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Review Article

Polyhydroxyalkanoates: Role of *Ralstonia eutropha*

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Abstract

Polyhydroxyalkanoates (PHAs) are biopolymers, which can be used as a substitute for petrochemical plastics in diverse applications. However, these bioplastics are presently more costly than petrochemical plastics. Various researchers had investigated to exploit the use of economical waste streams derivative substrates. Waste frying oil is an ample cheap substrate which can be used without filtration in PHA production. *Ralstonia eutropha* is a handy organism for the production of PHA. Due to its similar physical properties to synthetic plastics, Polyhydroxyalkanoate (PHA) a biologically-synthesized plastic is attracting major interests in green material industries. Bioplastics are synthesized from renewable resources and they are degraded by aerobic microorganism to CO_2 and H_2O when disposed-off. For the commercialization of PHA; some important aspects like inexpensive carbon sources, selection of suitable bacterial strains, efficient fermentation and recovery processes should be taken into consideration.

Keywords: PHA (polyhydroxyalkanoate), PHB (polyhydroxybutyrate), *Ralstonia eutropha*, Bioploymer, Biodegradable Bioplastic

1. Introduction

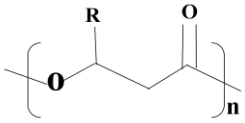
Polyhydroxyalkanoates (PHAs) are biodegradable biopolymers that serve as carbon and energy storage material in the cell and are accumulated by various microorganisms as inclusion bodies under certain unbalanced growth condition in presence of excess carbon[1,2]. PHA mainly consists of C, H and O that is derived from the metabolism of renewable resources like carbohydrates, lipids and other resources. In addition to PHA biosynthesis, these substrates are also catabolised for microbial growth and generation of energy. PHAs are biopolymers having property similar to that of synthetic plastics[3].

The name of the strain *Ralstonia eutropha* has been renamed to *Wautersia eutropha*[4] by the scientific community since 2004 and, in the same year, to *Cupriavidus necator*[5]. *Ralstonia* species has ability to accumulate a large quantity of polyhydroxyalkanoate (PHA) under unbalanced growth conditions where carbon and energy are in excess and protein synthesis reaction is the limiting factor[6]. PHA is usually seized intracellularly in the form of inclusion bodies; termed as granules. PHA is a family of polymers with over 150 types known to exist[7], all differentiated by the monomers incorporated into them. Wild-type *R. eutropha* produces short chain length (SCL) PHA like PHB [containing only the 3-hydroxybutyrate (3HB) or 4HB monomer], P (HB- *co* -HV) [containing 3HB and 3-hydroxyvalerate (3HV) or 4HV monomer]. Due to availability of genetic tools and ease of genetic manipulation of *R. eutropha*, strains are constructed that can produce short chain length (SCL) PHA and mixed chain length (SCL and MCL) PHA polymers[8,9,10,11]. PHAs are the plastic of the future, bioplastics are more sustainable as they degrade rapidly and synthesized from a renewable resource. Biodegradable plastics will solve the problem of plastics litter. The successful production of biodegradable plastics will lessen the burden placed on the environment by consumers. Bioplastics will protect our environment and save the life of the domestic animals. This review can help us to find new ways to face the challenges from plastic threatening the world environment.

2. Classification and Characteristics of PHA

PHA is usually composed of (*R*)-β-hydroxy fatty acids (Figure 1), where the '*R*' group varies from C_1 to C_{18}. The microbial bioplastics are classified (Table 1) according to different criteria[12]. Generally PHAs are classified on the basis of number of carbon atoms in the monomer units and these can be divided into three major groups:- (a) short chain length polyhydroxyalkanoates (SCL); which consist of 3 to 5 carbon (C_3-C_5) atoms, (b) medium chain length polyhydroxyalkanoates (MCL); consisting of 6 to 14 carbon (C_6-C_{14}) atoms and (c) long chain length polyhydroxyalkanoates (LCL); that contains of at least 15 carbon (C_{15}-C_{18}) atoms[13]. It is biodegradable in nature and is used in the production of bioplastics.

Figure 1: General structure of Polyhydroxyalkanoates (PHA)



R = CH_3 Polyhydroxybutyrate
R = CH_2CH_3 Polyhydroxyvalerate

PHAs are polyesters that are accumulated as carbon and energy source under nutrient limiting conditions in various microorganisms. Most of them have complete biodegradability and similar mechanical properties to petro-chemical plastics. The physical properties depend on their monomer units; hence biodegradable polymers have different properties depending on monomer units. PHA is a source for chiral compound synthesis and raw material for paint production. It can easily be depolymerised to give bi-functional, pure and active hydroxyl acids. They are used as osteosynthetic material for bone growth[14,15,16].

PHA can be produced from renewable resources like carbon dioxide, plant oil and sugars[17]. Palm oils are comparatively cheaper than most sugar and hence desirable for PHA production. Palm oil yields at least 2 fold[18] higher PHA because it contains higher carbon content per weight than simple sugars. Palm fruits are used for extraction of two different types of oils: (i) Palm Kernel Oil (PKO) which is obtained from kernel and (ii) Crude Palm Oil (CPO) which is obtained from mesocarp. Various types of waste and by-products are produced as a result of further physical and chemical refining processes.

Table 1: Classification of Microbial Bioplastics

S. No.	Criteria for Classification	Types	Description
1.	Monomer size	Short-Chain Length (SCL)	Contains (C_{3-} C_5) carbon atoms
		Medium-Chain Length (MCL)	Contains (C_{6-} C_{14}) carbon atoms
		Long-Chain Length (LCL)	Contains (C_{15-} C_{18}) carbon atoms
2.	Biosynthetic origin	Natural bioplastics	Produced by microorganisms from general metabolites (e.g. aliphatic PHAs like PHB)
		Semi synthetic bioplastics	Cannot be synthesised by the microbe and requires the addition to the culture broth of some precursors (e.g. PHAs containing aromatic monomers)
		Synthetic bioplastics	Obtained by chemical synthesis (e.g. synthetic thermoplastic polymers)
3.	Number of monomers in the polyesters	Homopolymeric bioplastic	Contains single monomer unit
		Heteropolymeric bioplastic (copolymer and terpolymers)	Contains more than one monomer unit
4.	Type of polyesters accumulated by the microbe	Unique	Single bio-plastic
		More than one	Mixed bio-plastics
5.	Chemical nature of the monomers	Bioplastic containing aliphatic fatty acid derivatives	Saturated or unsaturated monomers; linear or branched monomers; substituted or nonsubstituted
		Bioplastics containing aromatic fatty acid derivatives	-
		Bioplastics containing both aliphatic and aromatic fatty acid derivatives	-
		Bioplastics containing other different compounds	e.g. poly-γ-glutamic acid, poly-ε-L-lysine, poly-β-L-malic acid, polyglycolic acid, cianophicin

Ralstonia eutropha is a gram-negative soil bacterium found both in the soil and the water which is used in the bioremediation because of its ability to degrade a great number of chlorinated aromatic (chloroaromatic) and chemically related compounds. In the synthesis of PHA; the *Ralstonia eutropha* is used as the reference and Palmitic acid as testing material. *Ralstonia eutropha* is the most common bacteria that has the ability to produce PHB with the use of simple carbon substances like glucose, lactic acid and acetic acid as its carbon source and produces significant quantities of PHB within the cells. We can also use olive oil, corn oil and palm oil as the substrate for PHB synthesis in this strain, which could produce about 80% (w/w) PHB on dry cell weight. Common PHB molecules produced by such kind of bacteria are very brittle thermoplastic. Here the main focus is on producing bioplastic or even biofilm in *Ralstonia eutropha* and other bacteria to enhance the properties of the PHAs produced.

Methylobacterium also has ability to use methanol as a carbon substrate and these species are of much interest for production of PHB[19]. The use of methanol as a carbon substrate is significant because it is cheap carbon source and could reduce production costs. *Methylobacteria* are metabolically diverse and can grow on a number of carbon and nitrogen sources and they are also typically found naturally within the environment[20]. A better yield of PHB can be obtained from simple sugar carbon sources such as glucose or fructose. PHB is isolated through centrifugation then washing with water and at last performing centrifugation again. PHAs are more useful than homopolymers such as PHB because they possess more useful qualities for production of plastic. Palmitic acid, $CH_3(CH_2)_{14}COOH$ or hexadecanoic acid is one of the most common saturated fatty acids found in animals and plants. Keeping this in mind, Polyhydroxyalkanoates have received extensive interest as renewable-resource based biodegradable and biocompatible plastics with a wide range of potential application.

R. eutropha is an industrially significant organism and some of its advantageous traits are discussed in Table 2. Due to its similar physical properties to synthetic plastics and features like bio-based, biodegradable, and biocompatible; PHA which is a biologically-synthesized plastic is attracting major interests in green material industries. Thus being more environment friendly; PHA is considered as an alternative to chemically synthesized plastics and has many applications in industry, household, medical, etc. *R. eutropha* can grow and produce PHA autotrophically using mainly CO_2, H_2 and O_2 as the main growth substrates from a range of matters including animal and plant oils (such as corn oil, lard, palm oil, palm kernel oil, tallow); individual compounds (such as alcohols, organic acids, triacylgylcerols, sugars) and waste products of olive and palm oil production, sugar, alcohol and hydrolysis industries[9,21,22,23] etc. A genome-scale metabolic network model has been developed that confirms the significance of C/N ratio and culture pH for *R. eutropha* (strain H16) in PHB biosynthesis[17]. There are many methods to produce value added products from *R. eutropha* cultures using feedstock and products from this feedstock[24]. The manipulation of the carbon flux in the organism is the property of *R. eutropha* physiology studies. There are two methods used for carbon flux manipulation- (i) genetic alteration of enzymes, genes and pathways present in the bacterium and (ii) altering the timing and type of feed stocks used to synthesize the product.

Table 2: Characteristics of *Ralstonia eutropha* strain that make it useful for production of Bio-based products

S.No.	Characteristic/trait	Relevance in production of biological materials	References
1.	Adjustable polymer material properties	Produces distinct type of polymers having medium and longer length monomers through fermentation process controls	11,18,25
2.	Autotrophic growth	Utilizes CO_2 for production of biopolymers and other products	26,27
3.	Carbon source utilization range	Produces value added products like agricultural and food processing waste streams using plant oils or other inexpensive carbon sources	23,27,28,29
4.	Genetically manipulable	Constructs *R. eutropha* strains that has potential for production of different types of bio-based compounds including many types of PHA	8,9,30
5.	Non-pathogenic /biocompatible	Used to produce biopolymers for medical materials and devices and medical compounds	31,32
6.	Resistant to some toxic compounds	Is carbon monoxide resistant and can produce biopolymers from toxic mixtures like syngas; can also potentially produce biopolymers from phenol	33,34
7.	Robust carbon storage pathway	Produces intracellular biopolymers with a high productivity and purity	11,18,25

3. Nature of PHA

3.1 Biodegradable Nature

Biodegradability is the important characteristic of PHA. PHAs are degraded naturally by various microorganisms using 3-hydroxybutyrate-oligomer hydrolases and PHA depolymerases. The activities of these enzymes vary and depend on the environmental conditions and the composition of the polymer. The PHB (polyhydroxybutyrate) which is another representative of the PHA family degrades in few months (in anaerobic sewage) to years (in seawater). While processing biopolymers, thermal degradation point is the important criteria that we must know[35]. The decomposition of PHB (Biopol) starts[36] at 246·3°C. The degradation of PHA is accelerated by the presence of UV light. PHAs are biocompatible in nature; which means that they impart no toxic effects on living organisms. PHA leaves no visible, toxic residue when degraded. During the biological degradation by composting it yields CO_2, water, inorganic compounds, and biomass at a rate consistent with other compostable materials. The benefits of bioplastics include enrichment of the soil when they turn into compost, the reduced risk of injuries to the animals when they ingest non-degradable materials, decreased landfills space requirement and the ability to recycle the remaining components to recover cost of production[37].

The degradation of bioplastic mainly depends on the crystalinity, composition or surface area of the PHA material and also on the temperature, moisture, pH and nutrient supply available to the microbial community. The variety of environments including soil, fresh water and salt water contains various microorganisms that can utilize PHA as a growth substrate. The co-polymer of hydroxybutyrate and hydroxyvalerate is degraded in containers of garden soil by microbes typically occurring in such environments. The degradation rate is also relatively rapid.

3.2 Renewable nature and life cycle

The "Bio-based nature" of PHA is illustrated considering the fact that these materials are produced by the bio-synthetic action of living microbial cells based on renewable resources. Their entire life cycle is independent from the availability of fossil resources. The biodegradation and synthesis of PHAs are totally embedded into the carbon-cycle. This Bio-based nature of PHA is crucial provoking the considerable and increasing attention devoted to these materials. Using well-established methods of life cycle analysis (LCA) for comparing the production of biopolymers and conventional polymers, it has been reported that more energy would be needed during the life cycle of PHA[38] from crop growing to molding the final product, than in the life cycle of conventional plastics[39,40,41,42]. However, the petrochemical plastics production is fully developed[35] while the fermentation process to make PHA is far from optimization.

4. Biosynthetic approaches

Ralstonia eutropha is used as model organism for studying synthesis and accumulation of PHA which is produced by a variety of bacteria as carbon and energy source. Palm oil which is a major agricultural product in South Asia is used as feedstock. Strains of *R. eutropha* were engineered so that they can express and accumulate high levels of PHA copolymers (3-hydroxybutyrate and 3-hydroxyhexanoate) when grown on palm oil. This transgenic strain expressed a PHA synthase gene from bacteria *Rhodococcus aetherivorans I24*. The amount of 3-hydroxyhexanoate present in PHA is regulated by regulating the level of acetoacetyl-CoA reductase (PhaB) activity in the transgenic strains. Microarray experiments were performed to study the gene expression during growth on palm oil and the results provide insights which allow for additional improvement to be made in transgenic strains. Fermentation method was employed to study the growth of *R. eutropha* on palm oil and to measure the amount of carbon source utilized. In one of these methods, glycoprotein gum Arabic is used as emulsifier for palm oil. Palm oil fermentation was also carried out using unemulsified oil that represents more industrial culture conditions. In the recovery process, Methyl isobutyl ketone was used for extraction of PHA and polymer precipitation was done by addition of alkenes[43]. In another study Lee and his co-worker carried out PHA biosynthesis in one-stage culture in shake flasks[44].

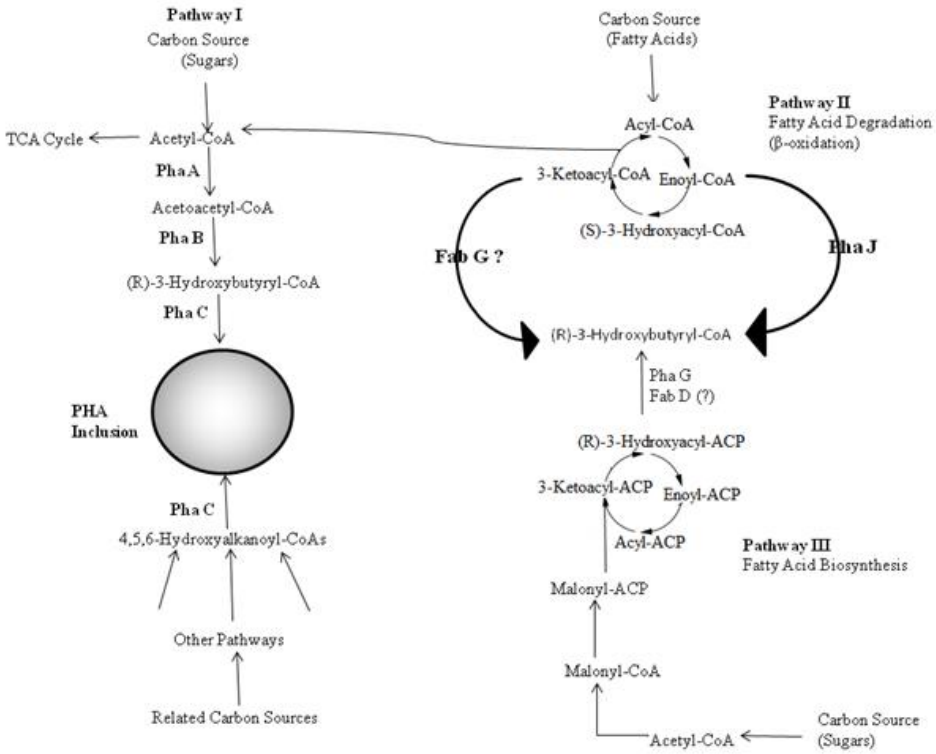
The biosynthetic pathway of P (3HB) consists of three enzymatic reactions catalyzed by three different enzymes[14]. The first reaction consists of the condensation of two acetyl-CoA molecules into acetoacetyl-CoA by enzyme β-ketoacylCoA thiolase (which is encoded by the gene *phb*A). The second reaction includes the reduction of acetoacetyl-CoA to (*R*)-3-hydroxybutyryl-CoA by an enzyme acetoacetyl-CoA dehydrogenase (which is encoded by the gene *phb*B). Lastly, the (*R*)-3-hydroxybutyryl-CoA monomers are polymerized into PHB by enzyme P(3HB) polymerase (which is encoded by the gene *phb*C).

5. Role of *R. Eutropha* and metabolic pathways for PHA biosynthesis from various carbon sources

The carbon source is one of the factors that determine the type of PHA[2]. The microorganisms can utilise various carbon sources ranging from simple carbohydrates to inexpensive, complex waste effluents like beet/cane molasses[45, 46] to alkanes[47], plant oils[48] and fatty acids of plant oils[49,50,51] for production of PHA. On the basis of the types of monomer integrated into PHA, different metabolic pathways are involved in the generation of these monomers. Figure 2 summarizes the various metabolic pathways that are known to supply monomer units for PHA biosynthesis. *R. eutropha* have been extensively studied among the bacteria for the production of PHA. The two acetyl-CoA moieties are condensed to acetoacetyl-CoA by β-ketothiolase (PhaA)[52] in *R. eutropha*. The product on reduction by an NADPH-dependent reductase (PhaB) produces the (R)-isomer of 3-hydroxybutyryl-CoA (Figure 2, Pathway I). But in *R. rubrum*; the reductase which is an NADH-dependent isoenzyme produces the (S)-isomer of 3-hydroxybutyryl-CoA. The two enoyl-CoA hydratases[53] then covert the (S)-type to the (R)-type isomer which is usually accepted by polymerizing enzyme (PHA synthase). *R. eutropha* can utilise various carbon sources for growth and PHA production. Linko and his coworkers reported that *R. eutropha* has capability for producing P (3HB) from lactic acid[54]. *R. eutropha* also produces

P (3HB) homopolymer from even carbon numbered n-alkanoates, while odd-carbon numbered n-alkanoates accumulates copolymers of 3HB and 3HV[55]. Studies on *R. eutropha* for PHA production under autotrophic conditions also suggest that it can utilize carbon dioxide for the production of P (3HB)[56]. *R. eutropha* also has ability to synthesize PHA from some specialized carbon sources such as 1, 4 - butanediol, γ-butyrolactone and 4-hydroxybutyric acid which give rise to the integration of 4HB monomers along with 3HB[57, 58].

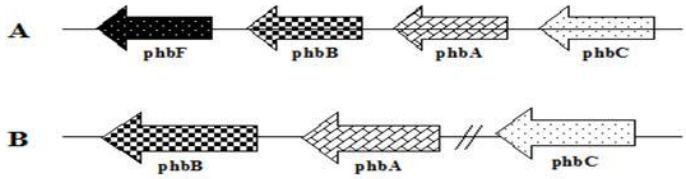
Figure 2: Metabolic pathways for PHA biosynthesis. PhaA, β-ketothiolase; PhaB, NADPH-dependent acetoacetyl-CoA reductase; PhaC, PHA synthase; PhaG, 3-hydroxyacyl-ACP-CoA transferase; PhaJ, (R)-enoyl-CoA hydratase; FabD, malonyl-CoA-ACP transacylase; FabG, 3-ketoacyl-CoA reductase.



6. Genes involved in PHA biosynthesis

There are more than 20 types of PHA synthesis operons that have been cloned and analyzed from various bacteria[59] and there is considerable divergence in the proteins required for PHA biosynthesis pathways. The arrangement of the genes and enzymes involved in the biosynthesis of PHA varies from organism to organism. The bacterial genome contains the PHA synthase genes and genes for other proteins associated with the metabolism of PHA in the form of cluster and since the genes are arranged in clusters, their organization differs from species to species (Figure 3).

Figure 3: Molecular arrangement of PHA synthase genes[59], which encodes PHA synthases of type I. phbC, gene encodes PHA synthase; phbA, gene encodes β-ketothiolase; phbB, gene encodes acetoacetyl-CoA reductase.



The genes associated (Table 3) with the enzymes for MCL PHAs formation are designated as *pha*, and those associated with enzymes for SCL PHAs formation are designated as *phb*. The genes coding for proteins involved in the biosynthesis of PHA are designated in alphabetical order as *phaA* (ketothiolase), *phaB* (acetoacetyl-CoA reductase), *phaC* (PHA synthase), *phaG* (3- hydroxyacyl-acyl carrier protein-CoA transacylase), *phaJ* (enoyl-CoA hydratase) and so on. The genes required for the degradation of PHA are designated in reverse alphabetical order such as *phaZ* for PHA depolymerases, *phaY*, *phaX*, *phaW* etc. The gene *phaP* refers to phasins. The gene product i.e. protein is indicated with first letter in upper case e.g. β-ketothiolase is written as *PhaA*[60].

After the cloning of the PHA synthase operon of *Ralstonia eutropha*, 54 different PHA synthases were cloned (plus one partial PHA synthase gene from *Pseudomonas* sp.) from 44 different microorganisms whose PHA synthases primary structures are available. Rehm and Steinbuchel have reviewed the strategies to clone the genes and the organization of the PHA synthase genes and other genes encoding proteins related to PHA metabolism[60].

Table 3: **Genes responsible for production of different PHA types in different species/strain of *Ralstonia eutropha*[61]**

S.No.	Species/Strain	Gene(s)	Protein	Method	References
1.	*Ralstonia eutropha* PHB-4	*phaC-egfp*	PHB synthase fused to EGFP	Fluorescence Microscopy; Fusion (PHB granules, located in the cell pole or the periphery; fusion also located in the CM 5 to 10 min after transfer to fresh medium)	61
2.	*Ralstonia eutropha*/rec. *Escherichia coli*	*phaC-egfp*	PhaC-EGFP	Fluorescence Microscopy (PHB granules, located in the cell pole or the periphery)	6,62
3.	*Ralstonia eutropha* H16	*phaZd* (*phaZd1*)	PhaZd	Western Blotting	63
		phaZc (*phaY*)	Oligomer hydrolase: PhaZb	Western Blotting; (partially soluble)	64
		phaZb (*phaZ2*, *phaY*)	Oligomer hydrolase: PhaZb	Western Blotting; (partially soluble)	65
		phaZa1-egfp	PhaZa1-EGF	Fluorescence Microscopy	66
		phaZa1	iPHB depolymerase, PhaZa1	Western Blotting	67
		phaR	PhaR	Western Blotting; Electron Microscopy Immunogold Technique	68
		phaP1, *phaP3*, *phaP4*	PhaP1, PhaP3, PhaP4, Bkt	2D-PAGE; Fluorescence Microscopy	69,70
		phaP	Phasins: PhaP	Western Blotting	71,72
		-	PhaC (PhbC)	In the absence of PHB, PhaC is in the soluble fraction	73
		phaC (*phbC*)	PHB synthase	Activity; Electron Microscopy Immunogold Technique	74, 75,76,77

7. Extraction and recovery of PHA

When the desired quantity of PHA has been prepared, the next step is the extraction and recovery of the product. The extraction process is carried out with a halogenated solvent such as chloroform and a PHA non-solvent such as methanol, using which PHA gets precipitated[78]. Generally preference is given to a recovery process which is more environments friendly and less hazardous in nature. Unfortunately other lipid extraction method like mechanical method of oilseeds is not effective in the case of PHA. There are many studies that have been done on PHA extraction. The extraction method includes the mechanical disruption of the cells[79], the use of organic solvents[80] as well as enzymatic or chemical digestion[81,82] of the tightly granule bound proteins (phasins)[83]. But these extraction processes are costly, incompetent or complex when scaled up.

7.1 Extraction from bacterial biomass

The Extraction and purification of PHA polymers from biomass presents a technical challenge. There are two common practices used for extraction of bioplastic from bacteria[84]. The first method is conventional method which utilizes the insolubility of PHA in methanol and solubility in chloroform[78]. After harvesting, lipids and other lipophilic components from the bacterial cells are removed by refluxing in hot methanol followed by solubilisation of PHA in warm chloroform. PHA from chloroform solvent is recovered by precipitation by adding methanol or by solvent evaporation. Although this method provides PHA of high purity yet a huge quantity of hazardous solvent is needed to repeat the same process. Thus this method is not eco-friendly and inappropriate for mass production of bioplastic[85]. The second method is intended to avoid the use of organic solvents where the bacterial cells are treated with a blend of enzymes (including lysozymes, proteases and nucleases) and detergents to remove cell walls, proteins and nucleic acids leaving behind PHA intact[85].

Heinrich and his co-worker showed a simple method which can be used at industrial scale for high purity PHA extraction and recovery from cells of *Ralstonia eutropha* H16 using sodium hypochlorite[86]. They developed a method for large scale isolation of poly-(3HB) from dried cell mass, which (i) recovers product of high quality (ii) is simple and cost effective as it mediates efficient separation of the polymer from the lysed cells without the use of any additional chemicals or a large scale centrifuge (iii) limits the total volume of extraction and (iv) presents technical solutions to challenges that appear with the upscale of extraction process with sodium hypochlorite.

The other methodology proposed by Loo and Sudesh are- (1). Synthesis of medium chain length of Poly- (R)-3- hydroxyalkanote from *Ralstonia eutropha* as reference material and Palmitic acid as test sample, (2). Formation of biofilm and testing of samples on three point tests for the quality of Poly-(R)-3- hydroxyalkanote and (3). Chemical, enzymatic and biological degradation of medium chain length of Poly-(R)-3-hydroxyalkanote. Thus from these proposed work the expected outcome is the successful production of biodegradable plastics by using palm oil as raw material. By using this approach the cost of bioplastics can be reduced and also it saves the life of the domestic animals and protects the environment from the problem of plastic hazards, landfill, and plastic litter[17].

7.2 Extraction from Non-PHA Cell Mass (NPCM)

Also the extraction and purification of bioplastic from non-PHA cell mass (NPCM) presents poses yet another challenge as both NPCM and PHA granules are in solid phase. Jacquel and his co-workers have extensively explained two strategies for the isolation and purification of bacterial PHA. The two strategies implemented in the downstream processing for PHA recovery are PHA solubilization and NPCM dissolution[89]. In PHA solubilization method, the PHAs are suspended in appropriate organic solvents and treated for further processing while in NPCM dissolution method, NPCM is removed by chemical agents leaving behind PHA granules in the solid state. The resultant liquid and solid phases are then separated by centrifugation and filtration[90] etc.

7.3 Extraction by non-solvent

One can also proceed with one approach which utilizes a system with a marginal non-solvent so that the use of harsh solvents is reduced. The marginal non-solvent itself is incapable of dissolving a substance, but when it is the solute of another solvent then it becomes capable of dissolving the substance. If PHA is dissolved in a chemical solvent such as acetone, accompanying oils (seed extraction) would become marginal non-solvent as PHA will not be dissolved in the oil directly. But if the original solvent is removed then the PHA as a solute already dissolved in another solution will remain just suspended in the oil[89]. However, after the removal of the chemical solvent if a marginal non-solvent is present then it will prevent the PHA from forming into an undesirable gel; the PHA can then be precipitated from the marginal

non-solvent. Thus this system provides the dual benefits- (i) it removes hazardous solvents at an earlier step and (ii) forms higher-quality PHA crystals[89].

7.4 Extraction by enzymatic treatment

Another method that can be applied for the purifying PHA is the use of enzymes like lysozymes, nucleases and proteases. Uncontaminated PHA can also be obtained from *Pseudomonas putida* using EDTA, proteases, SDS detergent and trace amount of lysozymes[90]. Some other method employs the degradation of the surrounding materials thus eliminating biomass that could be used in other value-adding processes. These degradation products through gasification could be transformed into a source of revenue. The synthetic gas (hydrogen and carbon monoxide) can be obtained through gasification reactions and high temperatures treatment of the left over carbohydrates and degraded carbon compounds that can be burnt as an energy source, thus making enzymatic degradation more economically feasible[90].

8. Applications of bioplastics

a) It has been found that under unfavorable conditions many microorganisms accumulate Polyhydroxybutyrate (PHB) intracellullarly; which is a biodegradable thermoplastic polyester. But in PHB production, about 40% of the total production cost is utilized for raw material. Thus a cheaper carbon source is required to replace this high PHB production cost. So an attempt to produce PHB using bacteria was made to ferment sap from oil palm biomass. This research was divided into three different steps- 1) extraction of sugars from oil palm fronds (OPF), 2) Bioplastic fermentation in which PHB is produced from fermentable sugar by bacteria such as *Comamonas putranensis*, *Bacillus* sp. and *Pseudomonas* sp., 3.extraction,purification and characterization of PHB from cell[91].

b) PHA as a low end–material is used in packaging, paper coatings and containers and high end-value products such as artificial cardiac valve and suture.

c) Medium Chain length polyhydroxyalkalonates (MCL-PHAs) are blended with different rubbers to give different polymers having 90 °C melting point.

d) Palm oil is used for PHAs production and rhamnolipids simultaneously[92] using *Pseudomonas aeruginosa* IFO3924.

e) These polyesters are used for medical applications such as fracture fixation, implants, drug-delivery vehicles, treatment of narcolepsy and alcohol addiction, cell microencapsulation, or as precursors of molecules with analgesic, anti-rheumatic, chemopreventive, radiopotentiator, antihelmintic or anti-tumuoral properties.

f) For power generation by incorporation of PHA production with methane production from palm oil mill effluent treatment.

g) The potential application of PHA within medical and pharmaceutical industry is due to their biodegradability and biocompatibility nature. PHA is used as packaging film in containers, paper coatings and bags. For chemical synthesis of optically active compounds they are used as stereo-regular compounds that act as chiral precursor[15]. PHA is used in cosmetics; the hydrophobic property enables PHA to be used in dye removal through adsorption in textiles waste water.

9. Conclusions

PHAs are the plastic of the future, bioplastics are more sustainable as they degrade rapidly and synthesized from a renewable resource. The system needs further development by which these plastics are created before they become economically competitive. In particular, plant plastids are needed which can tolerate greater diversions of their resources and cheaper extraction methods that deliver pure and undamaged products. Many important achievements have already been made. Biodegradable plastics will solve the problem of plastics litter. The biodegradable plastics must be composted and completely degraded in landfills. The successful production of biodegradable plastics will lessen the burden placed on the environment by consumers. Bioplastics will protect our environment and save the life of the domestic animals. Quality analysis of produced bioplastics will determine whether it can be substituted for the existing non-biodegradable plastic. Bioplastic has double return for the energy used in their manufacture, first as a fuel source and second as a useful item. This means that at the end of their life we should burn them to pick up energy which could then be used to produce new materials. *R. eutropha* is a handy organism and have been extensively studied among the bacteria for the production of PHA. It can utilise various carbon sources for growth and PHA production. *R. eutropha* is capable of producing P (3HB) from various carbon sources like animal and plant oils, sugars, alcohols, organic acids like acetic acid, hydroxybutyric acid, lactic acid and even carbon numbered n-alkanoates; it also utilizes carbon dioxide under autotrophic conditions for producing P (3HB). The above proposed schemes are the perfect quest of the plastic revolution to keep the environment safe from the plastic hazards. This review can help us to find new ways to face the challenges from plastic threatening the world environment.

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