

PHA-producing bacteria can be divided into two groups according to the culture conditions required for PHA synthesis. First group is constituted by the wild type forms such as *Ralstonia eutropha* (>80%), *Alcaligenes latus* (>75%), and *Pseudomonas oleovorans* (>60%) that requires limited essential nutrient(s) for the production of PHAs and quantity of PHAs (ranging from 50% to 80% of the dry cell mass) [51]. The second group synthesizes PHAs alongside with the growth in the cultivation medium. Bacteria's in this group includes *Alcaligenes latus* and recombinant *E. coli* containing the PHA biosynthetic genes.

Genetic engineering strategies have proven miraculous in the field of microbiology, as it has been used to reduce the generation time of the recombinant microbes and simplify PHA recovery. Most remarkable example is construction of several refactored microbes producing PHA is among these Recombinant *E. coli* has been of the most favored host developed by harboring the PHAs biosynthesis gene. It has the ability to growth fast facilitate short generation time, best understanding in genetics and biochemistry, abroad substrate utilization ability. Example molasses and whey, and easy purification of the polymer from *E. coli* contribute to its popularity [54,55]. Recombinant *E. coli* harbouring PHB biosynthetic genes from *C. necator* were able to accumulate P(3HB) with a yield of 80–90% dcw in fed-batch cultivation while a P(3HB) content of 76% dcw was obtained in a pH-stat fed-batch culture [56]. Moreover, possibilities for the formation of 'polymeric blends' such as blending of P(3HB) with mcl-PHA has also been investigated in recombinant *Pseudomonas oleovorans* [57]. Three key enzymes for biosynthesis of PHAs are b-ketothiolase, NADPH-dependent acetoacetyl-CoA reductase, and PHA synthase encoded by

genes *phaA*, *phaB*, and *phaC*, respectively [7]. Factors regulating PHAs synthesis are phasins protein, it bind directly to PHA and possibly to the PHA-cycling enzymes, PHA synthase and/or PHA depolymerase [58,59]. The second type of regulator is *PhaR* implicates in negative regulation of a phasin protein [60,61]. It was found that P(3HB) acts as an inducer for *phaP* expression in a *PhaR*-mediated regulatory system. By direct binding *PhaR* interacts with PHA. *PhaR*-mediated regulatory mechanism may be responsible for PHA accumulation in cells is common in SCL PHA-producing bacteria [62] it was also found that mutation in PHB synthesis enzyme acetyl coenzyme A (CoA), ketothiolase (*PhaA*) and NADPH-linked acetoacetyl-CoA reductase (*PhaB*), revealed not only a PHB deficiency but also a deficiency in growth on C1 and C2 compounds [63].

## Waste materials as inexpensive substrate

The major cost of the production of PHA is decided by the type of medium that is used [64]. Thus, the selection of media is a vital factor as it not only provides optimal conditions for production of a variety of PHAs by different strains of bacteria but it also deals with high volumetric productivity to provide an end product that is economically feasible. The PHAs can be produced from a wide variety of substrates such as low cost renewable resources (for example, sucrose, starch, cellulose, triacylglycerols), fossil resources (methane, mineral oil, lignite, hard coal), by products (molasses, whey, glycerol), chemicals (propionic acid, 4-hydroxybutyric acid) and carbon dioxide [65].

Innovative processes are getting investigated for producing PHAs from a variety of low cost substrate such as waste water, municipal wastes, agricultural and industrial residues and their byproducts. Waste water would help to improve environment conditions as organic matter present in wastewater (in the form of organic pollutant) can be converted to useful substances or polyhydroxyalkanoates (PHAs) during various stages of its treatment to facilitate the removal of these pollutants [66-68].

**Plant oils:** Plant oils are derived from oil-bearing crops, such as soy bean, rapeseed, palm, sunflower, and corn. Lately, interest abounded to utilize these plant oils in the bio-based product industries, more specifically, for its efficient conversion into PHA that would make PHA based product's prices competitive with their petroleum counterparts. Plant oils have been investigated and were found to be most desirable carbon source for large scale PHA production. Recent studies have suggested that plant oils give higher yield for both, cell biomass and PHA production (0.6 to 0.8 g of PHA per g of oil) in comparison with other tested substrates such as sugars because of their complex mix of triglycerides [69]. Various plant oils have been used such as P(3HB) homopolymer and P(3HB3co35 mol% 3HHx) copolymer has been synthesized by *Cupriavidus necator* H16 and its recombinant strain (harboring the PHA synthase gene from *A. caviae*) using soybean oil as the sole carbon source [70,71]. Similarly, Lee and colleagues observed the production of 80% dcw P (3HB-co-3HV) from *C. necator* H16 when grown on plant oils such as palm kernel oil [72]. *C. testosteroni* has been studied for its ability to synthesize mcl-PHA from vegetable oils such as castor seed oil, coconut oil, mustard oil, cotton seed oil, groundnut oil, olive oil and sesame oil [73]. *C. necator* organism is also reported to produce PHAs from the waste frying oil [74]. Although these are some very good producers of PHAs, on average the PHA accumulations are still low. Thus, more explorations are needed regarding the development of superior vegetable oil utilizing strains and to further improve fermentation technology for the utilization of waste vegetable oils and other plant oils to further enhance growth and PHA accumulation using these substrates.

**Rice bran:** Rice is the most important cereal product of Asian countries and second largest produced cereal in the world. India and China are the largest producers of rice, as they account for more than one-third of global population supply. Rice bran is a byproduct of the rice milling process and it accounts for 8 -12 % of the rice kernel [75]. It is associated with environmental impact due to presence of impurities like arsenic and silica, as well as the presence of free fatty acids. Study conducted by Ting-Yen Huang et al. showed procurement of PHA successfully from extruded rice bran and corn starch from *Haloferax mediterranei* under a hyper-saline condition [76]. Using rice bran (a low cost agro-industry residue), 3.63 g/L of biomass, 1.75 g/L of intra-cellular polymer Polyhydroxyalkanoate, (PHA) and 1.2 g/L of extra-cellular polymer (exopolysaccharide, EPS) was produced by *Sinorhizobium meliloti* [77].

**Molasses:** Molasses is the low value, unpalatable and final residual syrup generated in sugar-refining mills after repeated sugar extraction by carrying out crystallization of sugarcane or sugar beet juice. It is mainly used as a cattle feed supplement, in specialized yeast propagation or as a flavoring agent in some foods [78]. Depending on the grades and sources, sugar molasses cannot be further used in foods or feeds and is, thus, ideal for consideration as an inexpensive carbon source for fermentative processes. According to Tate & Lyle Molasses Germany GMBH [79] the quantities of cane molasses and beet molasses generated worldwide in 2004 were 39 and 12 million tons, respectively. Molasses has negative environmental impact due to its accumulation during sugar processing, thus cane molasses and beet molasses are ideal for consideration as an inexpensive carbon source for PHA production which could alleviate its disposal problems. Soy molasses have also been tested as alternative substrate, which is a by-product of soybean processing industry. It contains various soluble carbohydrates especially sucrose, raffinose and stachyose [80].

A great deal of work has been carried out into the utilization of molasses for PHA production. Both Gram negative and Gram positive bacteria such as recombinant *E. coli* and *Klebsiella* strains [81], *R. eutropha* [82], *Bacillus* strains [83] has used molasses for PHA production.

Cane molasses was found to be an excellent substrate for the growth of *Bacillus megaterium* for subsequent PHA production by [83] a total biomass and PHA content of 72.7 g · L<sup>-1</sup> and 42% (wt/wt) [84] demonstrated the production of 61% dcw PHA using molasses as a complex feedstock in 2-stage CSTR [85] reported the production of P(3HB) at 3 g polymer L<sup>-1</sup> and 50% dcw P (3HB) content by *Bacillus megaterium* grown on beet molasses [86] have tested *Pseudomonas corrugata* on inexpensive soy molasses carbon substrate for the production of medium-chain-length PHA and achieved a cell dry mass concentration of 3.4 g L<sup>-1</sup>, with a PHB content of 5 -17% dcw.

**Dairy whey:** During cheese and casein production at the food processing industries, about 80-90% of the processed milk volume is converted to whey as a byproduct [87]. Whey is rich in lactose, proteins, lipids, and lactic acid [88]. It is the soluble part of milk that is separated from the casein during cheese manufacture and is stabilized by disulfide bonds which makes it insoluble in water. For smaller dairies, disposal of whey is cost demanding as only about half of the produced whey is converted into useful products such as human and animal feed while the rest is disposed of as waste causing environmental problems due to its high oxygen demand.

Many isolates of bacteria were evaluated for the production of different polyester members of the PHA family from lactose or cheese

whey as carbon source [89]. Recombinant *Escherichia coli* strains have been used for the production of PHA [90,91]. The production of a heteropolymer from whey by *Thermus thermophilus* HB8 has also been described [92]. Role of *Cupriavidus necator* has been extensively established as a platform for PHA production and it is one of the best industrially useful strain which is able to use cheap carbon sources. However it is unable to hydrolyse the disaccharide lactose and galactose which is the main sugar contained in whey [93] hydrolyzed milk whey in order to make glucose available for *C. necator*. Scientist have proposed an alternative process consisting of two steps for PHB production from milk whey: lactose was first converted to lactic acid by lactobacilli, and the resulting lactic acid was then used as carbon source by *R. eutropha* for PHA production [94]. Further scientist have developed recombinant strains of *C. necator* which are able to produce the polymer directly from lactose and or whey permeate in one step [95]. Hence, milk whey can be successfully used as carbon source for PHAs production which would significant reduce the production cost of the PHAs.

### Downstream processing

Recovery of PHA is another process that significantly affects the overall process economics. The development of a cheaper safer downstream process for the recovery of PHA will have considerable impact on industrial production of this versatile biopolymer. The extraction and the purification of bacterial polyhydroxyalkanoates are the key step of the process profitability in the fermentation system. The ideal method should lead to a high purity and recovery level at a low production cost.

Traditional PHA downstream processing methods requiring the use of solvents, enzymatic digestion, or mechanical disruption are expensive and impractical for industrial-scale recovery. A common practice to recover PHA is through the organic solvent extraction method. This process has many advantages over other cell disruption processes, For example solvent extraction do not degrade the polymer and can be useful for several medical applications by the elimination of Endotoxin which can be found in Gram negative bacteria [96]. However, the use of solvents creates hazards for the operators and for the environment which limits its success in pilot-plant and large scale processing [97]. To alleviate this short coming, an extraction method using non-halogenated solvent such as sodium hypochlorite has been used for the aqueous process [98]. Although the use of sodium hypochlorite significantly increased PHA degradation, polymer purity greater than 95% is achieved [96].

Mechanical cell disruption is widely used to release intracellular protein [99]. It is mainly favored due to economic advantageous such as it causes mild damage to the products and does not involve any chemicals making it environmental friendly. But, it also has drawbacks like it decrease the molecular weight of the biopolymer, long processing time and difficulty in scaling up.

Enzymatic digestion method is a gentle and selective alternative separation method, which has attracted the interest of many researchers [100]. It eliminates the need of hazardous solvents but the enzymes used are expensive and the process is also more complex and economically unattractive.

Due to the problem associated with solvent extraction process, much attention has been given to centrifugal fractionation as a simple and economical process for separating specific resin components from the recovered PHA. Continuous centrifugal fractionation gives 85%

of PHA with purity more than 95% [101,102]. Pretreatment of cells, mechanical disruption, solubilisation of non-PHA cell material by chemical or enzymatic treatment in the system, spontaneous liberation of poly(3-hydroxybutyrate), dissolved air flotation or air classification and extraction using supercritical CO<sub>2</sub> have been recently reviewed [103].

Over the years, a variety of PHA recovery methods have been developed, although none of the downstream procedures possess all the necessary requirements for a significant efficient and economical large-scale process [104]. The major drawbacks are cost, safety and scalability. Still, further researches have been made for the development of simple fermentation strategies as well as modification of recovery techniques in order to scale-up the PHA production.

## Conclusions and Outlook

As plastic materials are of such importance in our society and knowing that materials are a fundamental determinant of sustainability; currently PHAs have rapidly developed as potential substitute material to petro plastics due to its versatile property like biodegradable and biocompatible. Their structural versatility and characteristics of PHAs have been investigated and new areas of exploitation are being discovered due to its various applications in industries and medical field. The major drawback for extensive use of these polymers and its slow growth is their high production cost and inferior thermo-mechanical, physical and processing properties of biodegradable polymers compared with petrochemical plastics.

Consequently, scientists have shown immense progress in searching for new bacterial strains, creating new types of recombinant strains and tailoring various kinds of PHA to reduce the cost of production. Use of low-cost renewable carbon sources in fermentation process has the potential to reduce PHA production costs, which will certainly improve PHAs competitiveness and its production substantially, leading to an enhancement in its widespread usage as they confer no long term effects on environment and could be adorable at social and economic levels.

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