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AN INITIATIVE TO REDUCE POLYMER POLLUTION BY INTRODUCING BIOPOLYMER SYNTHESIZED BY MICROORGANISMS WITH THE USE OF VARIOUS ORGANIC WASTE WITH ECONOMICALLY EFFECTIVE AND BIODEGRADABILITY

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Abstract—The utilization of single-use polymer increases day by day and there is no alternative to resolve the problem of waste management till now. It is estimated that 1.1 to 8.8 million tonnes of polymer waste enters the ocean from coastal communities each year. Recent researches suggest that there could be more synthetic polymer than fish in the oceans by weight by the year 2050. White pollution (polymer pollution) has build up an island in between the pacific ocean according to recent studies. This accumulation of polymer waste can face a huge negative impact on both aquatic and terrestrial ecosystems. One recent study also shown that polymer pollution can cause cancer in humans. To overcome this problem, there is a requirement to think of another way to introduce a product which has a resemblance with synthetic polymer and has a feature that makes it degradable and economic. Various biopolymer are synthesized by microorganisms that could able to replace the synthetic one. The advantage of using a biopolymer is that it can be biodegradable, could manage the agricultural plant waste as well as environmental waste which is directly responsible for pollution. To fulfill the demand, various biopolymer have been designed all over the world. This review concludes various example of Biopolymer Films with analysis for effective suggestions against Polymer Pollution (White Pollution).

I. INTRODUCTION

Nowadays, the population is increasing day by day. Due to the increase in population, utilization of natural resources increasing with a decrease in the quantity of source and quality of the environment. Environmental degradation is the deterioration of the environment through depletion of resources such as air, water, and soil, the destruction of ecosystems, habitat destruction; the extinction of wildlife; and pollution. The main source of pollution is petrochemically synthesized polymer (Bhat R.A. et al., 2020).

1.1 Petrochemical polymer and their environmental impact.

The use of polymer material is synthetic polymer composites. These are derivatives of petrochemicals like alkenes and alkanes. The polymer or polymeric materials generally means organic polymers because it is large in number and the number of inorganic polymers is quite less. Synthetic Polymer takes about 500-1000 years for its degradation but the actual time of degradation is not known. The monomer, initiators, catalyst, fillers, plasticizers, coloring materials used for the manufacture of a synthetic polymer are highly poisonous and cause many terrible diseases both in animals as well as Humans (Hayden et al., 2013). Uncertainty these waste polymers are not properly removed from the environment and it may cause hazardous effects to our Ecosystem. A synthetic polymer is a lightweight, durable, low cost, and good texture, which is the cause of the amplification of polymer in different sectors. The waste polymer materials such as bags, bottles,



rejected electronic and electrical devices, toys, balls, packaging materials, home utensils, decorative materials, etc., are used extensively in urban areas and therefore cause of clogging the water bodies mostly like canals, rivers, lakes and water pipelines particularly in the urban areas and this trend is increasing day by day (Biswal T., 2020). The pollution caused by polymer is known as 'White pollution'.

To solve this problem, there is a need to produce an alternative way such as the production of a biodegradable polymer. The biodegradable polymer could be defined as a biopolymer that can decompose in the environment by the action of microorganisms such as bacteria, fungi, and algae. The advantage of using biodegradable polymer is its ability to undergo decomposition in a short period without any harmful effect on the environment. It is an eco-friendly polymer and made from potentially inexpensive raw material. Moreover, the manufacturing cost of a biodegradable polymer is less as compared to conventional polymer (Chander, 2019).

Biopolymer:

The term 'Biopolymer' includes chemically unrelated products that are synthesized by microorganisms under different environmental conditions. One important family of biomaterials is a biopolymer. The PHA bioaccumulation feature is common among the bacterial and archaeal domains with PHA-producing microbes occurring in more than 70 bacterial and archaeal genera. Bioaccumulated PHA is stored in the form of intracellular lipid granules inside the cell membrane. Acting as biocatalysts, these PHA-producing microorganisms enable the coupling of a myriad of carbon catabolic pathways together with PHA anabolic pathways, thereby playing a vital role in the modification of PHA production from various carbon sources (Luengo et al., 2003),

In 1923, Lemogine at the Institute Pasteur proved that aerobic spore-forming bacillus, formed quantities of 3-HBA in anaerobic suspensions. He continued to explore further and was successful in estimating quantitatively the amount of 3-HBA formed. Finally, in 1927, he was able to extract a substance from Bacillus using chloroform and prove that the material was a polymer of 3-HBA (Philip. S et al., 2007). The concept of biopolymer introduced into the world.

Current Scenario:

These biomaterials are synthesized by a different microorganism which is different in structure. There are types of Polyhydroxyalkanoates are present in the market which is a substitute for synthetic polymer as follows:

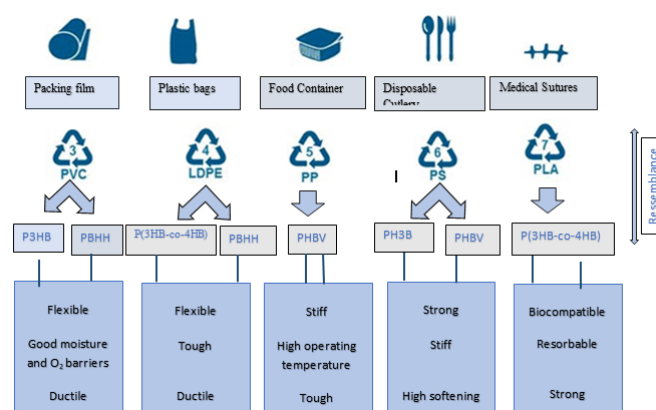
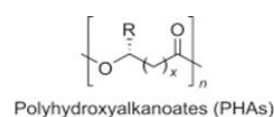


Figure1: Utilisation of Biopolymer in market

Polyhydroxy Alkanoates:

Polyhydroxyalkanoates are the polyesters which are synthesized by the microorganism. PHA are polyesters that are widely distributed in nature and accumulate intra-cellularly in micro-organisms in the form of storage granules with physicochemical properties resembling petrochemical polymer. These polymers are usually built from hydro-acyl-CoA derivatives via different metabolic pathways. Depending on their monomer composition, macromolecular structure, and physical properties most of them are biodegradable and biocompatible, which makes them extremely interesting from the biotechnological point of view (Luengo et al., 2003).

II. BASIC STRUCTURE OF PHA WHICH THEIR DIFFERENT STRUCTURE



	R Groups	Polymers	Abbreviations
X = 1	Hydrogen	Poly(3-hydroxypropionate)	PHP
	Methyl [-CH ₃]	Poly(3-hydroxybutanoates)	PHB
	Ethyl [-CH ₂ CH ₃]	Poly(3-hydroxyvalerate)	PHV
	Propyl [-(CH ₂) ₂ CH ₃]	Poly(3-hydroxyhexanoate)	PHHex
	Pentyl [-(CH ₂) ₄ CH ₃]	Poly(3-hydroxyoctanoate)	PHO
	Heptyl [-(CH ₂) ₆ CH ₃]	Poly(3-hydroxydecanoate)	PHD
X = 2	Hydrogen	Poly(4-hydroxybutyrate)	P(4HB)
X = 3	Hydrogen	Poly(5-hydroxyvalerate)	P(5HV)

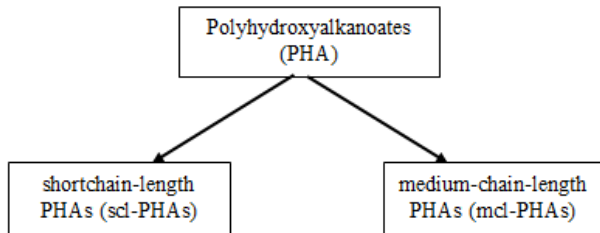
Figure 2: Different structures of Polyhydroxyalkanoates

PHA physiology:

Bacteria such as phototrophic bacteria, archaebacteria, Gram-positive and Gram-negative bacteria, aerobic and anaerobic



bacteria that extensively producing PHA, and these bacteria are also known to be taxonomical groups which comprise the diversity to synthesize different types of PHA (Yu., 2007).



PHA is divided into two subgroups according to their size. Different organisms synthesize different types of PHA. Some wild type strains of bacteria synthesize the copolymer of short-chain length PHA (scl-PHA) and Medium chain length PHA (mcl-PHA).

Carbon Source	Types of Carbon source	Bacterial strains	Bacterial Physiology	PHAs (g/L)	References
Industrial by-product					
Molasses	Sugar beet molasses	<i>Azotobacter vinelandii</i> UWD	Gram negative, Aerobic, Oval or Spherical	19-22 (PHB)	(Page., 1992)
		<i>Azotobacter vinelandii</i> UWD		36 (PHB)	(Chen., 1997)
	Sugar cane molasses	<i>Bacillus sp. Jma5</i> (<i>Bacillus subtilis</i>)	Gram positive, Rod shape, Aerobic/ facultative anaerobic	25-35% DW (PHB)	(Wu et al., 2001)
		<i>Bacillus megaterium</i> ATCC 6748	Gram positive, Rod shape,	PHB, 2.2 (43% CDW)	(Chaijamrus S et al., 2008)

	<i>Bacillus megaterium</i> BA-019	aerobic (multiple habitate)	PHB, 30.5 (42% CDW)	(Kulpreecha S. et al., 2009)
Soy molasses	<i>Pseudomonas corrugata</i>	Gram negative, Rod shape, Aerobic	mcl-PHA 5-17%	Solaiman DKY et al., 2006
	<i>Bacillus sp. CL1</i> (<i>Bacillus Cereus</i>)	Gram positive, Rod shape, Aerobic/ facultative anaerobic	PHAs, 90%	Full TD et al., 2006
Molasses	<i>Bacterial consortium</i>	Two or more bacterial strains are used, having different property of endosymbiotic or ectosymbiotic	PHAs, 0.37-0.5 Cmol/Cmol VFA	(Albuquerque MGE et al., 2007)
	<i>Bacterial consortium</i>		PHAs, 0.47-0.66 Cmol/Cmol VFA	Pisco AR et al., 2009 and Bengtsson S et al., 2010
Dairy By-product				
Whey and whey hydrolysates	Hydrolyzed soy and malt	Gram positive, Rod shape, Diverse habitate	PHAs, 18.42	Law K-H et al., 2001
	Cheese Whey	<i>Thermus Thermophilus</i> HB8	Gram negative, Rod shape, Aerobic	PHAs, 13.3-22.4% Chander., 2019
	Hydrolyzed whey	<i>Ralstonia eutropha</i> DSM545	Gram-negative, Rod shape, Facultative aerobic	PHBV, 2.25 Marangoni C et al., 2002
		<i>Pseudomonas hydrogenovora</i>	Gram negative, Rod shape, anaerobic	PHBV, 1.44 Koller M et al., 2008
	Recombinant <i>E. coli</i>	Gram negative, Rod shape,	PHB, 9.0	Nikel PI et al., 2006

Whey			Both aerobic and anaerobic				Xylose, glucose from sugar cane bagasse	<i>Burkhold eria cepacia</i> IPT 048 and <i>B. sacchari</i> IPT 101	Facultative aerobic	PHB, 34.8	Silva LF et al., 2005
	Recombi nant <i>E. coli</i>	Gram negative, Rod shape, Both aerobic and anaerobic	PHB, 5.2	Lee SY et al., 1997			Xylose and glucose	<i>E. coli</i> <i>PTS</i> mutant	Rod shape, Both aerobic and anaerobic	PHA, 0.476	Li R et al., 2007
	<i>Thermus thermoph iles</i> HB8	Gram negative, Rod shape, Aerobic	PHA, 0.51	Yellore et al., 1998			Wheat bran hydrolysa te	<i>Halomon as boliviensi s</i> LC1	gram-negative, rod-shaped, aerobe	PHB, 4	Van-Thuoc D et al., 2008
	<i>Methylob acterium</i> sp. ZP24	Gram-Negative, Rods and Cocci,	PHA, 2.6-5.9	Nath A et al., 2008			Cellulose, in tequila bagasse	<i>Saccharo phagus degradan s</i> ATCC 43961	Gram-negative, rod shaped, aerobic	PHA, 1.5	Munoz LEA et al., 2008
	<i>Methylob acterium</i> sp. ZP24	Aerobic	PHB, 6.12	Koller M et al., 2011			Formic acid, acetic acid, furfural and acid soluble lignin	<i>Ralstonia eutropha</i>	Gram-negative , Rod shape, Facultative aerobic	PHB, 6.1-6.8	Yu J et al., 2008
	<i>E. coli</i> GCSC 6576	Rod shape,	PHB, 109	Wong and Lee., 1998							
	<i>E. coli</i> GCSC 4401	Both aerobic and anaerobic	PHB, 96.2	Ahn WS et al., 2000							
	Plant sugar										
Lignocellulo sic raw materials	Hemicelll osic fraction of poplar wood	<i>Pseudom onas pseudoav a</i> (Burkhol deri cepacia ATCC 17759)	PHB, 6.57	Bertrand J-L et al., 1990		Other sugars	Orange peel	Bacillus subtilis NCDC06 71	Gram positive Rod shape Aerobic	2.04 g/L	Umesh et al., 2017
		Cassava starch					NR	NR	QU	(Chander, 2019)	
		Banana Peel starch					NR	NR	QU	(Chander, 2019)	
	Xylose; xylose with propionic acid	<i>Burkhold eria cepacia</i> ATCC 17759	PHB; PHBV, 1.6-3.7	Ramsay JA et al., 1995 and Young FK et al., 1994		Starch based raw material	Corn starch	NR	NR	PLA, QU	(Chander, 2019)
		Potato starch					NR	NR	QU	(Chander, 2019)	
	Xylose with levulinic acid	<i>Burkhold eria cepacia</i> ATCC 17759	Gram-negative ,	PHBV, 1.3-4.2		Fatty Acid Waste					
	Hemicell ulosic hydrolysa tes	<i>Burkhold eria cepacia</i> ATCC 17759				Rod shape,	PHB, 2.0	Keenan TM et al., 2006	Unsaponi fed olive oil	<i>Aeromon as caviae</i>	Gram-negative, rod shaped, facultative anaerobic



Fats, vegetable oils and waste cooking oils						Soybean oil	<i>C. necator</i> H16 and recombinant strains	Gram-negative, Rod shape,	PHA, 102	Kahar P et al., 2004
	Lard, butter oil, olive oil, coconut oil, soybean oil	<i>Pseudomonas aeruginosa</i> and <i>Pseudomonas resinovorans</i>	Gram-negative, Rod shape, Aerobic	mcl-PHA, 2.1	Ashby RD et al., 1998		<i>C. necator</i> mutants	Facultative aerobic	PHA, 1	Tsuge T et al., 2009
	Olive oil, corn oil and palm oil	<i>C. necator</i> (also known as <i>R. eutropha</i> , <i>A. eutropha</i>)	Gram-negative, Rod shape, Facultative aerobic	PHB, 3.4	Thakor N et al., 2005 and Fukui T et al., 1998	Jatropha	Marine bacteria, SM-P-3M	Gram-negative, Rod shape, Aerobic	PHA, 0.306	Ng K-S et al., 2011, Shrivastav A et al., 2010 and Allen A et al., 2005
	Olive oil, corn oil and palm oil	<i>P. putida</i> ; <i>C. necator</i> .	Gram-negative, Rod shape, aerobic/ Facultative aerobic	PHA, 1.6	Chaudhry W et al., 2011	Linseed oil	<i>Pseudomonas aeruginosa</i>	Gram-negative, rod-shaped,	PHA, 1.8, 50.2% CDW	Bassas M et al., 2008
	Castor seed oil, coconut oil, mustard oil, cottonseed oil, groundnut oil, olive oil, and sesame oil	<i>Comamonas testosteroni</i>	gram-negative, rod shape, aerobic	The polymer contained HA units with 6 to 14 carbon atoms, 87.5% CDW	Ntaikou I et al., 2009 and Thakor N et al., 2005	Brassica carinata oil	<i>Pseudomonas aeruginosa</i>		PHA, 5%	Fuller RC et al., 1999
						Waste cooking oil	<i>Pseudomonas aeruginosa</i>		PHA, 2.3	Fernández D et al., 2005
							<i>Pseudomonas aeruginosa</i>		PHA, 5.4	Haba E et al., 2007
							<i>Pseudomonas aeruginosa</i>	Aerobic or adoptive anaerobic	PHA, 3.43	Costa S et al., 2009
						Spent palm oil	<i>Cupriavidus necator</i>	Gram-negative, Rod shape, Facultative aerobic	P(3HB-co-4HB), 4.4	Rao U et al., 2010
						Waste vegetable oil	<i>Pseudomonas</i> sp. strain DR2	Gram-negative, rod-shaped, Aerobic or adoptive anaerobic	PHA, 23.5% CDW	Song JH et al., 2008
	Lard and coconut oil	<i>Pseudomonas putida</i>	Gram-negative, Rod shape, Aerobic	PHA, 0.9-1.6	Solaiman DKY et al., 2001	Polyhydroxyalkanoates are synthesized by different microorganisms with different substrate and different conditions for their survival. Mostly bacteria takes part for PHA synthesis. Different microorganisms undergoes in different cell cycle that results the different PHA structures. The table given below shows which microorganisms need which substrate and synthesize PHA in which amount (Du et al., 2012).				
	Coconut oil and tallow	<i>Pseudomonas saccharophila</i>	gram-negative, rod-shaped, anaerobic	mcl-PHA, 0.8	Solaiman DKY et al., 2001					
	Palm kernel oil, palm olein, crude palm oil and palm acid oil	<i>C. necator</i>	Gram-negative,	PHA, 3.3	Loo C-Y et al., 2005					
	Palm kernel oil	<i>C. necator</i>	Rod shape,	P(3HB-co-3HV-co-3HHx); 6.79	Bhubalan K et al., 2008					
		<i>C. necator</i>	Facultative aerobic	PHA, 2.5	Lee W-H et al., 2008					
		<i>Pseudomonas stutzeri</i>	Gram-negative, rod-shaped, facultative anaerobe	PHA, 1.4	He W et al., 1998	III. MICROBIAL STRAIN <i>Ralstonia eutropha</i> is a Gram-negative bacterium that is non-spore-forming. Many of the Gram-negative bacteria are pathogenic but not all, so this strain is nonpathogenic and is				



used for water remediation from numerous previous years. They are facultative aerobes, as a significance, they could survive in both aerobic as well as anaerobic conditions. *R. eutropha* is rod-shaped (bacilli) with two flagella and two membranes. There are some strains which are showing different characters of the same species example: *R. eutropha* JMP 134 strain could survive in multiple habitats but *R. eutropha* H16 has a specialized habitat. However both strains could not survive in non-halophilic (non-salty) environment and they require optimal temperature i.e., 30°C (Hidayat et al., 2019).

3.1 Source for Microbial growth

There are many sources from which they could use that carbon for many metabolic reactions as well as PHA synthesis (Tan et al., 2014):

Source	Types	Discription
	fructose, maltose, lactose, xylose, arabinose, glucose,	Saccharides are rich in Carbon, easily available and degradable by microbes present in environment
Saccharides		
Alkanes	hexane, octane, dodecane, etc	Alkanes contain a single covalent bond that is easily breakable by any enzyme
Alkanoic acid	acetic acid, propionic acid, butyric acids, valeric acid, lauric acid, oleic acid, etc.	Alkanoic acids are those in which alkanes contain positive charge on it which shows acidic behavior. Some microbes need carbon from acids as they survive in acidic habitats

Alcohols	methanol, ethanol, octanol, glycerol, etc.	Alkanes contains one or more OH group (alcoholic groups), they are known as alcohols. Some microbes utilize alcohol for carbon sources
Gases	CH ₄ and CO ₂	Microbes are able to use atmospheric gases as their carbon source

Table 4: Substrate that are utilised by microorganisms for synthesizing PHA

Microorganisms need Carbon as its basic source for survival. Carbon is not present freely, for that these microbes contain some enzymes which hydrolyze the bonds that are present in macromolecules. For carbon sources, they needed macromolecules from which they could use that for various metabolic processes (Tan et al., 2014).

Most of the microbes are growing in the waste material because they are rich in carbon with providing optimum conditions for the growth of that microbes. Waste materials like Waste frying oil, vinegar waste, waste fats, food waste, agricultural waste, domestic wastewater, plant oil mill effluents, crude glycerol from biodiesel production, plastic waste, landfill gas, etc. (Tan G et al., 2014).

3.2 Process Parameters involved in PHA production

3.2.1 Growth substrates

PHA are polyhydroxyalkanoates which are rich in carbon. For PHA synthesize through microorganisms, they need major carbon sources in media. In aerobic conditions, microorganisms synthesize ATP as their energy source but in anaerobic conditions, oxygen is absent and another essential component, for that microorganisms synthesize PHA and store that granule in the cell as their stock energy for survival. Consequently, Carbon is very important for PHA synthesis. Any type of Carbon source could be used for PHA synthesis as mentioned above. There is another vital role of Carbon in microbial cells are cell maintenance, cell biomass synthesis, and cell metabolism. Different microbial strains and organisms utilizing different souces for synthesizing a large amount of PHA. Based on large scale production, suitable microbial strains are used. PHA production not only depends



on microbial strains but it also depends on carbon source parameters which are: cost and feasibility of substrate. Different parameters are responsible for PHA production but Carbon source is very important as the basic need of organisms for the growth of cells as well as cell metabolism (Raza et al., 2019).

3.2.2 Nitrogen sources and limitation

After the Carbon source, Nitrogen's concentration also affects the PHA synthesis. Nitrogen is the basic component of amino acids and amino acids are present in protein like enzymes. Whether Nitrogen amount increases which increase the activity of enzymes that are responsible for cell division. When the process of cell division occurs, no PHA accumulation in cells takes place. Nitrogen shows an inverse effect on PHA synthesis. For PHA accumulation in cells, nitrogen concentration must limit because if the nitrogen amount would be high then enzymes responsible for cell replication would be active which results in an increase in cell number through cell division. But if the nitrogen amount would be limited then enzymes would be inactive and cells would not undergo in the division process. On that time enzymes responsible for PHA synthesis took part in the accumulation of PHA molecules in the cell. For limiting nitrogen concentration various salts are added in media which are amides (NH₂), ammonium sulfate (NH₄)₂SO₄, ammonium nitrate/ nitrogen, ammonium bicarbonate, ammonium carbonate, ammonium chloride, polyamide poly-γ-glutamate (PGA), urea, nitrates, and sodium nitrate, etc (Raza et al., 2019).

3.2.2.1 Carbon to nitrogen ratio

For any microorganisms and plants secondary metabolite production yield, Carbon to Nitrogen ratio is essential. This is the basic study for the introduction of any organism's nature. C/N ratio is different for different organisms as well as strains of a particular organism. This ratio has to be controlled for PHA synthesis which generally affects the size of PHA and bacterial cell numbers. Most microorganisms need a 21:1 C/N ratio in normal habitat but it would differ according to need. For increasing C/N ratio, various reagents are used like acetic acid, propionic acid, butyric acid, and an equivalent amount of ammonium sulfate (Huschner et al., 2014).

3.2.3 Effect of pH and Temperature

The major concern of any organism's survival, pH, and temperature plays a vital role. Because of these two important factors, researchers describe the concept of optimum conditions for the growth and survival of an organism. These two parameters affect the cell viability at different temperature and pH by either rupturing the cells and their components like cell organelles, DNA, RNA, proteins, etc or maintaining metabolic reactions and macromolecules which are involved. For *Ralstonia eutropha* pH should be in the range 5.5- 7.8 and the temperature is between 30-40°C. The reason for maintaining pH and temperature is that they affect the

extracellular environment conditions which significantly affects enzymatic activity. A study shows at neutral pH i.e. 7, PHA synthesis occurs in large amounts and microorganisms show high yields. Microorganism's growth depends on media composition but our desired products depend on such parameters (Raza et al., 2019).

3.2.4 Dissolved oxygen demand

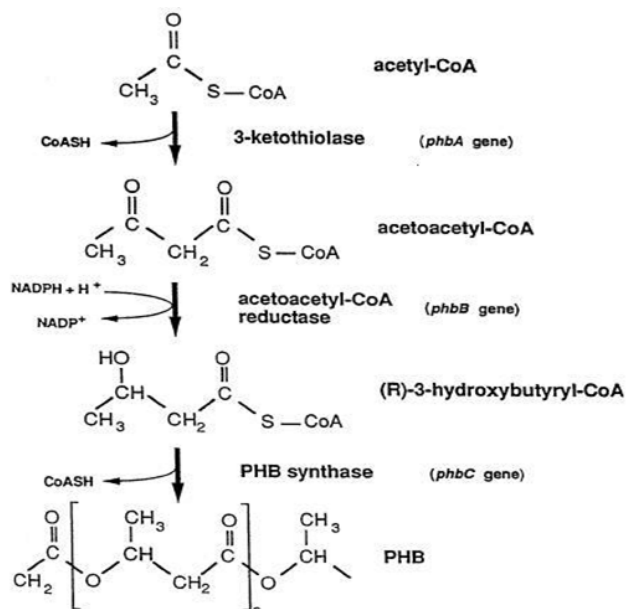
A study describes that *Ralstonia eutropha* is a facultative anaerobe which means an organism could survive in both aerobic and anaerobic conditions. For aerobic conditions, the organism needs oxygen for their growth and metabolic reactions in normal conditions. For the synthesis of adenosine triphosphate (ATP), oxygen plays an important role. ATP is responsible for the bio-oxidative respiration of organisms which provides energy for metabolic reactions. In the fermentation process, conditions would maintain to anaerobic but organisms need oxygen in less demand for further reactions. For maintaining that environment dissolved oxygen plays a vital role in the supply of oxygen through media to organisms (Raza et al., 2019).

3.2.5 Concentration of phosphorus

Phosphorus is essential for the synthesis of ATP, macromolecules like carbohydrates, proteins, fats, and DNA backbone, etc. It plays an important role in cell repair and maintains the growth rate of cells. Phosphorus also acts as a buffer which maintains the pH of media when added to media. Phosphorus optimized the fermentation media when changing the concentration in media for obtaining a proper yield of the product. It shows a reverse effect on PHA synthesis, for that phosphorus would be limit and added in a lesser amount (Raza et al., 2019).

3.3 Fermentation:

Fermentation is the process that takes place inside the cells. This process includes many enzymes for breaking down large molecules into smaller in the absence of oxygen in the environment. Some facultative aerobes/anaerobes undergo these reactions for their survival in both aerobic as well as anaerobic conditions. The metabolic reaction produces many macromolecules for their growth however these synthesized macromolecules play a vital role in the production of PHA. PHA molecules are accumulated inside the cell and this PHA is considered as monomers for Biopolymer Film. The organisms contain two types of enzymes from which one is for the synthesis of PHA and another is for the degradation of PHA. The below chart shows the synthesis of PHA by fermentation reaction (Yu., 2007).

Figure 1: PHB synthesis pathway of *R.eutropha*

(El-Nahrawy, Sahar. (2009). Study on poly-β-hydroxybutyrate in some bacteria. 10.13140/RG.2.2.24647.50080.)

The second reaction also takes place to produce a copolymer poly (3- hydroxybutyrate- Co 3- hydroxy valerate), P3HB3HV while propionic acid is present which merges 3-hydroxy valerate monomers with PHA backbone. This copolymer is produced with the action of the second enzyme β-ketothiolase which condense different molecules i.e., acetyl Co-A and propionyl Co-A to produce β-ketovaleryl- CoA which works as a precursor of 3HV.

3.4 PHA recovery from cell:

For any industrial or lab-scale production of any product, downstream processing must be required for the extraction and purification of the desired product to produce its end product. Downstream processing is used in many stages required from extraction to purified level of product. For the purification of PHA from a cell, several steps take place. For undergoing the process, the product's location should be known. According to the location of the product whether the product present inside/ outside the cell, the method would be used for suitability for the product (Tan et al., 2014). For PHA recovery from cell biomass, downstream processing is done in several steps:

1. Cell wall/ membrane lysis
2. Solubilization and Purification of PHA
3. Precipitation of PHA.

Cell lysis	Solubilization and	Precipitation
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	purification	
Mechanical methods	Solvent recovery	Solvent precipitation
Non mechanical methods	Chemical recovery	Ice cold ethanol
Others	Enzymatic recovery	Ice cold methanol

Table 5: Downstream process techniques for PHA isolation

Mechanical methods are very effective methods for cell lysis. It includes two types of force for cell disruption which are solid shear and liquid shear. In solid shear bead mill and the fresh press is used for cell lysis and in liquid shear high-pressure homogenization and ultrasonication is used. Other methods are used which are autoclave, lyophilization, and microwave which contain high temperature and pressure. This leads the cell lysis by increasing membrane porosity with bond degradation (Tan et al., 2014).

Solubilization and purification could be done by solvent recovery, chemical recovery, and enzymatic recovery. For PHA purification some specific solvent and chemicals are used which decreases the cost of the product yield but biohazardous for health and the environment. Enzymatic recovery produces less hazardous waste but because of particular enzyme use, cost increases (Tan et al., 2014).

Solvent	Recovery : purity	Chemical digestion	Enzymatic digestion
Chloroform : 61°C, 1h	68% : 96-98%	Sodium hypochlorite	Suspend in specific buffer
Methylene chloride: 40°C, 24h	25% : 98%	Chloroform	Optimize the enzymatic activity and centrifuge
1,2-dichloroethane: 83°C, 30 min.	68% : 98%	Sodium hypochlorite + chloroform	
Acetone (for scl-PHA)	65% : 96%		90% purity

Table 6: Use of solvents for PHA isolation

For scl-PHA acetone is used for extraction, filtration, and product workup which provides great yield as same as chloroform. Acetone is a good solvent because it is highly recyclable and non-reactive (no structural change either of polyester of itself) but major disadvantages are cost and produce a negative impact on the environment (Tan et al., 2014).

Chemical digestion is generally done by many salts like sodium hypochlorite, sodium chloride, lime, etc are used as cell solubilizer. A study shows that sodium hypochlorite is very well used in chemical digestion, it gives high yield but



disturbs the structure of biopolyester. For neutralizing this concern by maintaining its structure, chloroform is used with it which increases the digestion with solvent extraction (Tan et al., 2014).

Example: *P. putida* biomass sample using methanol and acetone in the pretreatment process results with high yield i.e., 94% with no detectable molecular mass (Tan G, et al., 2014).

IV. BIOSYNTHESIS OF BIOPOLYMER FILM

After purification and characterization, these PHA is used for further processing and producing a chain by combining with each other with the help of some blenders. Blenders are that organic material that has crosslinking capabilities to polymerize any organic monomers. PHA is acting like monomeric units for biopolymer film. This biopolymer chain provides the rigidity and tensile strength which could compete with a synthetic polymer. These are organic and could be easily decomposed by the microorganisms itself when it comes in contact with them (Brzeska et al., 2019).

Biodegradability of any biopolymer chain depends on blends which are to be used for producing a long chain of biopolymer. The blend itself could increase and decrease the degradability of the biopolymer chain. Blending material should be organic at that much which could degrade at a certain time limit. However many blends are available nowadays which provide the proper tensile strength with the major advantage of biodegradability. Blends are polyurethanes, cellulose acetate, lignin, etc (Brzeska et al., 2019).

V. AGING OF BIOPOLYMER FILM

The aging of biopolymer film means exposure to environmental conditions after a while changes its structure either chemically or physically. Chemical changes occur degradation of biopolymer film by the oxidation process. Physical changes occur includes the change in molecular weight additives which affects the film properties. This is due to the addition of blending items that are not able to remove easily." Kinetic solids " are glass type material which are thermodynamically unstable and which changes time to time. In the aging process, changes in transport and mechanical properties are directly affected by the physical properties like enthalpy and volume reduction (Thomas et al., 2013).

The aging provides the knowledge of product use, time limit, and starting of degradation. This parameter of characterizing the biopolymer film is very important for production. The product which has to be produced has some age for versatility, for checking its age there is some test which has to be complete in the lab before undergoing another step i.e., biodegradation (Thomas et al., 2013).

5.1. Aging tests

There are numerous methods which are used nowadays for characterizing the age of biopolymer film which are as follows(Thomas et al., 2013):

5.1.1 Environmental aging test:

The environmental aging test is a natural test in which the sample is placed in natural environmental conditions for several months. Whenever the sample is to be check, it shows various observations like crack formation, chalking, color change, etc which could be visualized with the naked eyes with some observations on the microscopic level, checked with different instruments like IR, mechanical test, etc. These observations not always show a positive effect, it also shows a negative effect if the product age is less than the assumption. If material starts degradation before its time limit in the normal environment then it shows a negative effect because then that product could not be useful for normal environmental conditions (Thomas et al., 2013).

5.1.2 Artificial Aging tests

In the Artificial aging test, natural environment condition is maintaining in a laboratory. This increases the aging but the major disadvantage is it doesn't contain microorganisms and sun exposure which are present in the open environment (Thomas et al., 2013).

5.1.3 Accelerated Aging Tests

The accelerated aging test is using nowadays because in this test two or more parameters could be modified at the same time. For example, for the hydrothermal aging test, both temperature and humidity maintain at a particular time. The accelerated aging test could maintain some parameters as natural environmental but not all which shows major drawbacks to use it and sometimes shows failure in experimentation (Thomas et al., 2013).

VI. DEGRADATION OF BIOPOLYMER FILM

Biodegradation is a process in which polymeric materials undergo chemical change through the action of enzymes that are secreted by living microorganisms such as bacteria, fungi, and algae. The process of biodegradation comprises two phases; the initial phases (primary biodegradation) and the second phase (ultimate biodegradation). During the initial phase, the material undergoes disintegration which is significantly associated with the deterioration in physical properties such as discoloration, embrittlement, and fragmentation. The second phase is assumed to be the ultimate conversion of polymer fragments, after being broken down to molecular sizes, to CO₂, water, cell biomass when the plastic material is exposed to aerobic conditions as well as CH₄, CO₂, and cell biomass in the case of anaerobic conditions. The primary and ultimate biodegradation must occur within a



specific time at a specific rate to avoid the accumulation of plastics in the environments (Singh et al., 2018).

The American Society for Testing and Materials (ASTM) and the International Organization for Standardization (ISO) define (Singh et al., 2018):

a. Degradation as “An irreversible process leading to a significant change of the structure of a material, typically characterized by a loss of properties (e.g. integrity, molecular weight, structure or mechanical strength) and/or fragmentation. Degradation is affected by environmental conditions and proceeds over a period of time comprising one or more steps”. According to the ASTM definition,

b. Biodegradable plastic is “A degradable plastic in which the degradation results from the action of naturally occurring microorganisms such as bacteria, fungi and algae”

c. Compostable plastic is “a plastic that undergoes biological degradation during composting to yield carbon dioxide, water, inorganic compounds and biomass at a rate consistent with other known compostable materials and leaves no visually distinguishable or toxic residues”.

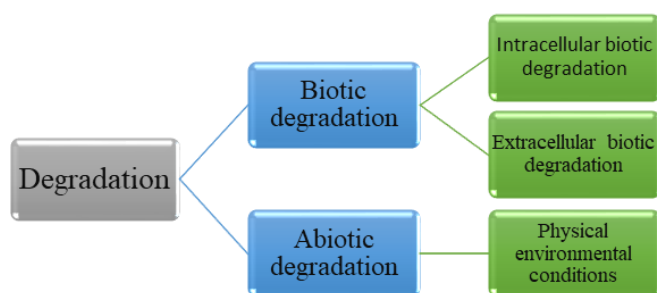


Figure 2: Types of Degradation of Biopolymer

Biodegradation are mainly classified into two division (Sharma and Mudhoo., 2011):

1. Abiotic Degradation
2. Biotic Degradation

Abiotic degradation of biopolymer film is the degradation that occurs without any microbial activities. This degradation takes place due to physical environmental conditions such as photo-oxidation, hydrolysis, oxidation, and photolysis. As it is an environmental activity that degrades partially and leaves small fragments in it which would be further used for microorganisms as their food in biotic degradation (Sharma and Mudhoo., 2011).

Biotic degradation occurs through the action of biological mechanisms. Due to biodegradation, plastics may be partially degraded (into smaller fragments) or completely degraded (mineralized) into simple molecules such as methane (CH₄), carbon dioxide (CO₂), and water (H₂O). The degradation fate of a plastic depends upon the environmental conditions and the number and types of microorganisms involved in the

degradation process. Biodegradation is enhanced if there is the presence of ester, ether, or amide bonds in the structure of the plastic. Several factors affecting biodegradation are given in the table (Sharma and Mudhoo., 2011).

Microbiological parameters	the diversity and distribution of microbes, activity, adaptability of microbes
Physicochemical properties	temperature, pH, humidity, oxygen level, nutrients, activators, inhibitors, etc
Material properties	Plastic composition, molecular weight, molecular weight distribution, crystallinity, glass transition temperature, porosity, hydrophobicity, steric configuration, etc
Material processing	the type of processing, surface characteristics, material thickness, additives, fillers, and coatings

Table 7: Factors affecting Biodegradation of Biopolymer

For effective and efficient biodegradation following elements are essential: appropriate microorganisms environmental conditions conducive for the growth of microorganisms vulnerability of the plastic to microbial attack. Biodegradation is essentially attributed to the role of enzymes produced and secreted by microorganisms which leads to mineralization of plastics to CO₂, H₂O, etc. The enzymes are responsible for the breakdown of these high molar mass products, for transport into cells followed by its utilization in the cell. The essential characteristics that an enzyme must possess include (Sharma and Mudhoo., 2011):

- accessibility to polymer for its activity
- ability to readily act on polymers at the specific site of action.

Microorganisms utilize the polymers as a carbon source and occasionally as a nitrogen source, because of the enzymatic activity on these unusual substrates. The limited reports on microbial activity on polyethylene are attributed to no points of attack in its structure, except at the termination of the carbon chain, as the repeating C–C bonds are not amenable to attack (Sharma and Mudhoo., 2011)..

As discussed earlier that PHA which is the basic monomeric unit of biopolymer film is synthesized by microorganisms in the absence of material that is required for the synthesis and growth of organisms. These microorganisms have dehydrogenase enzymes that could degrade the PHA molecules as their energy source. Microorganisms from the families' pseudonocardiaceae, Micromonosporaceae, Thermomonosporaceae, Streptosporangiaceae, and



Streptomycetaceae predominantly degrade PHA in the environment (Roy., 2006).

Biofilm degradation occurs by microorganisms in two different methods which are as follows (Sharma and Mudhoo., 2011):

1. Intracellular degradation
2. Extracellular degradation

Intracellular Degradation is the active degradation (mobilization) of an endogenous storage PHA by the accumulating bacterium itself. Enzymes responsible for such intracellular degradation of PHA are intracellular PHA depolymerase (i-PHA depolymerase). A dehydrogenase acts on the latter and oxidizes it to acetylacetate and a β -ketothiolase acts on acetylacetate to break it down to acetyl-CoA. The β -ketothiolase enzyme plays an important role in both the biosynthesis and the biodegradation pathways. This type of degradation takes place intracellularly (Philip et al., 2007).

Extracellular Degradation Reports on biodegradation of PHA-based materials in the environment are also available. This is due to the enzymes secreted by microorganisms into the external environment, i.e. extracellular degradation. Thus, extracellular degradation is the utilization of an exogenous polymer using extracellular PHA depolymerase (e-PHA depolymerase) not necessarily produced by the accumulating microorganism (Sharma and Mudhoo., 2011). Extracellular depolymerase degrade PHA present in the environment. The bacteria, algae, fungi present in the environment attack the polymers on the surface. These microbes secrete extracellular enzymes that solubilize the polymer and these soluble products are then absorbed through their cell wall and utilised (Philip et al., 2007).

VII. CONCLUSION

This study shows the production of new approach which helps to reduce white pollution. White pollution increasing the risk of environmental quality. For eliminating these, biopolymers were introduced years ago which resemble the quality of polymers. The major drawback is, these biopolymers are not that much feasible because of its high cost. Biodegradation is done by microbes but it takes a lot of time which is not effectively degraded. For the concern of these two parameters i.e., cost effective and biodegradability, each and every aspect should be known and studied well while production. This study helps which parameters affect two major concern. In the review, there is a vast knowledge of that parameters which should be needed to be modified. That parameters include maintain the environmental effects, steps of production, microbial needs for growth and production of Biopolymer monomers, blends use for crosslinking the two monomers for generation film, aging of biopolymer film, and the very important that is biodegradability.

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