

**POLY-HYDROXYALKANOATES (PHA): BIOLOGICAL COMPETITION TO PLASTIC WORLD****Tanuja Murab*¹, Ragini Gothalwal¹, Nidhi Tripathi²**¹Department of Biotechnology and Bioinformatics center, Barkatullah University, Bhopal, Madhya Pradesh, India²Department of Biotechnology and Biochemistry, Career College, Bhopal, Madhya Pradesh, India*Corresponding author: tanujamurab16@gmail.com**ABSTRACT**

Plastics are the most extensively used synthetic polymer due to its multi-specialized application due to its versatile nature and properties which make them useful in various fields ranging from construction of massive monuments, machinery parts, packaging products, kitchen appliances, day to day tools to children toys and, it has a high demand but being a mineral based polymer, it is difficult to balance supply to demand ratio. Moreover, its xenobiotic properties make it difficult to degrade plastics products thereby causing deleterious effect to environment and humans in the long run. There is constant need to find an alternative to this polymer which has similar properties to that of plastics but are biodegradable. One such promising class of bio-based polymer is Polyhydroxyalkanoate also known as PHA, green-plastic or biodegradable plastic polymer which fulfils the requirement and is not recalcitrant in nature and the use of polyhydroxyalkanoate is thus a step closer to green and clean earth. This review is an attempt to understand Polyhydroxyalkanoates or PHAs by summarising these aspects in order to understand the great potential thereby studying and understanding the complexities involved in PHA production starting from varied properties to its metabolism and their application.

Keywords: Biodegradable, Polyhydroxyalkanoate, Recalcitrant, Xenobiotic.**1. INTRODUCTION**

The intervention of humans and their man-made products in the nature has drastically deteriorated the quality of life and environment. The most prominent among these materials are manufacturing of plastic based products. Urbanisation and industrialization are the two major contributors of plastic pollution, especially in big cities. Plastic materials are inevitable part for humans in this modern era, due to its versatile nature it can be moulded into complex products ranging from basic household utensils and gadgets to heavy duty materials for constructional purpose, from transportation vehicles to packaging products, from healthcare products to communication equipments. Most plastics, which are currently used, generally derived from mineral oil and are fossil fuel (non-renewable resource) and thus cause environment implications due to its recalcitrant properties towards microbial decay [1-3]. This property make its use more prominent due to its longevity and durability which is highly demanded in manufacturing industries and thereby replacing metal and glass products but in the long run when it comes to degradation of plastic based waste products the same

property makes it highly undesirable as it sustains in the environment for hundreds of years and causes serious problems to environment as well as to living beings [4]. Synthetic mineral based plastics currently exploited and used in masses are mostly polymers that can be moulded and designed by tweaking the original groups or side chains through addition of new groups or by branching and rendering them to become a non-biodegradable material. The high demand of these synthetic polymers have generated a high production of waste that have been accumulated on land and water causing pollution thus harming the soil quality as well as the portability of drinking water. It has also become a threat to marine population by deploying their toxicity in the long run [2, 5]. The irresponsible methods of wastes disposal of these polymers have caused a serious problem to the ecosystem. The methods such as land filling, recycling, incineration etc. are inconsistent and not at par with the current amount of plastic waste generated and hence the need of the hour is to find out some alternatives the reason being either these are highly expensive as in case of recycling or completely a waste of natural resources as it is in case of land filling that destroys the soil further

by releasing toxins of these synthetic polymers into the soil and also percolating into the water-table. These polymers also clog the pipelines during outburst of rain and thus causing sanitation problems too. Thus an alternative to these synthetic polymers are required that aren't recalcitrant but show similar qualities desirable for various industrial as well as for commercial applications and also aids in conserving the fossil fuel for the future.

The polymers have been classified on the basis of their source and renewability into four categories (Fig. 1) out of which the natural and biodegradable polymers are the main focus of scientific studies due to being natural as well as biodegradable. Depending upon the sources polymers can also be classified into four groups further listed below:

1. Derived from living organism and are denoted as biopolymers these include natural fibres such as cotton, wool, silk, cellulose, starch, rubber, lignin, PHAs etc. [6]
2. Artificially using chemical method of polymerization using monomers that are present in nature or derived from nature such as Polytrimethyl Glycol, Poly lactic acid or PLA etc.
3. Mineral oil based/Fossil fuel derived/Petrochemically derived that includes, Polyethylene, Polypropylene terephthalate, PET etc.
4. Combination of both mineral based and renewable living organism based polymers or their derivatives such as Starch-Polyvinyl Alcohol (SPA).

The main focus of study is on PHAs polyhydroxyalkanoates. These major class of polymer that are natural polymers derived from living organisms and are easily extracted using various biotechnological tools. These polymers show promising results when it comes to the variability, quality and moulding ability of these

polymers which are present in conventional mineral based polymers except these are biodegradable and also prevents the exploitation of fossil fuel for their generation and hence are also eco-friendly thus getting them categorised into family of Green Plastics along with PBS and PLA [2, 7].

2. PROPERTIES OF POLYHYDROXYALKANOATES

2.1. Physical Properties of Polyhydroxyalkanoates

PHAs are water insoluble as they are moderately hydrophobic, thermoplastic that is nontoxic in nature, soluble in organic solvents. Their properties vary due to their length and a monomeric group present within these polymers and also depends upon them being a homopolymer or a heteropolymer or the branched structure present and Table 1. depicts the physical properties of PHA. Thus they can be plastic or elastic having variable recrystallization time period. Most (SCL-Short chain length) are brittle and are not ideal for preparing of plastics while the (MCL-Medium chain length) (PHAs) are ideal for creating plastic materials and the qualities can be varied depending upon the carbon source given to the potential microbial colonies while the (LCL-Large chain length) PHAs are elastic polymers and can be a better suited for making rubber like materials [5, 8, 9]. The variability in properties of PHAs are dependent upon the carbon sources provided to the microorganism, extraction method and duration of cultivation. [60] The crystallization property depends on the Glass transition temperature of PHA that is reported to be around 2°C. Melting point of PHA ranges between 150 to 180°C above which it degrades into crotonic acid and other oligomers and hence is non-volatile in nature. The tensile strength of PHA has been reported to be between 10 to 50 MPa. [10].

Table 1: Physical Properties of Polyhydroxyalkanoates (PHAs) [10, 13, 14]

SN	Properties of PHA	
1.	Glass transition temperature (T_g)	2°C
2.	Crystalline degree (X_{cr})	40-60%
3.	Elongation of break (ϵ)	1-15%
4.	Melting temperature (T_m)	160-175°C
5.	Young Modulus (E)	1-2 GPa
6.	Tensile strength (σ)	15-40 MPa
7.	Water Vapour Transition Rate (WVTR)	2.36 g.mm/m ² .day
8.	Oxygen Transition Rate (OTR)	55.2cc.mm/m ² .day
9.	Degradation temperature	180°C
10.	Resistance to UV light	Good

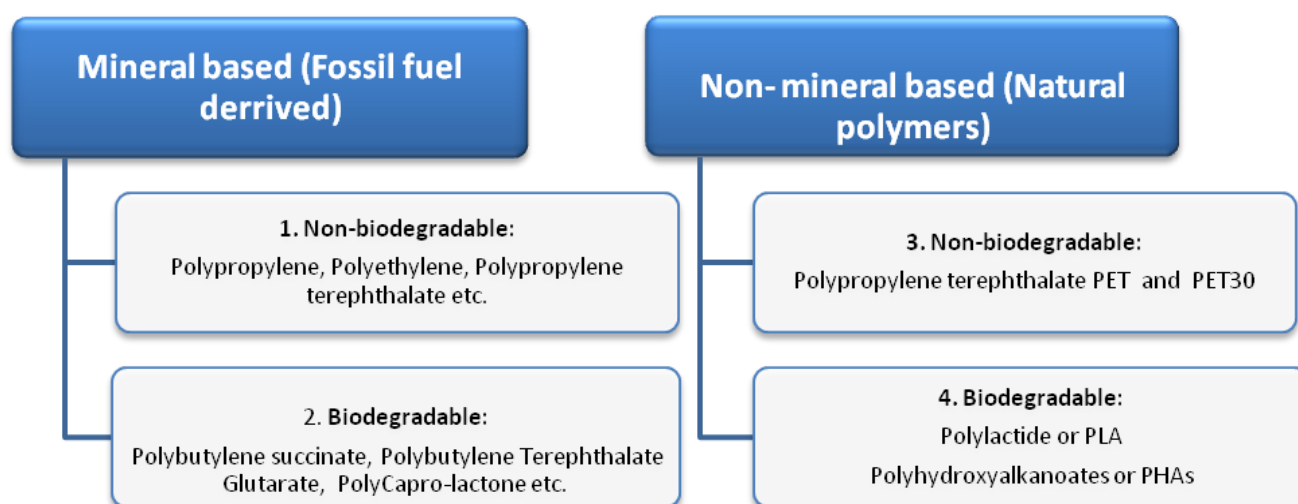


Fig. 1: Classification of polymers based on their source and renewable properties

The most studied PHA molecule is PHB or polyhydroxybuterate which comes in the class of short chain length molecules which are crystalline, having brittle quality and are thermoplastic in nature these are found as a homopolymer and sometimes as a copolymer with other PHA monomers such as Poly-HV (Hydroxy valerates), Poly-HH (Hydroxy hexanoate) and form biopolymers and even tetrapolymers displaying variable properties and exploited for the same. The variability is achieved by application of various substrates ranging from small chain length utilizing sugars simple lipids and alcohols utilizing acetyl-Co-A pathway yielding PHB and its tetramers while complex and long chain lipids and waxes. Various microbes are employed ranging for algae which yield the maximum amount of PHA, to bacteria that are both Gram positives and Gram negatives such as Bacillus, Pseudomonas, Areomonas sps. etc. and also extremophiles have been reported to form PHAs [11,12]. Further modified PHAs are also produced referred as quadripolymers synthesized having 4 different types of monomeric units and are produced using genetic engineering has been made possible which show further more variable qualities needed in polymer industries and are in demand.

2.2. Bio-chemical Properties of Polyhydroxyalkanoates

PHAs are polyoxoesters of hydroxyalkanoates found within the living system. These are high molecular weight polymers. It has been reported that the chemical properties and structural integrity which leads to formation of crystals are dependent upon the

monomeric unit of PHAs [15]. PHAs are optically active and exhibit piezoelectric effect. PHAs can further be classified biochemically into various types depending on various parameters and qualities into different types and has been summarized in Table 2. PHAs are lipid granules (liquid polymer) which are mobile or in amorphous forms stored as reserve or inclusion bodies surrounded by monolayer of enzymes namely polymerases and depolymerises present within cells of living organisms naturally under normal condition in some while during stress conditions in other organisms as depicted in Fig.2. Polyhydroxyalkanoates consist of hydrophobic coiled chain and water and this form a plasticizer. The core is surrounded by hydrophilic enzymes along with certain structural proteins as stipulated by scientists and termed as Carbonosomes. Polyhydroxyalkanoates are helical molecules containing helical PHAs stabilized by Hydrogen bonds formed between carbonyl groups between the individual monomeric units of 2-5 or 6 or more carbon containing monomers. The main function of PHAs within an organism is storage of energy reserves at time of emergency i.e. starvation but further studies have revealed that PHAs also play an important role in spore formation in bacteria, Nitrogen fixation etc. have also been studied as a defensive barricade when exposed to hazardous environment [11].

These are biodegradable, biocompatible and are either thermoplastic or elastic depending upon the organism and carbon source available to them and almost hundred and fifty types have been identified and more are being discovered or genetically modified or

bioengineered through GMOs using recombinant DNA technology [16-19]. PHAs are polymerization product formed by esterification of hydroxyalkanoic acids as a condensation reaction of these carboxyl groups in presence of intracellular enzymes present within the organism. PHAs are the only biopolymers that are produced and degraded by living cells. PHAs have been isolated till date from various organisms ranging from prokaryotic and eukaryotic organisms but the mass

production is most feasible with the microbes ranging from algae, fungi, archae bacteria and bacteria ranging from Gram positive to Gram negatives.[20-23] The ground breaking work of utilizing microorganisms for production of PHAs was done by microbiologist M. Lemoigne in 1926 and has paved a way for applying his knowledge to identify and study many of microbial groups in mass production in the future thereof.

Table 2: Classification of Polyhydroxyalkanoate based on various Biochemical Parameters.

Classification criteria	Type	Description	References
<i>Monomer size</i>	Short chain length (SCL-PHA)	Contains C3-C5 carbon atom	[24]
	Medium Chain Length (MCL-PHA)	Contains C6-C14 carbon atom	[5], [3]
	Long chain length (LCL-PHA)	Contains C15-C18 carbon atom	
<i>Biosynthetic origin</i>	Natural bioplastics	Produced by microbes utilizing general metabolites	[4]
	Semi synthetic bioplastics	Cannot be synthesized by the microbe and requires the addition to	[25]
	Synthetic bioplastics	Obtained by chemical synthesis (e.g. synthetic thermoplastic)	
<i>Number of monomers in the polyesters</i>	Homopolymeric bioplastic	Contains single monomer unit	[3]
	Hetropolymeric bioplastic	Contains more than one type of monomer unit	[14]
<i>Type of polyesters accumulated by microbe</i>	Unique	Single bio-plastic	[3]
	More than one	Mixed bio-plastic	
<i>Chemical nature of the monomers</i>	Bioplastic containing aliphatic fatty Acid derivatives	Saturated or unsaturated monomers; linear or branched monomers; substituted or nonsubstituted	
	Bioplastics containing aromatic fatty acid derivatives	-	[3]
	Bioplastics containing both aliphatic and aromatic fatty acid derivatives	-	[26]
	Bioplastics containing other different compounds	e.g. poly- γ -glutamic acid, poly- ϵ -L-lysine, poly- β -L-malic acid, polyglycolic acid, cyanophycin	

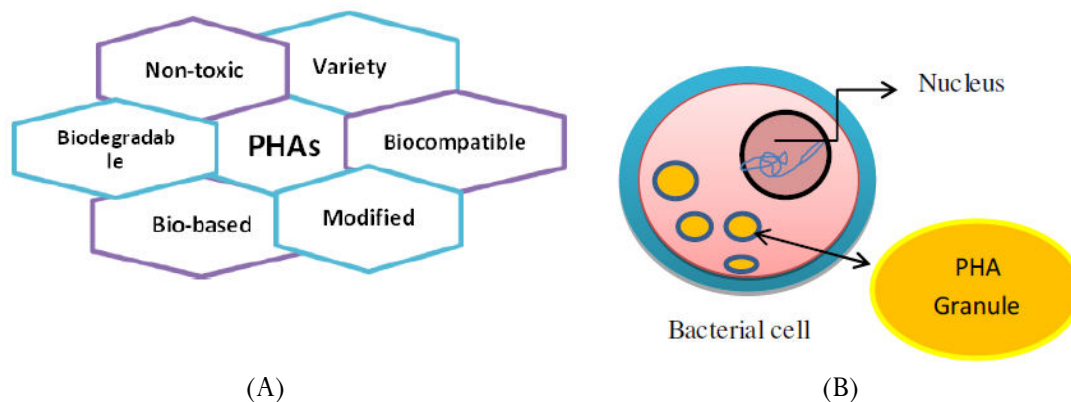


Fig. 2: A) Schematic diagram of properties that makes polyhydroxyalkanoates a good replacement to mineral based plastics. B) Diagram representing of Bacterial Cell and PHA granules.

3. BIOSYNTHESIS OF POLYHYDROXYALKANOATES

Polyhydroxyalkanoates are synthesized as a storage reserve by both eukaryotes as well as prokaryotes ranging from plants and animals as well as bacteria, archaeobacteria and blue green algae. In plants the percentage of PHA production is low and higher percentage of PHA to be synthesized occurs at the cost of some kind of abnormalities in plants has been reported [12] The accumulation of PHAs in bacterial cells is as high as 90% of total dry weight of cell and thus more emphasis is on the bacterial biomass for mass production. In bacteria various pathways are present to utilize different kinds of carbon sources some of these are listed in Table 3. There are 8 pathways out of these 3 pathways namely Pathway I, Pathway II and Pathway III have been studied in detail.

In Pathway I it was reported that sugar or carbohydrates was utilized by bacteria as substrate such as glucose, sucrose, starch, molasses, etc. due to presence of enzymes namely Beta-ketolases, Acetoacetyl Co-A reductase, Polyhydroxyalkanoate synthetases and the enzymes listed in various pathways have been listed in Table 4. Pathway II employs fatty acid as substrate which upon degradation joins pathway IV which utilizes

Butenoyl Co-A or Crotonoyl Co-A as substrate and gets further degraded to Acetoacetyl Co-A which is a path followed to form by pathway I to yield PHA. Fatty acids may also undergo breakdown through Beta oxidation pathway and yield PHA. The genes and enzymes responsible have been described by [13,27,28]. Pathway V utilizes Enoylacyl carrier protein which aids in synthesis of type II fatty acid synthesis to yield PHA. Pathway VI is following alkanolactone degradation such as 2-Deceno-t 5-lactone has been studied by Hiroshi Nago in 1993 and such compounds can also yield monomers of PHA which can be employed by microbial system to yield PHA. Pathway VII and VIII was reported the utilization of alcohols in yielding polyhydroxyalkanoate in detail along with the enzymes involved in Table 4. [13]. Polyhydroxyalkanoates can be produced by utilization of a variety of renewable resources that are either waste products left after food processing or are non-food sources. Since waste products such as whey, glycerol, etc. can also be employed it aids the industrial and municipal corporations by aiding in waste management as well as it helps in economical aspect also as the raw material used is cheap and easily available thus being renewable as well as biodegradable[13,28].

Table 3. List of Bacteria that produces Polyhydroxyalkanoates

S.N.	Bacteria	Carbon Source	PHA Produced	References
1	<i>Escherichia coli</i> (Mutant)	Glucose Glycerol Sucrose Ethanol Palm oil Molases	(UHMW)PHB	[12]
2	<i>Pseudomonas oleovorans</i>	Octanoic Acid	mcl-PHAs	[12]
3	<i>Pseudomonas stutzeri</i>	Glucose Soyabean oil Alkanoates Alcohols	mcl-PHAs	[12]
4	<i>Pseudomonas fluorescens</i> A2a5 B	Sugarcane liquor	PHB	[29]
5	<i>Pseudomonas putida</i> KT2440	Glucose Glycerol Citrate	PHA	[30]
6	<i>Pseudomonas sp.</i>	Corn oil	PHA	[31]
7	<i>Pseudomonas putida</i> . KT2442	Glucose	P(HB-co-HHx)	[32]
8	<i>Pseudomonas mediterranea</i>	Glycerol Crude	mcl-PHA	[33]
9	<i>Pseudomonas corrugate</i>	Glycerol	mcl-PHA	[33]
10	<i>Pseudomonas sp.</i> G101	Saponified waste palm oil	mcl-PHA	[34]
11	<i>Pseudomonas aeruginosa</i> BP C1	Glucose	3-HV-co-5-HD	[35]
12	<i>Escherichia coli</i> (Recombinant)	Glucose Xylose	3-HB or 4-HB or 2-HV	[36]

13	<i>Escherichia coli</i> (Recombinant)	Glucose	P(3HB-co-2HB-co-LA)	[37]
14	<i>Ralstonia eutropha</i> (Recombinant)	Phenyl propionate Phenyl butyrate	P(3HB-co-3H3PhP)	[38]
15	<i>Cupravidus nector</i> (Recombinant)	Sucrose	PHBHHx	[39]
16	<i>Cupravidus taiwanensis</i>	Fructose Gluconate Glycerol Maltose	PHB	[11]
17	<i>H mediterranei</i> DSM1411	Hydrolyzed whey	3HB-co-3HV- co-4HB P(3HB-co-3HV-co-4HB)	[40]
18	<i>Pseudomonas aeruginosa</i> ATCC27853	Heptadecanoic Nonadecanoic Heneicosanoic	3-hydroxyvalerate, 3-hydroxyheptadecanoate	[41]
19	<i>Pseudomonas mosselii</i> TO7	Crude glycerol	mcl-PHA	[42]
20	<i>Cupravidus nector</i> DSM4058	Crude Glycerol	3HB	[42]
21	<i>Alcaligenes latus</i>	Sugar beet molasses	PHB	[40]
22	<i>Burkholderia cepacia</i>	Soybean	PHA	[43]
23	<i>Cupravidus nector</i>	Soybean	PHA	[43]

Table 4: Summary of Genes and Enzymes responsible for Polyhydroxyalcanoate synthesis required in the 8 pathways [13, 28]

SN	PHA Pathway	Enzymes Involved	Genes
1	Pathway 1	Enzyme: Beta-ketolase Enzyme: Aceto-acetyl Co-A Reductase Enzyme: PHA Synthase Enzyme: PHA depolymerase.	Gene: Pha A Gene: Pha B Gene: Pha C Gene: Pha Z
2.	Pathway 2	Enzyme: 3-Keto acetyl Co-A reductase, Epimerase Enzyme: R-Enoyl-CoA hydratase Enzyme: Acetyl CoA oxidase, Acetyl Co-A hydratase	Gene: Fab G Gene: Pha J
3	Pathway 3	Enzyme: 3-Hydroxyacyl-ACP-Co-A transferase, Malonyl CoA-ACP transacylase Enzyme: NADP dependent acetoacetyl Co-A reductase	Gene: PhaG Gene: Fab D
4	Pathway 4	Enzyme: Succinic semialdehyde dehydrogenase	Gene: Suc D
5	Pathway 5	Enzyme: 4-Hydroxybutyrate dehydrogenase Enzyme: 4-Hydroxybuterate Co-A:Co-A transferase, Lactonase	Gene: 4HbD Gene: Orf Z
6	Pathway 6	Enzyme: Hydroxyacyl Co-A Synthase Alcohol dehydrogenase	Unknown
7	Pathway 7	Enzyme: Cyclohexanone dehydrogenase Enzyme: Cyclohexanone monooxygenase Enzyme: Caprolactone hydrolase Enzyme: 6-Hydroxyhexanoate dehydrogenase Enzyme: 6-Oxohexanoate dehydrogenase Enzyme: Semialdehyde dehydrogenase, 6-Hydroxyhexanoate dehydrogenase, Hydroxyacyl-CoA synthase	Gene: Chn A Gene: Chn B Gene: Chn C Gene: Chn D Gene: Chn E

4. DEGRADATION OF POLYHYDROXYALKANOATES

Degradation of the Polyhydroxyalkanoates or PHAs have also been studied in order to mould polymers which show desirable qualities based on their

functionalities and hence their various agent responsible have been studied. Degradation is the break in the long polymer chain due to variety of factors that can accelerate or retard the degradation of the biopolymers including polyhydroxyalkanoate/PHA. The rate of

degradation is influenced by environmental factors such as exposure to air, water, light and heat as well as exposure to chemicals and microbes as well. All such factors affect the durability and longevity of PHA as well. The symptomatic changes observed during degradation is the change in chemical structure, its crystallization properties, molecular weight, morphology (such as discoloration, cracking, erosion) etc. [28] Fig.3. Showcases the various modes of degradation of PHAs namely physical, chemical and biological. Based on these properties PHAs can be modified in accordance with the requirement in varies fields and thus employed in various applications.

5. APPLICATION OF POLYHYDROXYALKANOATES

Due to the versatile and unique nature of polyhydroxyalkanoates experiments are being done constantly to employ it in various fields ranging from medical, pharmaceuticals, bio-fuel, cosmetics etc. enlisted in Table 5. The nature of monomeric units and linear structure of PHAs is responsible for its thermoplastic nature along with the varied properties like optic activity, ant-oxidative nature and most importantly its biocompatibility and biodegradability making them highly useful polymer in the long run.

Table 5: Potential application of PHA in various fields.

PHA based Industries	Application and Examples	References
Packaging industry	PHBV used in Europe for packaging food materials Manufactured by P&G, Biomers, Metabolix, Polyone.	[47]
Medical Industries	<i>Drug Delivery System</i> Nanocapsules/Microsphere/Nanoparticles treatment of diseases and disorders.	[48]
	PHB & PHBV drug delivery system.	[49]
	ii) <i>Steroid Delivery System</i>	[50]
	iii) <i>Vaccine Delivery System</i> PHB integrated vaccine delivery of staphylococcus enterotoxin to Gut Associated Lymphoid Tissue (GALT)	[51]
	iv) <i>Cancer Treatment</i> : Rhodamine B isothiocyanate (RBITC) target delivery.	[52]
	v) <i>Brain disorder treatment and Memory enhancer</i> : PHB substrate for regeneration of mitochondria preventing neural damage or cell death. Parkinson's and Alzheimer's cure in future Neural cell regeneration	[53] [54]
	vi) <i>Prevents osteoporosis</i> : Preventing decrease in bone	[48]
	vii) <i>Medical Devices</i> : grafts like cartilage grafts, repair patches, cardiovascular patches and sutures, stents and pins and are Non-toxic.	[49]
viii) <i>Surgical film Generation</i> P3HBV) films are non-cytotoxic and bio-safe for <i>in vitro</i> 3T3 fibroblast cells and <i>invivo</i> in mice	[51] [7,52]	
Tissue culture	Human mesenchymal stem cells attached better on PHA heteropolymer P(3HB-4HB-3HHx) film	[7, 53-55]
Bio-Surfactants	PhaR show bactericidal activity	[56]
Paper Industry	PHB stable latex and is used in paper industries	[57]
As Biofuel	(3HBME) yield heat of combustion of 30KJ/g and 20KJ/g respectively and can be employed as biofuel.	[40, 58] [59]
Cosmetic industry	PHAs rapidly absorb and retain oil, used in cosmetic oil-blotting films. Metabolix company manufactures PHhBV copolymer for shampoo bottles.	[60] [61-62]
Drug Abuse alcoholism	Gamma-hydroxybuterate pharmacologically active for treating alcoholism.	[63]
Nanoparticle for Protein purification	PHA granule or nanoparticles associated protein phasin used for protein purification employing recombinant technology.	[64]
Healthy food additives	<i>Feed Additives</i> : PHA a biocontrol agent degraded through gastrointestinal tract of animals is used to prevent Salmonella infections in pigs and poultry birds, fishes and crustaceans.	[13]
Textile Industries	Polyhydroxy butyrate copolymer (PHBV) "Biopol" Zeneca developed thermoplastic, biodegradable surgical fibre.	[50] [65]
Pharmaceutical Industries	(3HB) used in synthesis of antibiotics such as carbapenem, macrolode, elaiophylidene, etc.	[13]

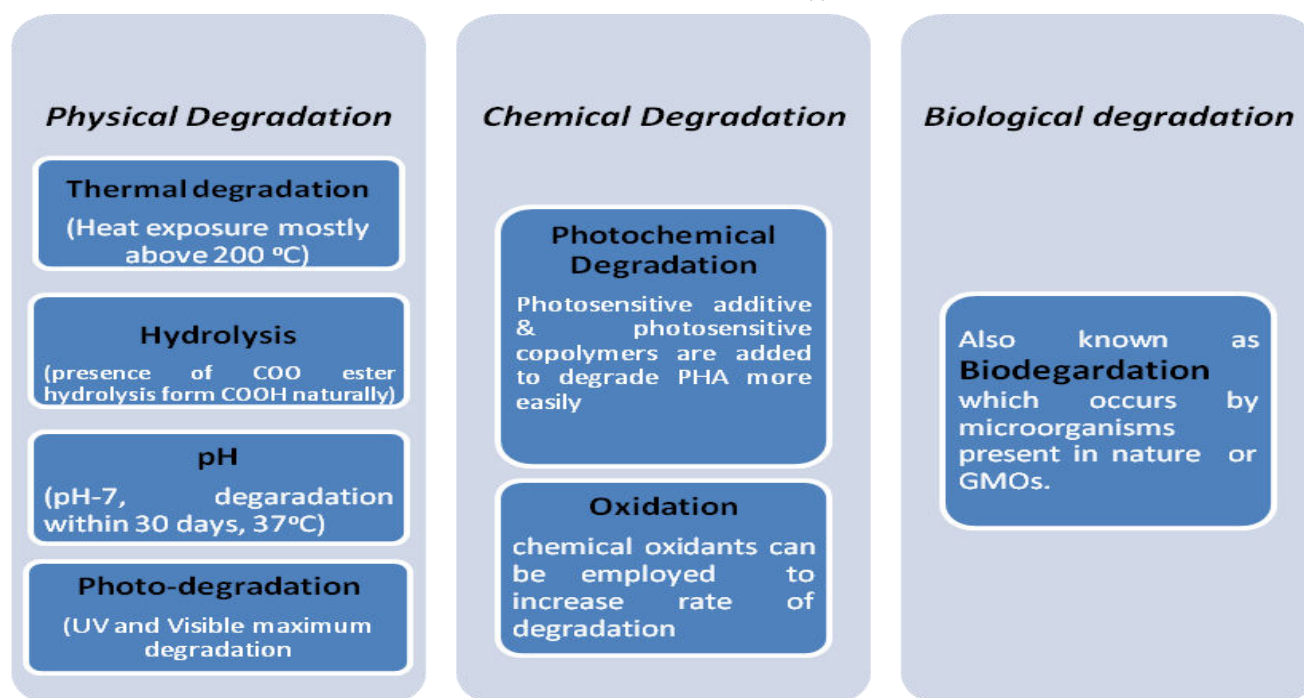


Fig. 3: Various agents responsible for PHA degradation [14.44-46]

6. CONCLUSION

The study and development of PHA will aid in the replacement of mineral oil based plastic material and also help in the issue of shortage of the same. It will aid in sustainable development of the eco-friendly biodegradable polymers which in long run is a step towards preservation and conservation of the environment. Due to the diverse use of PHA in various fields such as fuel industry, medical industry, packaging industry, and textile industries etc. have already been explored but the major downfall of PHA is the choice of PHA producing diversity selected as we know that different diversities yield different PHAs and to find the ideal microbial population as per the desirable polymer or plastic required is the primary reason for limited success for PHA use and mostly the most common PHA produced are PHB a SCL-PHA polymer that is brittle and lacks the quality desired in a plastic. The solution to this problem is to optimize the media and to select the ideal diversity and further to improve the diversity to engineer such an organism which show a diverse production of PHA as per our requirement but this process is time consuming and bit costly. And other problem faced in the process of mass production of PHA and its successful replacement to the conventional plastic is that the cost of production is three times the cost of production of conventional mineral plastics. The study of the properties, metabolic pathway and synthesis

mechanism will help us to identify the cost-effective methods of mass production of PHAs by bacterial population in the future. As its multifaceted application should not be denied due to its versatility, types and unique properties it has become the most desirable biopolymer with respect to its counterparts. In this review we want to showcase the properties and mechanism by getting an insight on the metabolic mechanism and the broad array of applications in detail.

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Conflict of interest

The authors do not have any conflict or interest.

8. REFERENCES

1. Jassim HM, Mahmood OT, Ahmed SA. *IntJ Eng. Trends and Tech.*, 2014; **7(1)**:18-25
2. Tan GYA, Chen CL, Li L, Ge L, Wang L, Razaad IMN, Li Y, Zhao L, Mo Y, Wang JY. *Polymers*, 2014; **6(3)**:706-754.
3. Suriyamongkol P, Weselake R, Narine S, Moloney M, Shah S. *Biotec. Advances*, 2007; **25**:148-175.

4. Kourmentza C, Plácido J, Venetsaneas N, Burniol-Figols A, Varrone C, Gavala HN, Reis MAM. *Bioeng.*, 2017; **4(55)**:1-43.
5. Zhang X, Luo R, Wang Z, Deng Y, Chen GQ. *Biomacromol.*, 2009; **10(4)**:707-711.
6. Sangkharak K, Prasertsan P. *J gen and applmicrobio.*, 2012; **58**:173-182.
7. Ji GZ, Wei X, Chen GQ. *J Biomaterials Sci. Polymer.*, 2009; **20(3)**:325-339.
8. Bhuwal AK, Singh G, Aggarwal NK, Goyal V, Yadav A, *Int. J. of biomat.*, 2013:752-821
9. Bugnicourt E, Cinelli P, Lazzeri A, Alvarez V. *Express Polym. Let.*, 2014; **8(11)**:791-808.
10. Tang X, Chen EYX. *Nature Communications*. 2018; **9(2345)**:1-11
11. Koller M. 2018; *Ferment.4*: 1-30.
12. Verlinden RAJ, Hill DJ, Kenward MA, Williams CD. *J. of Appl. Microbio.*, 2007; **102**:1437-1449.
13. Chen GQ, Wu Q, Zhao K, Yu PH. *Chinese J. Polymer Sci.*, 2000; **18(5)**:389-396.
14. José dos Santos A, Valentina LVOD., Schulz AAH, Duarte MAT. *Ingeniería y Ciencia.*, 2018; **14(27)**:207-228.
15. Volova TG, Zhila NO, Shishatskaya EI, Mironov PV, Vasil'ev AD, Sukovatya AG, Sinskey AJ. *Polymer Science Ser. A.*, 2013; **55(7)**:427-437.
16. Garay LA, Boundy-Mills KL, German JB. *J. Agric. and Food Chem.*, 2014; **62**:2709-2727.
17. Jendrossek D. *J. of Bacteriol.*, 2009; **191(10)**:3195-3202.
18. Kunasundari B, Sudesh K. *Exp. Polym. Lett.*, 2011; **5(7)**:620-634.
19. Sharma PK, Munir R, Blunt W, Dartailh C, Cheng J, Charles TC, Levin DB. *Appl. Sci.* 2017; **7(242)**:1-20.
20. Burdon KL. *J. Bacteriol.*, 1946; **52(6)**:665-678.
21. Troschl C, Meixner K, Drosch B, Koller M. *Bioengineering (Basel).*, 2017; **4(2)**:26.
22. Wagle AR, Dixit YM, Vakil BV. *Int. J. Curr. Microbiol. App. Sci.*, 2016; **5(4)**:413-423.
23. Xuerong H, Yasuharu S, Yumi K, Teruyuki S, Shinji F, Takanori S, Takanori K, Kenji T. *J Biosci & Bioeng.*, 2014; **118(5)**:514-519.
24. Escapa IF, Morales V, Martino VP, Pollet E, Averous L, García JL, Prieto MA. *App. Microbio. and Biotec.*, 2011; **89**:1583-1598.
25. Rajeswari S, Prasanthi T, Sudha N, Swain RP, Panda S, Goka V. *World J. pharm. and pharmaceut. Sci.*, 2017; **6(8)**:472-494.
26. Ishii-Hyakutake M and Mizuno S. Tsuge T. *Polym.*, 2018; **10**:111-1124.
27. Diba H, Sifi-Kashani R, Tavakkoli S, Malek-Moh S. *Cur. World Env.*, 2015; **10(Special Issue(1))**:644-649.
28. Chen GQ, Wu Q, Zhao K, Yu PH. *Chinese J Polymer Sci.*, 2000; **18(5)**:389-396
29. Jiang Y, Song X, Gong L, *Enz. Microb. Technol.*, 2008; **42(2)**:167-172.
30. Wang Q, Nomura TC. *J. Biosci. Bioeng.*, 2010; **6**:653-659.
31. Chaudhry WN, Jamil N, Ali I, Ayaz MH, Hasnain S, *Ann. Microbiol.*, 2011; **61**:623-629.
32. Tripathi L, Wu LP, Chen J, Chen GQ. *Microb. Cell Fact.*, 2012; **11(1)**:44.
33. Palmeri R, Pappalardo F, Fragalà M, Tomasello M, Damigella A. Catara AF. *Chem. Eng. Trans.*, 2012; **27**:121-126.
34. Možejko J, Ciesielski S. *J. Biosci. Bioeng.*, 2013; **116(4)**:485-492.
35. Qu XH, Wu Q, Chen GQ. *J. Biomaterial Sci. Polym. Ed.*, 2006; **17**:1107-1121
36. Choi SY, Park SJ, Kim WJ, Yang JE, Shin J, Lee SY. *Nat. Biotec.*, 2016; **34**:435-440.
37. David Y, Joo JC, Yang JE, Oh YH, Lee SY, Park SJ. *Escherichea coli. Biotechnology Journal*, 2017; **12(11)**.
38. Mizuno S, Hiroe A, Fukui T, Rsuge T. *Polymer Journal*, 2017; **49**:557-565.
39. Arikawa H, Matsumoto K, Fujiki TW. *Appl. Microbiol. Biotechnol.*, 2017; **101(10)**:7497-7507.
40. Favaro L, Basaglia M, Casella S. *Biofuel, bioproduct and bioref.*, 2018, **13**:208-227.
41. Impallomeni G, Ballistreri A, Carnemolla GM, Rizzo MG, Nicolò MS, Guglielmino SPP. *Int. J. Biol. Macromol.*, 2018; **108**:608-614.
42. Liu MH, Chen YJ, Lee CY. *Biosci. Biotechnol. Biochem.*, 2018; **82(3)**:532-539.
43. Rodrigues PR, Nunes JMN, Lordelo LN, Druzian JI. *Braz. J. Chem. Eng.*, 2019; **36(01)**: 73- 83.
44. Brandl H, Bachofen R, Mayer J, Wintermantel E. *Canadian J. Microbio.*, 1995; **41(1)**:143-153.
45. Kunioka M, Doi Y. *Macromolecules*, 1990; **23**:1933-1936.
46. Ong SY, Chee JY, Sudesh K. *J Siberian Federal Univ. Bio.*, 2017; **10(2)**:211-225.
47. Chea V, Couss YHA, Peyron S, Kemmer D, Gontard N. *J. appl. Polymer Sci.*, 2015; **133(2)**: 41850:8-16.

48. Ray S, Kalia VC. *Indian J. Microbio.*, 2017; **57(3)**:261-269.
49. Gangrade N, Price JC. *J. Microencapsulation*, 1991; **8(2)**:185-202.
50. Ee JH, Hammond CJ, Meulbroek JA, Staas JK, Gilley RM, Tice TR. *J. Controlled Release*, 1990, **(1-3)**:205-214.
51. Yao YC, Zhan XY, Zhang J. *Biomater.*, 2008; **29(36)**:4823-4830.
52. Zhao L, Zhang C, Lv L, Dong Y, Chen Z, Zhang X. Shanxi University, Peop. Rep. China. 2009; **7**.
53. Chan RT, Russell RA, Marçal H, Lee TH, Holden PJ, Foster LJ. *Int. J. Polymer Sci.*, 2011:1-9.
54. Chang HM, Wang ZH, Luo HN, Xu M, Ren XY, Zheng GX, et al. *Brazilian J. Medical Biological Res.*, 2014; **47(7)**:533-539.
55. Taguchi K, Tsuge T, Matsumoto K, Nakae S, Taguchi S, Doi Y. *RIKEN Review: Focused on Ecomol., Sci. Res.* 2001; 71-74.
56. Ma HK, Li SY, Wu Q, Chen JC. *J Biotech.*, 2013; **166(2)**:34-41.
57. Bourbonnais R, Marchessault RH. *Biomacromol.*, 2010; **11 (4)**:989-993.
58. Zhang X, Luo R, Wang Z, Deng Y, Chen GQ. *Biomacromol.*, 2009; **10(4)**:707-711.
59. Watts BBB, Bellerose RJ, Chang MCY. *Nature Chem Biol.*, 2011; **7**:222-227.
60. Sudesh K, Loo CY, Goh LK, Tadahisa IT, Maeda M. *Macromol. Biosci.*, 2007; **7(11)**:1199-1205.
61. Ojumu TV, Yu J, Solomon BO. *Afri. J. Biotec.*, 2011; **43(1)**:18-24.
62. Gadgil BST, Killi N, Rathna GVN. *Med. Chem. Commun.*, 2017; **8**:1774-1787.
63. Caputo F, Vignoli T, Maremmanni I, Bernardi M, Zoli G. *Int. J. Environ. Res. Public Health*, 2009; **6(6)**:917-1929.
64. Maestro B, Sanz JM, *Microb. Biotechnol.*, 2017;**10 (6)**:1323-1337.
65. Numata K, Doi Y. *Polymer J.*, 2011; **43**:642-647.