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

Shivangi Shrivastava
School of Biotechnology
RGPV, Bhopal, Madhya Pradesh, India

Chahal Kailashiya
Department of Biotechnology
MITS, Gwalior, Madhya Pradesh, India

Dr Mritunjai Singh
Asst. Professor, School of Biotechnology
RGPV, Bhopal, Madhya Pradesh, India

Dr Archana Tiwari
Director, School of Biotechnology
RGPV, Bhopal, Madhya Pradesh, India

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 imact on both aquatic and terrestial 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:
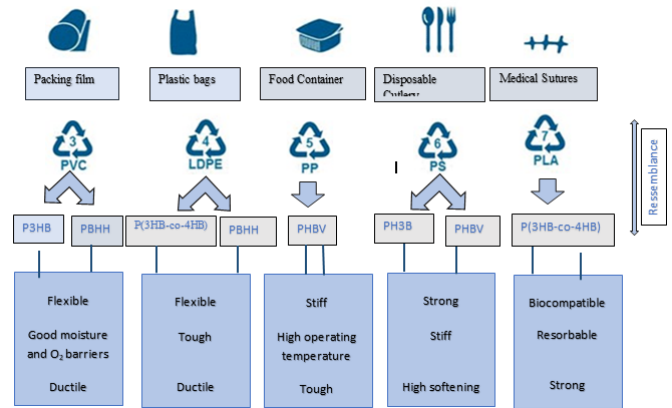

Figure1: Utilisation of Biopolymer in market

Polyhydroxy Alkanoates:

Polyhydoxyalkanoates 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

Polyhydroxyalkanoates (PHAs)
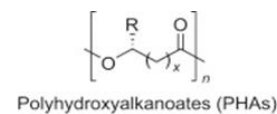
|	R Groups	Polymers	Abbreviations
X = 1	Hydrogen	Poly(3-hydroxypropionate)	PHP
	Methyl [-CH$_3$]	Poly(3-hydroxybutanoates)	PHB
	Ethyl [-CH$_2$CH$_3$]	Poly(3-hydroxyvalerate)	PHV
	Propyl [-(CH$_2$)$_2$CH$_3$]	Poly(3-hydroxyhexanoate)	PHHex
	Pentyl [-(CH$_2$)$_4$CH$_3$]	Poly(3-hydroxyoctanoate)	PHO
	Heptyl [-(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).
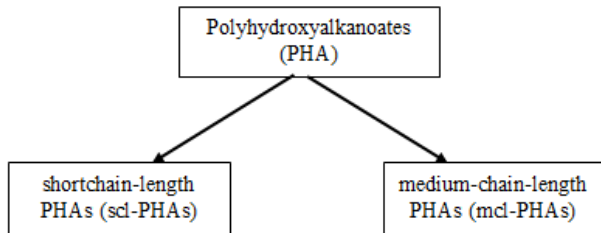
PHA is divided into two subgroups according to their size. Different organisms synthesize different types of PHA. Some wild type strains of bacteria synthesize the copolymer of short-chain length PHA (scl-PHA) and Medium chain length PHA (mcl-PHA).

Carbon Source	Types of Carbon source	Bacterial strains	Bacterial Physiology	PHAs (g/L))	References
Industrial by-product					
Molasses	Sugar beet molasses	*Azotobacter vinelandii UWD*	Gram negative, Aerobic, Oval or Spherical	19-22 (PHB)	(Page., 1992)
		Azotobacter vinelandii UWD		36 (PHB)	(Chen., 1997)
	Sugar cane molasses	*Bacillus sp. Jma5 (Bacillus subtilis)*	Gram positive, Rod shape, Aerobic/ facultative anaerobic	25-35% DW (PHB)	(Wu et al., 2001)
		Bacillus megaterium ATCC 6748	Gram positive, Rod shape,	PHB, 2.2 (43% CDW)	(Chaijamrus S et al., 2008)
	Soy molasses	*Bacillus megaterium BA-019*	aerobic (multiple habitate)	PHB, 30.5 (42% CDW)	(Kulpreecha S. et al., 2009)
		Pseudomonas corrugata	Gram negative, Rod shape, Aerobic	mcl-PHA 5-17%	Solaiman DKY et al., 2006
		Bacillus sp. CL1 (Bacillus Cereus)	Gram positive, Rod shape, Aerobic/ facultative anaerobic	PHAs, 90%	Full TD et al., 2006
	Molasses	*Bacterial consortium*	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)
		Bacterial consortium		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	*Bacillus sp. HF-1, HF-2*	Gram positive, Rod shape, Diverse habitate	PHAs, 18.42	Law K-H et al., 2001
	Cheese Whey	*Thermus Thermopiles HB8*	Gram negative, Rod shape, Aerobic	PHAs, 13.3-22.4%	Chander., 2019
	Hydrolyzed whey	*Ralstonia eutropha DSM545*	Gram-negative , Rod shape, Facultative aerobic	PHBV, 2.25	Marangoni C et al., 2002
		Pseudomonas hydrogenovora	Gram negative, Rod shape, anaerobic	PHBV, 1.44	Koller M et al., 2008
	Recombinant *E. coli*		Gram negative, Rod shape,	PHB, 9.0	Nikel PI et al., 2006

Category	Substrate	Organism	Characteristics	PHA (type, value)	Reference
Whey			Both aerobic and anaerobic		
		Recombinant *E. coli*	Gram negative, Rod shape, Both aerobic and anaerobic	PHB, 5.2	Lee SY et al., 1997
		Thermus thermophiles HB8	Gram negative, Rod shape, Aerobic	PHA, 0.51	Yellore et al., 1998
		Methylobacterium sp. ZP24	Gram-Negative, Rods and Cocci, Aerobic	PHA, 2.6-5.9	Nath A et al., 2008
		Methylobacterium sp. ZP24		PHB, 6.12	Koller M et al., 2011
		E. coli GCSC 6576	Rod shape,	PHB, 109	Wong and Lee., 1998
		E. coli GCSC 4401	Both aerobic and anaerobic	PHB, 96.2	Ahn WS et al., 2000
Plant sugar					
Lignocellulosic raw materials	Hemicellulosic fraction of poplar wood	*Pseudomonas pseudoava* (Burkholderi cepacia ATCC 17759)		PHB, 6.57	Bertrand J-L et al., 1990
	Xylose; xylose with propionic acid	*Burkholderia cepacia ATCC 17759*	Gram-negative , Rod shape,	PHB; PHBV, 1.6-3.7	Ramsay JA et al., 1995 and Young FK et al., 1994
	Xylose with levulinic acid	*Burkholderia cepacia ATCC 17759*		PHBV, 1.3-4.2	
	Hemicellulosic hydrolysates	*Burkholderia cepacia ATCC 17759*		PHB, 2.0	Keenan TM et al., 2006

Category	Substrate	Organism	Characteristics	PHA (type, value)	Reference
	Xylose, glucose from sugar cane bagasse	*Burkholderia cepacia* IPT 048 and *B. sacchari* IPT 101	Facultative aerobic	PHB, 34.8	Silva LF et al., 2005
	Xylose and glucose	*E. coli PTS* mutant	Rod shape, Both aerobic and anaerobic	PHA, 0.476	Li R et al., 2007
	Wheat bran hydrolysate	*Halomonas boliviensis* LC1	gram-negative, rod-shaped, aerobe	PHB, 4	Van-Thuoc D et al., 2008
	Cellulose, in tequila bagasse	*Saccharophagus degradans* ATCC 43961	Gram-negative, rod shaped, aerobic	PHA, 1.5	Munoz LEA et al., 2008
	Formic acid, acetic acid, furfural and acid soluble lignin	*Ralstonia eutropha*	Gram-negative , Rod shape, Facultative aerobic	PHB, 6.1-6.8	Yu J et al., 2008
	Wheat straw	132 strains used including 2 *bacillus* strain and 4 lactic acid bacteria	Not identified	PHB, 1.23	(Chander, 2019)
Other sugars	Orange peel	Bacillus subtilis NCDC0671	Gram positive Rod shape Aerobic	2.04 g/L	Umesh et al., 2017
Starch based raw material	Cassava starch	NR	NR	QU	(Chander, 2019)
	Banana Peel starch	NR	NR	QU	(Chander, 2019)
	Corn starch	NR	NR	PLA, QU	(Chander, 2019)
	Potato starch	NR	NR	QU	(Chander, 2019)
Fatty Acid Waste					
	Unsaponified olive oil	*Aeromonas caviae*	Gram-negative, rod shaped, facultative anaerobic	mcl-PHA, max. 96 wt%	Ashby RD et al., 1998 and Cromwick AM et al., 1996

Substrate	Microorganism	Characteristics	PHA, amount	Reference
Fats, vegetable oils and waste cooking oils — Lard, butter oil, olive oil, coconut oil, soybean oil	*Pseudomonas aeruginosa* and *Pseudomonas resinovorans*	Gram-negative, Rod shape, Aerobic	mcl-PHA, 2.1	Ashby RD et al., 1998
Olive oil, corn oil and palm oil	*C. necator* (also known as *R. eutropha, A. eutropha*)	Gram-negative, Rod shape, Facultative aerobic	PHB, 3.4	Thakor N et al., 2005 and Fukui T et al., 1998
Olive oil, corn oil and palm oil	*P. putida; C. necator.*	Gram-negative, Rod shape, aerobic/ Facultative aerobic	PHA, 1.6	Chaudhry W et al., 2011
Castor seed oil, coconut oil, mustard oil, cottonseed oil, groundnut oil, olive oil, and sesame oil	*Comamonas testosteroni*	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
Lard and coconut oil	*Pseudomonas putida*	Gram-negative, Rod shape, Aerobic	PHA, 0.9-1.6	Solaiman DKY et al., 2001
Coconut oil and tallow	*Pseudomonas saccharophila*	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	*C. necator*	Gram-negative, Rod shape, Facultative aerobic	PHA, 3.3	Loo C-Y et al., 2005
Palm kernel oil	*C. necator*		P(3HB-co-3HV-co-3HHx); 6.79	Bhubalan K et al., 2008
	C. necator		PHA, 2.5	Lee W-H et al., 2008
	Pseudomonas stutzeri	Gram-negative, rod-shaped, facultative anaerobe	PHA, 1.4	He W et al., 1998
Soybean oil	*C. necator H16* and recombinant strains	Gram-negative, Rod shape, Facultative aerobic	PHA,102	Kahar P et al., 2004
	C. necator mutants		PHA, 1	Tsuge T et al., 2009
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
Linseed oil	*Pseudomonas aeruginosa*		PHA, 1.8, 50.2% CDW	Bassas M et al., 2008
Brassica carinata oil	*Pseudomonas aeruginosa*		PHA, 5%	Fuller RC et al., 1999
Waste cooking oil	*Pseudomonas aeruginosa*	Gram-negative, rod-shaped, Aerobic or adoptive anaerobic	PHA, 2.3	Fernández D et al., 2005
	Pseudomonas aeruginosa		PHA, 5.4	Haba E et al.,2007
	Pseudomonas aeruginosa		PHA, 3.43	Costa S et al., 2009
Spent palm oil	*Cupriavidus necator*	Gram-negative, Rod shape, Facultative aerobic	P(3HB-co-4HB), 4.4	Rao U et al., 2010
Waste vegetable oil	*Pseudomonas sp.* strain DR2	Gram-negative, rod-shaped, Aerobic or adoptive anaerobic	PHA, 23.5% CDW	Song JH et al., 2008

Polyhydroxyalkanoates are synthesized by different microorganims 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).

III. MICROBIAL STRAIN

Ralstonia eutropha 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℃ (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
Saccharides	fructose, maltose, lactose, xylose, arabinose, glucose,	Saccharides are rich in Carbon, easily available and degradable by microbes present in environment
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	CH4 and CO2	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 synthesize. 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 (NH_2), ammonium sulfate ($(NH_4)_2SO_4$, 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 synthesize of PHA by fermentation reaction (Yu., 2007).
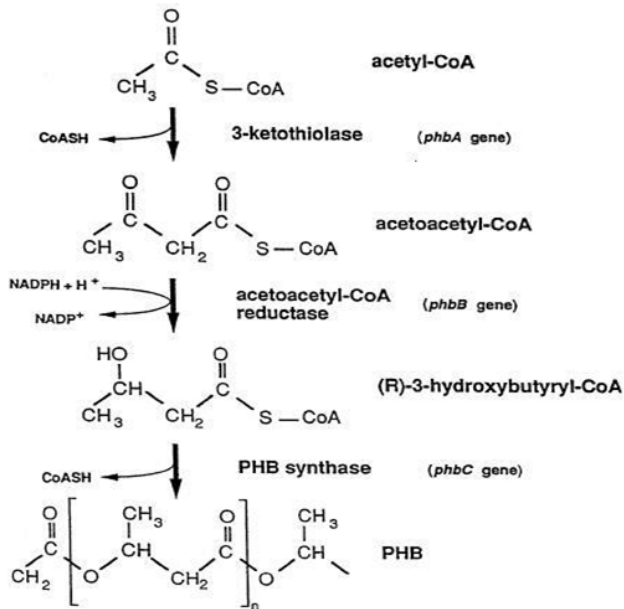
Figure 1: PHB synthesis pathway of R.eutropha

(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
	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-dichroloethane: 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 combing 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 CO_2, water, cell biomass when the plastic material is exposed to aerobic conditions as well as CH_4, CO_2, 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".
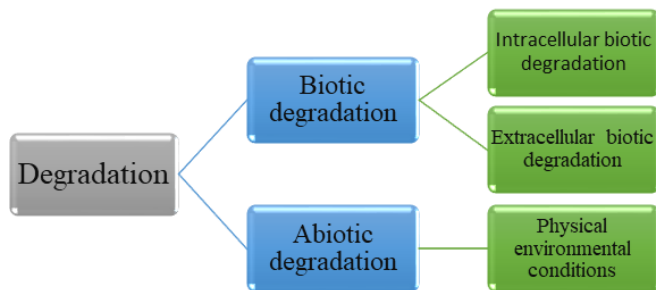
Figure 2: Types of Degradation of Biopolymer

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 (CH_4), carbon dioxide (CO_2), and water (H_2O). 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 eleminating these, biopolymers were introduced years ago which ressemblance the quality of polymers. The major drawback is , these biopolymers are not that much feasable 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 biodegradablity, 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.

VIII. References

1. Arcos-Hernandez M.V., Laycock B., Pratt S., Donose B.C., Nikolie M.A.L., Luckman P., Werker A., and Lant P.A., Biodegradation in a soil environment of activated sludge derived polyhydroxyalkanoate (PHBV). Polymer Degradation and Stability, 2012.

2. Cai Z., Hou C., and Yang G., Characteristics and blending performance of electroactive polymer blend made with cellulose and poly (3-hydroxybutyrate). Carbohydrate Polymers, 2012; 87: 650-657.

3. Chan R.T., Garvey C.J., Marcal H., Russell R.A., Holden P.J., and Foster L.J.R., Manipulation of Polyhydroxybutyrate Properties through Blending with Ethy-Cellulose for a Composite Biomaterial. International Journal of Polymer Science, 2011.

4. Chander M., Microbial Production of Biodegradable Plastics from Agricultural Waste. International Journal of Research and Analytical Reviews, 2019; 6(2): 2349-5138.

5. Du C., Sabirova J., Soetaert W., Lin S.K.C., Polyhydroxyalkanoates Production from Low-cost Sustainable Raw materials. Current Chemical Biology, 2012; 6:1.

6. Gustafsson J., Landberg M., Batori V., Akesson D., Taherzadeh M.J., and Zamani A., Development of Bio-Based Films and 3D Objects from Apple Pomace. Polymers 2019; 11: 289.

7. Hohne G., Hemminger W.F., and Flammersheim H.J., Differential Scanning Calorimetry. Springer, 2003.

8. http://www.ncbi.nlm.nih.gov

9. https://www.researchgate.net/figure/Fig-II-3-Pathway-of-PHB-synthesis_fig2_312934859

10. https://www.researchgate.net/figure/Updated-prices-of-bioplastics_tbl5_266850155

11. Iriani E.S., Permana A.W., Yuliani S., Kailaku S.I., and Sulaiman A.A., The effect of Agricultural waste Nanocellulose on The Properties of Bioplastic for Fresh Fruit Packaging. Earth and Environmental Science, 2019; 309: 012035.

12. Kaur M., Aggrawal N.K., Kumar V., and Dhiman R., Effects and Management of Partheniumhysterophorus : A Weed of Global Significance. International Scholarly Research Notices, 2014.

13. Kim J.S., Lee Y.Y., Kim T.H., A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. Bioresource Technology, 2015; 08:084.

14. Lavanya D., Kulkarni P.K., Dixit M., Raavi P.K., Krishna L.N.V., Sources of Cellulose and their Applications-A Review. International Journal of Drug formulation and Research, 2011; 2(6): 2229-5054.

15. Luengo J.M., Garcia B., Sandoval A., Naharro G., and Olivera E.R., Bioplastics from microorganisms. Current Opinion in Microbiology, 2003; 6: 251-260.

16. Mohanty A.K., Wibowo A., Misra M., and Drzal L.T., Development of Renewable Resource- Based Cellulose

Acetate Bioplastic: Effect of Process Engineering on the Performance of Cellulosic Plastics. Polymer Engineering and Science, 2003; 43: 5.

17. Mousavioun P., George G.A., and Doherty W.O., Environmental degradation of lignin/poly (hydroxybutyrate) blends. Polymer Degradation and Stability, 2012; 97: 1114-1122.

18. Mudenur C., Mondal K., Singh U., Katiyar V., Production of Polyhydroxyalkanoates and its Potential Applications. Advances in Sustainable Polymer, 2019.

19. Raza Z.A., Tariq M.R., Majeed M.I., Banat I.M., Recent developments in bioreactor scale production of bacterial polyhydroxyalkanoates. Bioprocess and Biosystems Engineering, 2019; 42: 901-919.

20. Ruiz C., Kenny S.T., Narancic T., Babu R., and Connor K.O., Conversion of waste cooking oil into medium chain polyhydroxyalkanoates in a high cell density fermentation. Journal of Biotechnology, 2019; 8:20.

21. Samer M., Khalefa Z., Abdelall T., Moawya W., Farouk A., Abdelaziz S., Soliman N., Solah A., Gomaa M., Mohamed M., Bioplastics production from agricultural crop residues. Agricultural Engineering International: CIGR Journal, 2019; 21(3): 190-194.

22. Saratale G.D., Oh M.K., Characterization of poly-3-hydroxybutyrate (PHB) produced from Ralstonia eutropha using an alkali-pretreated biomass feedstock. International Journal of Biological Macromolecules, 2015; 80: 627-635.

23. Sartori T., Tibolla H., Prigol E., Colla L.M., Cost J.A.V.C., and Bertolin T.E., Enzymatic Saccharification of Lignocellulosic Residues by Cellulases obtained from Solid State Fermentation Using Trichodermaviride. BioMed Research International, 2015; 342716:9.

24. Sepe P., and Limited R.T. Thermal Analysis of Polymers, Rapra Technology Limited.

25. Shrivastav A., Mishra S.K., Pancha I., Jain D., Bhattacharya S., Patel S., and Mishra S., Biodegradability studies of polyhydroxyalkanoate (PHA) film produced by a marine bacteria using Jatropha biodiesel byproduct as a substrate. World Journal of Microbiology and Biotechnology, 2011; 27: 1531-1541.

26. Sudesh K., Abe H., Doi Y., Synthesis, structure and properties of polyhydroxyalkanoates : biological polyesters. Progress in Polymer Science, 2000; 25: 1503-1555.

27. Verlinden R.A.J., Hill D.J., Kenward M.A., Williams C.D., and Radecka I., Bacterial synthesis of biodegradable polyhydroxyalkanoates. Journal of Applied Microbiology, 2007; 102:1437-1449.

28. Wang G., Cheng G., Ma S., Cai Z., and Zhang L., Crystallization behaviour, mechanical properties, and environmental biodegradability of poly (β-hydroxybutyrate)/cellulose acetate butyrate blends. Journal of Applied Polymer Science, 2003; 89: 2116-2122.

29. Webb H.K., Arnott J., Crawford R.J., and Ivanova E.P., Plastic Degradation and its Environmental Implications with special Reference to poly (ethylene terepthalate). Polymers, 2013; 5:1-18.

30. Yu J., Stahl H., Microbial utilization and biopolyester synthesis of bagasse hydrolysates. Bioresource Technology, 2009; 99: 8042-8048.

31. Yu L., Dean K., and Li L., Polymer blends and composites from renewable resources. Progress in Polymer Science, 2006; 31: 576-602.

32. Yustinah, Hidayatet N., Alamsyah R., Roslan A.M., Hermansyah H., Gozan M., Production of Polyhydroxyalkanoate from Oil Palm empty fruit bunch (OPEFB) hydrolysates by Bacillus cereus suaeda B-001. Biocatalysis and Agricultural Biotechnology, 2019; 18: 101019.

33. Mary Siji.K., Pillai P.K.S., Amma D.B., Pothen L.A., Thomas S., Handbook of Biopolymer-Based Materials: From Blends and Composites to Gels and Complex Network, 2013; 26: 777-799.

34. Bhatt R., Patel K., Trivedi U., A Handbook of Applied Biopolymer Technology: Synthesis, Degradation and Applications. 2013; 10: 51: 35: 311- 331.

35. Bhat R. A., Qadri H., Wani K. A., Dar G.H., Mehmood M. A., Innovative Waste Management Technologies for Sustainable Development. IGI Global: International Publisher of Information Science and Technology Research, 2020; 4: 52-81.

36. Singh O.V., Chandel A. K., Sustainable Biotechnology- Enzymatic Resources Renewable Energy , Springer International Publishing AG, 2018; 15: 399-421.

37. Volova T.G., Boyandin A.N., Vasiliev A.D., Karpov U.A., Prudnikova S.V., Mishukova O.V., Boyarskikh U.A., Filipenko M.L., Rudnev V.P., Xuan B.B., Dung V.V., Gitelson I.I., Biodegradation of polyhydroxyalkanoates (PHAs) in tropical coastal waters and identification of PHA-degrading bacteria. Polymer Degradation and Stability. 2010; 95: 2350-2359.

38. Kumar B.S., Prabakaran G., Production of PHB (bioplastics) using bio-effluent as substrate by Alcaligens eutrophus. Indian Journal of Biotechnology. 2006; 5: 76-79.

39. Raza Z A, Abid S, Banat I M. Polyhydroxyalkanoates: Charaterizations, production, recent developments and applications. Internatinal Biodeterior Biodegradtion, 2018; 126: 45-56.

40. Cui Y W, Shi Y P, Gong X Y. Effects of C/N in the substrate on the simultaneous production of polyhydroxyalkanoates and extracellular polymeric substances by Haloferax mediterranei via kinetic model analysis. Journal of Chemical Society, 2017; 7:18953–18961.

41. Martinez G A, Rebecchi S, Decorti D, Domingos J M B, Rio D D, Bertin L, Porto C D, Fava F. Towards multi-purpose biorefinery platforms for the valorisation of red grape pomace: production of polyphenols, volatile fatty acids, polyhydroxyalkanoates and biogas. Green Chemistry, 2016; 18:261–270.

42. Albuquerque M, Torres C, Reis M. Polyhydroxyalkanoate (PHA) production by a mixed microbial culture using sugar molasses: effect of the influent substrate concentration on culture selection. Water Resource, 2010; 44:3419–3433.

43. Kellerhals M B, Kessler B, Witholt B, Tchouboukov A, Brandl H. Renewable long-chain fatty acids for production of biodegradable medium-chain-length polyhydroxyalkanoates (mcl-PHAs) at laboratory and pilot plant scales. Macromolecules, 2000; 33:4690–4698.

44. Israni N, Shivakumar S. Evaluation of upstream process parameters influencing the growth associated PHA accumulation in Bacillus sp. Journal of Scientific and Industrial Research, 2015;74:290–295.

45. Masood F, Abdul-Salam M, Yasin T, Hameed A. Effect of glucose and olive oil as potential carbon sources on production of PHAs copolymer and tercopolymer by Bacillus cereus FA11. 3 Biotechnology, 2017; 7:87–101.

46. Shahid S, Mosrati R, Ledauphin J, Amiel C, Fontaine P, Gaillard J L, Corroler D. Impact of carbon source and variable nitrogen conditions on bacterial biosynthesis of polyhydroxyalkanoates: evidence of an atypical metabolism in Bacillus megaterium DSM 509. Journal of Bioscience and Bioengineering, 2013; 116:302–308.

47. Park S J, Jang Y A, Noh W, Oh Y H, Lee H, David Y, Baylon M G, Shin J, Yang J E, Choi S Y, Lee S H, Lee S Y. Metabolic engineering of Ralstonia eutropha for the production of polyhydroxyalkanoates from sucrose. Biotechnology and Bioengineering, 2015; 112:638–643.

48. Eggink G, Steinbuchel A, Poirier A, Witholt B. International symposium on bacterial polyhydroxyalkanoates. NRC Research Press, Toulouse, 1997.

49. Byrom D. Production of poly-β-hydroxybutyrate: poly-β-hydroxyvalerate copolymers. FEMS Microbiology Letter, 1992; 103:247–250.

50. Pandian S R, Deepak V, Kalishwaralal K, Rameshkumar N, Jeyaraj M, Gurunathan S. Optimization and fed-batch production of PHB utilizing dairy waste and sea water as nutrient sources by Bacillus megaterium SRKP-3. Bioresource Technology, 2010; 101:705–711.

51. Kim S W, Kim P, Lee H S, Kim J H. High production of poly-β-hydroxybutyrate (PHB) from Methylobacterium organophilum under potassium limitation. Biotechnology Letter, 1996; 18:25–30.

52. Khosravi-Darani K, Mokhtari Z B, Amai T, Tanaka K. Microbial production of poly(hydroxybutyrate) from C1 carbon sources. Applied Microbiology and Biotechnology, 2013; 97:1407–1424.

53. Muhr A. Biodegradable latexes from animal-derived waste: biosynthesis and characterization of mcl-PHA accumulated by Ps. citronellolis. Reactive and Functional Polymers, 2013; 73:1391–1398.

54. Akiyama M, Taima Y, Doi Y. Production of poly(3-hydroxyalkanoates) by a bacterium of the genus Alcaligenes utilizing long-chain fatty acids. Applied Microbiology and Biotechnology, 1992; 37:698–701.

55. Shay E G. Diesel fuel from vegetable oils: status and opportunities. Biomass Bioenergy, 1993; 4:227–242.

56. Rincon J, Camarillo R, Rodriguez L, Ancillo V. Fractionation of used frying oil by supercritical CO2 and cosolvents. Industrial and Engineering Chemistry Research, 2010; 49:2410–2418.

57. Jiang G. Carbon sources for polyhydroxyalkanoates and an integrated biorefinery. International Journal of Molecular Science, 2016; 17:1157.

58. Gumel A, Annuar M, Heidelberg T. Growth kinetics, effect of carbon substrate in biosynthesis of mcl-PHA by Pseudomonas putida Bet001. Brazilian Journal of Microbiology, 2014; 45:427–438.

59. Kumar M, Singhal A, Verma PK, Thakur I S. Production and characterization of polyhydroxyalkanoate from lignin derivatives by Pandoraea sp. ISTKB. Journal of the American Chemical Society, 2017; 2:9156–9163.

60. Hao J, Wang X, Wang H. Overall process of using a valerate-dominant sludge hydrolysate to produce high-quality polyhydroxyalkanoates (PHA) in a mixed culture. Journal of Natural Products, 2017; 7:6939–6943.

61. Yu J, Si Y. A dynamic study and modeling of the formation of polyhydroxyalkanoates combined with treatment of high strength wastewater. Environmental Science and Technology, 2001; 35:3584–3588.

62. Begun G, Palko A, Brown L. The ammonia-ammonium carbonate system for the concentration of nitrogen-15. Journal of Physical Chemistry, 1956; 60:48–51.

63. Hu D. Biosynthesis and characterization of polyhydroxyalkanoate block copolymer P-3-HB-b-P-4-HB. Biomacromolecule, 2011; 12:3166–3173.

64. Rehm B H. Bacterial polymers: biosynthesis, modifications and applications. Nature Reviews Microbiology, 2010; 8:578.

65. Sreekanth M, Vijayendra S, Joshi G, Shamala T. Effect of carbon and nitrogen sources on simultaneous production of α-amylase and green food packaging polymer by Bacillus sp. CFR 67. J Food Sci Technol, 2013; 50:404–408.

66. Zhao D. Improving polyhydroxyalkanoate production by knocking out the genes involved in exopolysaccharide biosynthesis in Haloferax mediterranei. Appl Microbiol Biotechno, 2013; l 97:3027–3036.

67. Xu Z, Dai X, Chai X. Effect of influent pH on biological denitrification using biodegradable PHBV/PLA blends as electron donor. Biochem Eng J , 2018; 131:24–30.

68. Liu C, Luo G, Wang W, He Y, Zhang R, Liu G. The effects of pH and temperature on the acetate production and microbial community compositions by syngas fermentation. Fuel, 2018; 224:537–544.

69. Poblete-Castr I, Escapa I E, Jager C, Puchalka J, Lam J M C, Schomburg D, Prieto M P, Dos Santos V A P. The metabolic response of P. putida KT2442 producing high

levels of polyhydroxyalkanoate under single-and multiple-nutrientlimited growth: highlights from a multi-level omics approach. Microb Cell Fact, 2012; 11:1–21.

70. Kang M, Peng S, Tian Y, Zhang H. Effects of dissolved oxygen and nutrient loading on phosphorus fluxes at the sediment–water interface in the hai river estuary, China. Mar Pollut Bull, 2018; 130:132–139.

71. Third KA, Newland M, Cord-Ruwisch R. The effect of dissolved oxygen on PHB accumulation in activated sludge cultures. Biotechnol Bioeng, 2003; 82:238–250.

72. De Almeida A, Giordano AM, Nikel PI, Pettinari MJ. Effects of aeration on the synthesis of poly(3-hydroxybutyrate) from glycerol and glucose in recombinant Escherichia coli. Appl Environ Microbiol, 2010; 76:2036–2040.

73. Korkakaki E, Van Loosdrecht MC, Kleerebezem R (Impact of phosphate limitation on PHA production in a feastfamine process. Water Res 126:472–480

74. Albuquerque M G E, Eiroa M, Torres C, Nunes B R, Reis M A M. Strategies for the development of a side stream process for polyhydroxyalkanoate (PHA) production from sugar cane molasses. J Biotechnol, 2007; 130: 411-21.

75. Page WJ. Production of polyhydroxyalkanoates by Azotobacter vinelandii UWD in beet molasses culture. FEMS Microbiol Rev, 1992; 103: 149-57.

76. Chen G-Q, Page WJ. Production of poly-b-hydroxybutyrate by Azotobacter vinelandii in a two-stage fermentation process. Biotechnol Tech, 1997; 11: 347-50.

77. Pisco AR, Bengtsson S, Werker A, Reis MAM, Lemos PC. Community Structure Evolution and Enrichment of GlycogenAccumulating Organisms Producing Polyhydroxyalkanoates from Fermented Molasses. Appl Environ Microb, 2009; 75: 4676-86.

78. Bengtsson S, Pisco AR, Reis MAM, Lemos PC. Production of polyhydroxyalkanoates from fermented sugar cane molasses by a mixed culture enriched in glycogen accumulating organisms. J Biotechnol, 2010; 145: 253-63.

79. Wu Q, Huang H, Hu G, Chen J, Ho K P, Chen G Q. Production of poly-3-hydroxybutrate by Bacillus sp. JMa5 cultivated in molasses media. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol, 2001; 80: 111-8.

80. Chaijamrus S, Udpuay N. Production and characterization of polyhydroxybutyrate from molasses and corn steep liquor produced by Bacillus megaterium ATCC 6748. Agric Eng Int: The CIGR E J, 2008;X.

81. Kulpreecha S, Boonruangthavorn A, Meksiriporn B, Thongchul N. Inexpensive fed-batch cultivation for high poly(3-hydroxybutyrate) production by a new isolate of Bacillus megaterium. J Biosci Bioeng, 2009; 107: 240-5.

82. Solaiman DKY, Ashby RD, Hotchkiss Jr AT, Foglia TA. Biosynthesis of medium-chain-length Poly(hydroxyalkanoates) from soy molasses. Biotechnol Lett, 2006a; 28: 157-62.

83. Full TD, Jung DO, Madigan MT. Production of polyhydroxyalkanoates from soy molasses oligosaccharides by

new, rapidly growing Bacillus species. Lett Appl Microbiol, 2006; 43: 377-84.

84. Law K-H, Leung Y-C, Lawford H, Chua H, Lo W-H, Yu P. Production of polyhydroxybutyrate by Bacillus species isolated from municipal activated sludge. Appl Biochem Biotech, 2001; 91-93: 515-24.

85. Koller M, Bona R, Chiellini E et al. Polyhydroxyalkanoate production from whey by Pseudomonas hydrogenovora. Bioresource Technol, 2008; 99: 4854-63.

86. Lee SY, Middelberg APJ, Lee YK. Poly(3-hydroxybutyrate) production from whey using recombinant Escherichia coli. Biotechnol Lett, 1997; 19: 1033-5.

87. Wong HH, Lee SY. Poly-(3-hydroxybutyrate) production from whey by high-density cultivation of recombinant Escherichia coli. Appl Microbiol Biotechnol, 1998; 50: 30-3.

88. Ahn WS, Park SJ, Lee SY. Production of poly(3-Hydroxybutyrate) by fed-batch culture of recombinant Escherichia coli with a highly concentrated whey solution. Appl Environ Microb, 2000; 66: 3624- 7.

89. Marangoni C, Furigo Jr A, de Aragão GMF. Production of poly(3- hydroxybutyrate-co-3-hydroxyvalerate) by Ralstonia eutropha in whey and inverted sugar with propionic acid feeding. Process Biochem, 2002; 38: 137-41.

90. Yellore, Desai. Production of poly-3-hydroxybutyrate from lactose and whey by Methylobacterium sp. ZP24. Lett Appl Microbiol, 1998; 26: 391-4.

91. Nath A, Dixit M, Bandiya A, Chavda S, Desai AJ. Enhanced PHB production and scale up studies using cheese whey in fed batch culture of Methylobacterium sp. ZP24. Bioresource Technol, 2008; 99: 5749-55.

92. Koller M, Hesse P, Salerno A, Reiterer A, Braunegg G. A viable antibiotic strategy against microbial contamination in biotechnological production of polyhydroxyalkanoates from surplus whey. Biomass Bioenerg, 2011; 35: 748-53.

93. Lin CSK, Luque R, Clark JH, Webb C, Du C. Wheat-based biorefining strategy for fermentative production and chemical transformations of succinic acid. Biofuels Bioprod Bior, 2012; 6: 88-104.

94. Munoz LEA, Riley MR. Utilization of cellulosic waste from tequila bagasse and production of polyhydroxyalkanoate (pha) bioplastics by Saccharophagus degradans. Biotechnol Bioeng, 2008; 100: 882- 8.

95. Bertrand J-L, Ramsay BA, Ramsay JA, Chavarie C. Biosynthesis of Poly--Hydroxyalkanoates from Pentoses by Pseudomonas pseudoflava. Appl Environ Microb, 1990; 56: 3133-8.

96. Ramsay JA, Hassan M-CA, Ramsay BA. Hemicellulose as a potential substrate for production of poly(-hydroxyalkanoates). Can J Microbiol, 1995; 41: 262-6.

97. Young FK, Kastner JR, May SW. Microbial Production of poly-- hydroxybutyric acid from d-xylose and lactose by Pseudomonas cepacia. Appl Environ Microb 1994; 60: 4195-8.

98.   Keenan TM, Nakas JP, Tanenbaum SW. Polyhydroxyalkanoate copolymers from forest biomass. J Ind Microbiol, 2006; 33: 616-26.

99.   Keenan TM, Tanenbaum SW, Nakas JP. Microbial formation of polyhydroxyalkanoates from forestry-based substrates. ACS Symposium Series, 2006; 921: 193-209.

100.   Silva LF, Taciro MK, Michelin Ramos ME, Carter JM, Pradella JGC, Gomez JGC. Poly-3-hydroxybutyrate (P3HB) production by bacteria from xylose, glucose and sugarcane bagasse hydrolysate. J Ind Microbiol Biot, 2004; 31: 245-54.

101.   Li R, Chen Q, Wang PG, Qi Q. A novel-designed Escherichia coli for the production of various polyhydroxyalkanoates from inexpensive substrate mixture. Appl Microbiol Biot, 2007; 75: 1103-9.

102.   Van-Thuoc D, Quillaguamn J, Mamo G, Mattiasson B. Utilization of agricultural residues for poly(3-hydroxybutyrate) production by Halomonas boliviensis LC1. J Appl Microbiol, 2008; 104: 420-8.

103.   Yu J, Stahl H. Microbial utilization and biopolyester synthesis of bagasse hydrolysates. Bioresource Technol, 2008; 99: 8042-8.

104.   Ashby RD, Foglia TA. Poly(hydroxyalkanoate) biosynthesis from triglyceride substrates. Appl Microbiol Biot, 1998; 49: 431-7.

105.   Cromwick AM, Foglia T, Lenz RW. The microbial production of poly(hydroxyalkanoates) from tallow. Appl Microbiol Biot, 1996; 46: 464-9.

106.   Ntaikou I, Kourmentza C, Koutrouli EC et al. Exploitation of olive oil mill wastewater for combined biohydrogen and biopolymers production. Bioresource Technol, 2009; 100: 3724-30.

107.   Chaudhry W, Jamil N, Ali I, Ayaz M, Hasnain S. Screening for polyhydroxyalkanoate (PHA)-producing bacterial strains and comparison of PHA production from various inexpensive carbon sources. Ann Microbiol, 2011; 61: 623-9.

108.   Thakor N, Trivedi U, Patel KC. Biosynthesis of medium chain length poly(3-hydroxyalkanoates) (mcl-PHAs) by Comamonas testosteroni during cultivation on vegetable oils. Bioresource Technol, 2005; 96: 1843-50.

109.   He W, Tian W, Zhang G, Chen G-Q, Zhang Z. Production of novel polyhydroxyalkanoates by Pseudomonas stutzeri 1317 from glucose and soybean oil. FEMS Microbiol Lett, 1998; 169: 45-9.

110.   kahar P, Tsuge T, Taguchi K, Doi Y. High yield production of polyhydroxyalkanoates from soybean oil by Ralstonia eutropha and its recombinant strain. Polym Degrad Stabil, 2004; 83: 79-86.

111.   Allen A, Anderson W, Ayorinde F, Eribo B. Biosynthesis and characterization of copolymer poly(3HB-3HV) from saponified Jatropha curcas oil by Pseudomonas oleovorans. J Ind Microbiol Biot 2010; 37: 849-56.

112.   Fernández D, Rodríguez E, Bassas M, et al. Agro-industrial oily wastes as substrates for PHA production by the new strain Pseudomonas aeruginosa NCIB 40045: Effect of culture conditions. Biochem Eng J 2005; 26: 159-67.

113.   Haba E, Vidal-Mas J, Bassas M, Espuny MJ, Llorens J, Manresa A. Poly 3-(hydroxyalkanoates) produced from oily substrates by Pseudomonas aeruginosa 47T2 (NCBIM 40044): Effect of nutrients and incubation temperature on polymer composition. Biochem Eng J 2007; 35: 99-106.

114.   Costa S, Lépine F, Milot S, Déziel E, Nitschke M, Contiero J. Cassava wastewater as a substrate for the simultaneous production of rhamnolipids and polyhydroxyalkanoates by Pseudomonas aeruginosa. J Ind Microbiol Biot 2009; 36: 1063-72.

115.   Rao U, Sridhar R, Sehgal PK. Biosynthesis and biocompatibility of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) produced by Cupriavidus necator from spent palm oil. Biochem Eng J 2010; 49: 13-20.

116.   Nikel PI, de Almeida A, Melillo EC, Galvagno MA, Pettinari MJ. New Recombinant Escherichia coli Strain Tailored for the Production of Poly(3-Hydroxybutyrate) from Agroindustrial By-Products. Appl Environ Microb 2006; 72: 3949-54.

117.   Fuller RC. Microbial inclusions with special reference to PHA inclusions and intracellular boundary envelopes. Int J Biol Macromol 1999; 25: 21-9.

118.   Fukui T, Doi Y. Efficient production of polyhydroxyalkanoates from plant oils by Alcaligenes eutrophus and its recombinant strain. Appl Microbiol Biotechnol 1998; 49: 333-6.

119.   Song JH, Jeon CO, Choi MH, Yoon SC, Park W: Polyhydroxyalkanoate (PHA) production using waste vegetable oil by Pseudomonas sp. strain DR2. J Microbiol Biotechnol 2008; 18: 1408- 1415.

120.   Solaiman DKY, Ashby RD, Foglia TA. Production of polyhydroxyalkanoates from intact triacylglycerols by genetically engineered Pseudomonas. Appl Microbiol Biot 2001; 56: 664-9.

121.   Solaiman DKY, Ashby RD, Foglia TA. Medium-Chain-Length Poly(-Hydroxyalkanoate) Synthesis from Triacylglycerols by Pseudomonas saccharophila. Curr Microbiol 1999; 38: 151-4.

122.   Loo C-Y, Lee W-H, Tsuge T, Doi Y, Sudesh K. Biosynthesis and Characterization of Poly(3-hydroxybutyrate-3-hydroxyhexanoate) from Palm Oil Products in a Wautersia eutropha Mutant. Biotechnol Lett 2005; 27: 1405-10.

123.   Bhubalan K, Lee W-H, Loo C-Y, et al. Controlled biosynthesis and characterization of poly(3-hydroxybutyrate-co-3-hydroxyva-lerateco-3-hydroxyhexanoate) from mixtures of palm kernel oil and 3HV-precursors. Polym Degrad Stabil 2008; 93: 17-23.

124.   Lee W-H, Loo C-Y, Nomura CT, Sudesh K. Biosynthesis of polyhydroxyalkanoate copolymers from mixtures of plant oils and 3- hydroxyvalerate precursors. Bioresource Technol 2008; 99: 6844- 51.

125.   Tsuge T, Yamamoto T, Yano K, Abe H, Doi Y, Taguchi S. Evaluating the ability of Polyhydroxyalkanoate

synthase mutants to produce P(3HB-co-3HA) from soybean oil. Macromol Biosci 2009; 9: 71-8.

126. Martinez-Toledo MV, Gonzalez-Lopez J, Rodelas B, Pozo C, Salmeron V. Production of poly--hydroxybutyrate by Azotobacter chroococcum H23 in chemically defined medium and alpechin medium. J Appl Microbiol 1995; 78: 413-8.

127. Bassas M, Marqués AM, Manresa A. Study of the crosslinking reaction (natural and UV induced) in polyunsaturated PHA from linseed oil. Biochemical Eng J 2008; 40: 275-83.

128. Ceyhan N, Ozdemir G. Poly-hydroxybutyrate (PHB) production from domestic wastewater using Enterobacter aerogenes 12Bi strain. Afr J Microbiol Res 2011; 5: 690-702.