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Impact of Microorganisms on the Production and Degradation Processes of the Natural Polymers, Polyhydroxyalkonates, For Replacement of the Dangerous Plastics

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Abstract: *The most dangerous problems these days are cleaning our earth from rubbish which is difficult to be degraded by time. Over the decades, the human population and the development is increasing rapidly all over the world in all field particularly in industrial field which is more vibrant than ever before. However, this development has contributed to the increased the amount of wastes rapidly. As a consequence, the earth is in danger since the environment has been ruined, and therefore, putting people's health in warning. With the passage of time, accumulation of massive amount of wastes especially of non-degradable waste in the world is threatened the environment and potential survival of many species of organisms. Developed countries such as China and USA have serious concerns about the rising number of non-biodegradable waste which is disposed of in landfills and oceans. Consequently, abundant countries have a novel research of projects in order to improve the ways how to disposal of pollutant residues from the biosphere and environment. Another solution is the use of biodegradable plastic which are safe ad ecofriendly materials. Polyhydroxyalkonates (PHA) produced by bacteria and could be an attractive to common plastics because it fit well with new waste management strategies. The use of PHA produced by bacterial fermentation as a commodity polymer is limited by its high production cost compared to some widely used petroleum derived plastics.*

Key words: *Plastic, pollutant, Polyhydroxyalkonates, Poly 3-hydroxybutyrate, bio- materials,*

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I. Problems of plastic and its dangerous components

In spite of the advantages, extensive uses of conventional plastic have serious drawbacks and considered as composed of toxic chemicals that contaminated air and water in the earth, causing many problems. The production and elimination of non degradable materials causes severe impact to environment especially in marine environment. It is major challenge faced countries these days is the ways to dispose the non degradable waste safely because of recycling alone not sufficient (Leonard 2018). The daily general non degradable wastes in the main cities in Saudi Arabia are around 7,000 tons, and 30% of the general wastes are non-biodegradable materials. In fact, non degradable wastes in general take up to 1,000 years to degrade in environment. The widespread use of plastics affect animals and human body and biomonitoring studies approved presence of steady-state concentration of plastics' components in the human body, thereby reflecting the ongoing balance of constant exposure, metabolism and excretion of these compounds (Calafat *et al.,* 2005). Detectable levels of bisphenol A have been found in the urine of 95% of the adult population (Vandenburg *et al.,* 2007). Health effects of plastic cause destructive effects on health and reproduction, early sexual maturation, decreased male fertility and aggressive behavior (Halden, 2010). Plastic packaging, bags and bottles are thrown away every day, and end up in trash sites as well as in forests, rivers, seas, and oceans around the world. Globally more than 200 species are known to be affected by marine rubbish including whales, seals, dugong, seabirds, turtles, crabs, sea snakes and sharks. Plastic pieces, floating in every square mile cause the death of over a million sea birds, whales, seals, dolphins, sea turtles and sharks (Derraik, 2002). It was reported that there is a need for replacement petroleum-based plastic with Bioplastics (Arikan and Ozsoy, 2015). This due to several reasons as: On burning traditional plastics, create toxic fumes which can be harmful to people's health and the environment. A conventional plastic consumes 65% more energy compared to bioplastic. Plastics are absolutely unsustainable, need a long time be degraded and cause huge damage to the environment. On contrast, Bioplastic is more sustainable, safe, mostly non toxic, need short period for degradation, saves 30-80% of the greenhouse gas emissions and returns to its natural state when buried in the ground increasing soil organic content thus increased nutrient retention with reducing chemical inputs and suppressing plant diseases. Despite the fact that polyethylene and Nylon 11 (NY11) can be produced from biomass or renewable resources, they are non-biodegradable. Acetyl cellulose (AcC) is either biodegradable or non-biodegradable, depending on

the degree of acetylation. Acetyl cellulose with a low acetylation can be degraded, while those with high substitution ratios are non-biodegradable (Tokiwa *et al*., 2009).

Biodegradable Polymers

Polyhydroxyalkonates, Poly (hydroxybutyrate, poly lactic acid (PLA) are biodegradable polymers**.** Lactic acid is produced by the group of lactic acid producing bacteria by fermenting hexoses into lactic acid. The purified lactic acid is used as the precursor for the chemical synthesis of PLA. Starch blends are produced from biomass or renewable resources, and are thus biodegradable. Also, bio-based materials include starch-based materials, protein based materials and cellulose-blended materials (Mekonnen *et al*., 2013). They can also be blended with conventional plastic such as polyethylene, polypropylene (Rajendran, *et al*., 20112) and poly (vinyl alcohol) to get better properties but they are only partially biodegradable. The residual petroleum-based plastic remains as broken pieces creating additional pollution (Tripathi, *et al.*, 2016).

Why Biodegradable Polymers

Over the past few years, owing to rising petroleum prices and many environmental concerns related to non-degradable material pollutions which have been developed rapidly (Thompson *et al* .,2009). Increasingly reduction of carbon dioxide emissions has become another reason for promoting bio-based plastics (Chee, *et al.*,2010). According to United Nations Environment Programmed (UNEP) in 2006, non degradable waste causes the death of up to a million seabirds, 100,000 marine mammals and countless fish through various impacts (European commission, 2011). Moreover, non degradable wastes could be harmful to human and lead to cancer risk. Example of release chemical are dioxins that are organic environmental pollutants released by burning of waste, especially Polyvinyl chloride (PVC).These dioxins, after being released into the air, are taken up by fish and animals and accumulate in fat. People are risky to dioxins by eating meat, dairy foods, eggs and fish (Chee *et al*., 2010, Rubin 2011, Mehdi Emadian *et al*., 2017). Solid wastes accumulation affects human health and cause greenhouse gas emissions. Recent technologies must be aimed towards the production and development of bio-green materials with no side effects on the environment for biotechnological applications.

Importance of Natural Polymers (Bio- Materials)

Bio-material produced from renewable resource is degraded aerobically by microorganisms to carbon dioxide and water, meaning that the material returns to its natural component when buried in the ground which safe and clean our environments. The word 'bio- materials' is used mainly for biodegradable polymers which can be degraded by microorganisms.

To produce bio-based materials completely look like plastic based petroleum, bacteria are employed to make the building block for polymers from renewable sources, including starch ,cellulose, fatty acids, and bio waste (Chee, *et al.* 2010). Actually, the word bio- materials can refer either to bio-based plastics synthesized from biomass and renewable resources such as Poly(lactic acid) (PLA) and Polyhydroxyalkanoate (PHA) or plastics produced from fossil fuel including aliphatic plastics like Polybutylene succinate (PBS), which can also be utilized as a substrate by microorganisms (Tokiwa *et al*., 2009; Mekonnen *et al*., 2013). For instance, utilizing starch as a renewable resource in production of packaging bio-plastic resulted in a lower consumption of non-renewable energy resources (-50%) and therefore less greenhouse gas emissions (-60%) when compared to the polystyrene packaging (Razza *et al*., 2015).

These polymers are well known for their diverse applications in industries, domestic appliances, transportation, construction, shelters, storage and packaging practices. Such polymers are differentiated according to their chemical nature, structural arrangement, physical properties and applications (Shah et al. 2008).

Polyhydroxyalkanoates (PHA), Poly-lactic acid (PLA), poly butylenes succinate (PBS), polyethylene, poly trimethylene terephthalate (PTT) and poly -p- phenylene (PPP) are the best studied polymers containing at least one monomer synthesized by bacterial transformation. Among them, PHA, PLA, and PBS are well known for their biodegradability whereas PE, PTT and PPP are probably less biodegradable (Chee *et al.*,2010). In past years, the bio- material properties, whether physical or chemical , and their applications have been studied and developed to reduce their cost or improve their properties (Tokiwa *et al.*, 2009).

Polyhydroxyalkonates the most famous bacterial polymers

Polyhydroxyalkonates is composed of large part, or even completely of renewable resources that is organic polymers with high molecular. These materials can be shaped under heat and pressure to materials used safely in biotechnological application. Polymers of PHA are produced by the microbes as storage molecules under stress conditions such as excess carbon and limiting nitrogen (Verma *et al*., 2002; Huisman and Medison, 1999). They are natural polymers produced mainly by certain strains of bacteria and can be used in many fields, packing, agriculture, food adulteries. These organic polymers have high molecular weight and can be molded

under heat and pressure (Fridovich-Keil, 2016). Biotechnological production of PHA from special bacterial isolates is recommitted due to their biodegradation Thus, in order to create a sustainable environment and prevent the possible disposal of recalcitrant non-biodegradable materials & wastes in the environment, production of biodegradable materials gained a lot of attention due to their biodegradability, meaning that the material returns to its natural state when buried in the ground (Rajendran *et al*., 2012, Patel *et al.*, 2011, Mehdi Emadian *et al* ., 2017).

Indeed, it became apparent that the PHA polymers could be derived from the microbial fermentation of natural substrates under various growth conditions (Basnett and Roy 2010). The French chemist Maurice Lemoigne was the first to discover that Gram-positive bacterium produced them as intercellular materials (Digregorio, 2009). Production of these biopolymers in an industrial scale is already well established. They have been produced using cheap carbon sources to achieve economical production (Sodergard and Stolt, 2002).

Poly 3-hydroxybutyrate

Poly 3-hydroxybutyrate (PHB) is the most common type PHA and was first described by Lemoigne, a French scientist in year 1925 (Chee *et al*., 2010). It is a wide spread intracellular storage compound typically in prokaryotic organisms. Since then, various bacterial strains among Gram positive and Gram negative bacteria and photosynthetic bacteria including cyanobacteria have been identified to accumulate PHB both aerobically and anaerobically (Chaitanya *et al.*, 2014). The recognition of the role of PHB as a bacterial storage polymer that possesses a function almost similar to starch and glycogen was accepted. Macrae and Wilkinson (1973) noticed that *Bacillus megaterium* initiated the accumulation of PHB homopolymer when the ratio of glucose to nitrogen in the culture medium was high and the subsequent intracellular degradation of PHB occurred in the absence of carbon and energy sources. The opinion of PHB monomer as the only constituent of this polymer changed after a year of its acceptance as bacterial storage material when other types of monomers were discovered.

History of Poly-β-hydroxybutyrate:

In the early 1920s, Lemoigne a microbiologist at Pasteur Institute in Paris isolated a polymer from *Bacillus megaterium* by chloroform extraction and demonstrated that it was a polyester of 3-hydroxybutyric acid and since then, the polymer has presented many challenges to microbiologists and biochemists who are interested in its physiological functions and metabolism (Aly et al., 2011, Balaji *et al.*, 2013).

The general, it was found that PHB is only one type in a huge family of polymers collectively known as polyhydroxy alkanoate (PHA). In 1974, PHB was isolated by chloroform extraction of activated sludge (Balaji *et al.*, 2013). The monomers that were detected in chloroform extracts of activated sewage sludge are 3-hydroxyvalerate (3HV) and 3-hydroxyhexanoate (3HH) as the major and minor constituents respectively. About a decade later following the identification of heteropolymers, the analysis of marine sediments by capillary gas chromatography revealed the presence of 3HB and 3HV as the predominant components among 11 other short chain 3-hydroxyalkanoate monomers (Balaji *et al.*, 2013). Likewise, research on finding new PHBs is also in the streamline. Among the 150 different types of polyhydroxy alkanoids identified so far, the homopolymer of hydroxybutyrate like PHB is widespread in different taxonomic group of prokaryotes including cyanobacteria (Aly et al., 2103, Balaji *et al.*, 2013). The properties of pure PHB including thermoplastic processibility, hydrophobicity, complete biodegradability and biocompatibility with optical purity have increasingly become of interest as a raw material for biodegradable plastics.

Chemical composition and physical properties of poly-β-hydroxybutyrates:

Poly-β-hydroxybutyrate is synthesized from acetyl coenzyme A (acetylco A) via three enzymatic reactions. 3-Ketothiolase converts two acetyl-coA molecules to one acetoacetyl co A molecule, NADPH dependent acetoacetyl co A reductase converts acetoacetyl co A to D-3-hydroxy butyryl co A and the last enzyme PHB synthase catalyzes linking of the D-3-hydroxybutyryl moiety to an existing PHB molecule via an ester bond (Chien *et al.*, 2007). However, β and many other PHA are composed of 3 hydroxy fatty acids. The pendant group varies from methyl carbon 1 to tridecyl carbon 13. Fatty acids with the hydroxyl group at position 4, 5 and 6 pendant group containing substituents or unsaturation's are known. PHB and PHV (poly 3 hydroxyvalerate) form a class of PHA and are typically referred to as short chain length PHAs (Chien *et al.*, 2007). Copolymers of PHB are formed when mixed substrates are used such as a mix of glucose and valerate. The microorganisms convert the substrates into small chain linked PHAs like poly (3 hydroxy-butyrate co 3 hydroxy valerate) PHBV or poly (3 hydroxy butyrate co 4 hydroxybutyrate) PHB4B.

Bacteria produce PHBs with average molecular mass of up to 4.6×10^6 Da with a polydispersity (Mw/Mn) of around 2.0 and the material characteristics of these biopolymers are similar to conventional plastics such as polypropylene. PHB homopolymer is a highly crystalline, stiff, but brittle material. When spun into fibers it behaves as a hard-elastic material (Chien *et al.*, 2007). Homopolymer PHB has a helical

crystalline structure which seems to be similar in various copolymers. Melting behavior and crystallization of PHB have been studied and the physical properties have been differentiated (Balaji *et al.*, 2013).

Degradation of Polyhydroxyalkonates and Poly 3-hydroxybutyrate

A degradable materials in which the degradation results from the action of naturally accruing microorganisms like bacteria, fungi and algae (Gnanavel *et al.* 2012). For this to occur oxygen and water is resulted and the plastic must be derived from biopolymer rather petro polymers degradable polymers have attracted a lot of attention in the recent years (Kržan 2012).

Polyhydroxyalkonates accumulated as intracellular food storage by wide variety of microorganism under carbon rich growth conditions and is mobilized during nutrient starvation period under the influence of an enzyme, PHB depolymerase (Klingbeil *et al.*, 1996, Nojima *et al.*, 1996). PHB depolymerases hydrolyze PHB as a substrate and form monomers or dimers that serve as nutrients for microorganisms (Nakayama *et al.*, 1985). Since PHB has been widely accepted as biodegradable polymer, it is necessary to evaluate its biodegradation, the role of polymer-degrading microorganisms, factors affecting the activity of PHB degradation and kinetics of PHB degrading enzyme (Wani *et al.*, 2016).There are number of bacteria isolates from soil and water seem to have significant production of polyhydroxyalkonates which can used as alternative polymers for many applications. However, the ability of biodegradation of polyhydroxyalkonates obtained from bacteria by quantity microorganisms are expected. The population of PHB-degrading microorganisms in soil has been calculated as 5% to 86% of the total population of microorganisms growing on PHB agar plates. In addition of the typical polymeric properties described above, an important characteristic of PHAs is their biodegradability. Microorganisms in nature are able to degrade PHAs by using PHA hydrolases and PHA depolymerizes. The activities of these enzymes may differ and depend on the composition of the polymer and the environmental conditions. The degradation rate of a piece of PHA is typically in the order of a few months to years (Huisman and Madison 1999).

Degradation of PHA and its copolymer has been investigated in different natural environments such as soils, composts, and natural waters (Mehdi Emadian *et al .,* 2017*).* More than 90 types of microorganisms including: aerobes, anaerobes, photosynthetic bacteria, archaebacterial and lower eukaryotic are responsible for the biodegradation and catabolism of bio plastics (Lee et al., 2005; Kumaravel *et al*., 2010; Accinelli *et al*., 2012). These microorganisms were isolated from several ecosystem likes oil, aerobic and anaerobic sewage sludge, fresh and marine water, estuarine sediment and air extensively in soil or compost materials (Sudesh and Abe, 2010).

The degradation of Polyhydroxyalkonates by bacteria or fungal species is recognized through the appearance of a clear zone surrounding the growth in a plate containing the materials as the only carbon source, followed by the consideration of the diameter for the biodegradation extension (Tezuka *et al*., 2004; Lee *et al.*, 2005; Ishii *et al*., 2008). Scanning electron microscopic (SEM) observations were great used by researches in order to determine the change of polymer structure due to the biological activity. For example, instability of PHA was observed by utilizing the SEM method verifying the PHA biodegradation (Wu, 2009, 2011; Phukon *et al*., 2012; Tachibana *et al*., 2013). Moreover, Fourier Transform Infrared (FTIR) spectroscopy was also employed to detect the change in bond intensity, which was caused by microbial degradation (Wu, 2011; Phukon *et al*., 2012.).

Polyhydroxyalkonates utilization by bacteria:

Bacterial species such as, *Ralstonia eutropha*, *Azotobacter beiijerinckii* and *Hydrogenomonas eutropha*, *Alcaligenes latus* biodegrade different biopolymers (Wang *et al*., 2009). They were mainly isolated from different soil environments and were capable of degrading the biopolymers. *Pseudomonas, Mycobacterium, Bacillus, Azospirilum, Comamonas, Streptomyces, Ilyobacter* used polyhydroxyalkonates as carbon source (Muhammadi *et al*., 2015). It has been reported that 39 bacterial strains of the classes Firmicutes and Proteobacteria can degrade PHB, polycaprolactone (PCL but not Poly (lactic acid) or polylactide (PLA) (Suyama *et al.*, 1998). Bacteria including *Bacillus*, *Pseudomonas*, *Klebsiella*, *Actinomycetes*, *Nocardia*, *Streptomyces*, *Thermoactinomyces*, *Micromonospora*, *Mycobacterium*, *Rhodococcus*, *Flavobacterium*, *Comamonas*, *Escherichia*, *Azotobacter*, and *Alcaligenes* played an important role in the biodegradation process (Jayasekara *et al.*, 2005, Gautam *et al.*, 2007).

Wani *et al.* (2016) reported the isolation and identification of novel poly-b-hydroxybutyrate (PHB) degrading bacterium *Stenotrophomonas* sp. RZS 7 and studies on its extracellular PHB degrading depolymerase enzyme. The bacterium was isolated from soil samples of plastic contaminated sites of municipal area in Shahada, Maharashtra, Western India. The bacterium grew well in minimal salt medium, containing PHB as the only source of nutrient and produced a zone of PHB hydrolysis on agar medium. An optimum yield of enzyme was obtained on the fifth day of incubation at 37°C and at pH 6.0. Further increase in enzyme production was

recorded with Ca^{2+} ions, while other metal ions like Fe^{2+} (1 mM) and chemical viz. mercaptoethanol severally affected the production of the enzyme.

Polyhydroxyalkonates utilization by Fungi

Various microorganisms isolated from soil environments utilized polyhydroxyalkonates as the carbon source. Fungi isolated from a variety of environmental samples were capable of polyhydroxyalkonates degradation like most species of *Penicillium* and *Aspergillus* (Lee, *et al*. 2005). Among the soil isolated fungi species responsible for biodegradation, *Penicillium* and *Paecilomyces farinosus* were mainly encountered (Kim, 2000).

Many reports have been published on the fungal degradation of PHA in the environment; however reports on the properties of the PHB depolymerases from fungi are relatively rare. Only few fungal PHB depolymerases have been purified and partially characterized to date (Shivakumar, 2013). Therefore, the biochemical properties of fungal PHB depolymerases are not well documented in comparison to those of bacterial PHB depolymerases (Shivakumar, 2013). Also, in another experiment five strains were isolated from soil as fungi able to degrade both poly (3-hydroxybutyrate) (PHB) and polycaprolactone (PCL), and one of the strains, D218, identified as *Paecilomyces lilacinus*, was selected (Oda *et al.,* 1995). In 10 days, the isolate D218 degraded PHB almost completely and 10% of the PCL, each at 0.1% in the culture media. Strain D218 excreted PHB and PCL depolymerases in media containing either PHB, PCL or PHB plus PCL. Both depolymerase activities were reduced when the medium was supplemented with either soluble starch, lactose or glucose (Oda *et al.,* 1995). The optimum conditions for PHB depolymerase were pH 6.5 to 7.5 at 50°C, while those for PCL depolymerase were pH 3.5 to 4.5 at 30°C. In the reaction mixtures used for the enzyme assays, the formation of 3-hydroxybutyrate from PHB and ε-caprolactone from PCL was confirmed by high performance liquid chromatography (Oda *et al.,* 1995).

Fusarium solani Thom produced maximum PHB depolymerase by 48 h when grown in medium containing 0.2%, w/v PHB, pH 8.0 at 25◦C. Statistical optimization studies using Plackett Burman design of PHB depolymerase production yielded maximum PHB depolymerase activity after 2 days as against 4 days in the unoptimized conditions with a 2 fold increase in activity (Shivakumar, 2013).

The highest PHB degradation in Petri dish by *A. fumigates* was at pH 5, 30°C and 7days (Aly *et al.,* 2017). In liquid medium, degradation by *A. fumigates* was studied using enzyme assay method (U/ml). All the experiments were performed enzyme activities were monitored. After 3 days of incubation, maximum PHB depolymerase production was at pH 5 and 30°C. In conclusion, PHB can be degraded in solid and liquid medium using fungal depolymerase enzyme.

Various microorganisms producing depolymerase enzymes to degrade PHB and poly-β-hydoxybutyrate- co-3-hydroxyvalterate (PHBV) (Roohi *et al.,* 2017). Fungi active in the biodegradation process are *Sporotrichum*, *Talaromyces*, *Phanerochaete*, *Ganoderma*, *Thermoascus*, *Thielavia*, *Paecilomyces*, *Thermomyces*, *Geotrichum*, *Cladosporium*, *Phlebia*, *Trametes*, *Candida*, *Penicillium*, *Chaetomium*, and *Aerobasidium* (Delort and Combourieu 2001). Another study by Vergara-Porras *et al.* (2011) produced blends of poly (β-hydroxybutyrate-co-bhydroxyvalerate) with poly (caprolactone) using melt mixing and solvent casting techniques. The biodegradation of blends was tested using *Penicillium funiculosum* fungal specie. The CO_2 production during biodegradation was measured. Biodegradation of blends varies according to the mixing technique and the proportion of bacterial polymers in the blends. Although lag phase was larger, solvent-casted blends were easier to degrade due to their porous surface and relative lower crystallinity. *P. funiculosum* morphology during biodegradation appeared to be related to carbon availability i.e. larger and more complex conidiophores, more phialides per conidiophore and the presence of double-phialides, were found in blends with higher PHAs proportion. *P. funiculosum* morphology was independent to the blending technique used. Hence, morphology of *P. funiculosum* could be useful as a reference for carbon bioavailability of the blends.

However, reports on the fungal degradation of these polyesters and the related hydrolytic enzymes are relatively rare and not well documented. Considering that fungi play a significant role in degrading natural organic substances in the ecosystem, such as cellulose, hemicellulose, and lignin, the fungal contribution to the biodegradation of polyesters in the environment (Kim and Rhee, 2003).

Polyhydroxyalkonates utilization by Actinomycetes

A thermophilic *Streptomyces* sp. isolated from soil can degrade not only PHB but also PES, PBS and poly [oligo (tetramethylene succinate)-co- (tetramethylene carbonate)] (PBS/C) (Tokiwa *et al.,* 2009). This actinomycete has higher PHB-degrading activity than thermotolerant and thermophilic *Streptomyces* strains from culture collections (Calabia and Tokiwa 2004). A thermotolerant Aspergillus sp. was able to degrade 90% of PHB film after five days cultivation at 50°C (Sanchez *et al.,* 2000). Furthermore, several thermophilic polyester degrading actinomycetes were isolated from different ecosystems (Tokiwa *et al.,* 2009). Out of 341 strains, 31 isolates were PHB, PCL and PES degraders and these isolates were identified as members of the

genus *Actinomadura*, *Microbispora*, *Streptomyces*, *Thermoactinomyces* and *Saccharomonospora* (Tseng *et al.*, 2007).

Factors affecting degradation of PHB:

The percentage of PHB-degrading microorganisms in the environment was estimated to be 0.5-9.6% of the total colonies (Suyama *et al.*, 1998). Majority of the PHB-degrading microorganisms were isolated at ambient or mesophilic temperatures and very few of them were capable of degrading PHB at higher temperature (Tokiwa *et al.*, 2009).

Tokiwa *et al.*, (2009) emphasized that composting at high temperature is one of the most promising technologies for recycling biodegradable plastics and thermophilic microorganisms that could degrade polymers play an important role in the composting process (Tokiwa *et al.*, 1992). Thus, microorganisms that are capable of degrading various kinds of polyesters at high temperatures are of interest.

The physical and chemical properties of polymer have a strong influence on the biodegradable capabilities of enzymes. As far molecular weight is concerned, low molecular weight polymer is favorable for biodegradation. The PHB is a high crystalline thermoplastic with a melting temperature around 178°C. When Tm of a polymer increases, then enzymatic degradability decreases. The rate of enzymatic erosion of PHB by depolymerase is also strongly dependent on the concentration of the enzyme. The surface conditions (surface area, hydrophilic, and hydrophobic properties), the first order structures (chemical structure and molecular weight distribution), and high order structures (crystallinity, and crystal structure) of polymers played important roles in the biodegradation processes. In general, polyesters with side chains are less assimilated than those without side chains. Besides these factors, environmental conditions in which microbial population is growing also plays crucial role for biodegradation (Roohi *et al.*, 2017).

PHB depolymerase:

Depolymerase play an important role in the metabolism of PHB in the environment (Aly *et al*., 2015). There are two types of PHB polymers: native and denatured PHB granules. Native PHB granules contain lipids and proteins thereby rapidly hydrolyzed by intracellular PHB depolymerase. On the other hand, denatured PHB granules are partially crystalline, which are not hydrolyzed by intracellular depolymerases but are degraded by extracellular enzymes into water-soluble products. Although intracellular PHB- depolymerases play a number of roles in the degradation of PHB, little is known about this enzyme due to complexity of its action. An intracellular PHB depolymerase system from *Rhodospirillum rubrum* which was used to degrade native PHB granules, isolated from *Bacillus megaterium* was reported (Romen *et al.*, 2004, Roohi *et al.*, 2017) while Kobayashi *et al.*, (1999) isolated two new species of thermophilic bacteria, namely, *Caldimonas manganoxidans* and *Schlegelella thermodepolymerans*, which are able to produce PHB depolymerase enzyme. Molecular weights of PHB depolymerases ranges from 40 to 90 KD with most depolymerases consisting of only one polypeptide chain. The enzyme exhibits alkaline isoelectric points and showed maximum activity between pH 7.5 to 9.5.

Many PHB depolymerases are inhibited by reducing agents, and most of them do not bind to ion exchangers. The genes responsible for the enzyme synthesis have been examined, and the primary structure of the PHB depolymerases have been determined for *Acidovorax* sp., (Kasuya *et al.*, 1999), *Alcaligenes faecalis*, *Comamonas acidovorans* (Hadad *et al.*, 2005) *Comamonas* sp., *Paucimonas lemoignei*, *Pseudomonas stutzeri*, and *Streptomyces filiatus* (Merrick and Doudoroff, 1964, Jendrossek *et al.*, 1996). Besides the bacteria, fungal depolymerases are also involved in PHB degradation (Gilkes *et al.*, 1991) but only few fungal PHB depolymerases have been purified and partially characterized till date (Roohi *et al.*, 2017).

The extracellular purified PHB depolymerase enzyme is usually made up of single polypeptide chain whose molecular weights ranges from 37 to 60 KDa. Its molecular structure comprises of two domains and one linker moiety connecting the two domains. One is catalytic domain meant for catalyzing substrate present at N-terminus and other is substrate binding domain responsible for binding of substrate molecule present at C-terminus (Roohi *et al.*, 2017). This structural similarity is represented by other depolymerizing enzymes such as cellulase, chitinase (Watanabe *et al*., 1990, Kellet *et al*., 1990) and xylanase, (Watanabe *et al*., 1990, Nojiri and Saito, 1997) which hydrolyze water-insoluble, complex polysaccharides. The catalytic domain contains a peculiar pentapeptide [Gly-X1-Ser-X2-Gly] sequence called lipase box, which is common for several serine hydrolases (Shinomiya *et al*., 1998). This serine forms a catalytic triad with 2 more amino acid, that is, aspartate and histidine (His-Ser-Asp) that forms actual catalytic centre for catalytic domain. According to the position of lipase box in the catalytic domain, extracellular PHB depolymerases can be classified into 2 classes. In class I enzymes, lipase box is located at the centre of the catalytic domain (as represented by PHB depolymerase of *Alcaligenes faecalis* AE122; *A. faecalis* T1; *Pseudomonas lemoignei* PhaZ1, PhaZ2, PhaZ3, PhaZ4, and PhaZ5; and *Pseudomonas stutzeri*). In class II enzymes, lipase box is adjacent to the N-terminus (as represented by PHB depolymerase of *Comamonas acidovorans*, *Comamonas teststeroni*, and *Streptomyces exfoliates*).

Substrate-binding domain specifically binds with water immiscible PHB polymer and is unable to bind with water-soluble (R)-3HB oligomers (Shinomiya *et al*., 1998).This domain is essential for the adsorption of PHB depolymerase onto the surface of PHB powder (Doi, *et al*., 1994). The linker region connecting catalytic and substrate-binding domains played structural role in maintaining an optimal distance between the 2 domains. The substrate specificity of PHB depolymerase has been investigated using various PHA homopolymers (Kasuya *et al.,* 1997, Hadad *et al.,* 2005). PHB depolymerase enzymes show relatively narrow substrate specificity for PHA hydrolysis.

Mechanism of PHB degradation by depolymerase enzymes

The enzymatic degradation of PHB polymer is heterogeneous reaction involving 2 steps, namely, adsorption and hydrolysis. First, the enzyme binds to the surface of the PHB material by the binding domain of PHB depolymerase, and the second step is hydrolysis of polyester chains by the catalytic domain of the enzyme (Roohi *et al.,* 2017). Adsorption study of substrate binding domain of PHB depolymerase with single crystals was investigated using immuno-gold labelling techniques. Here, it was found that enzyme binds on the entire surface of PHB single crystals and this binding increases the mobility of polyester chains along the crystal edge, which results in the formation of disordered PHB chains resembling the polymer chains in the amorphous phase, which are likely attacked by the active site of the enzyme. The PHB depolymerase enzymes are not able to make strong interaction with the irregular surfaces of copolymer crystals since the copolymer chains with second monomer units have loose loop folding on the surface of the crystals (Roohi *et al.,* 2017).

Generally, an increase in molecular weight results in decline of polymer degradability by microorganisms. High molecular weight results in sharp decrease in solubility making them unfavorable for microbial attack because bacteria require substrate to be assimilated through the cellular membrane and further degraded by cellular enzymes (Shah *et al.,* 2008). The PHB can be degraded either by the action of intracellular and extracellular depolymerases in PHB-degrading bacteria and fungi. Intracellular degradation is the hydrolysis of an endogenous carbon reservoir by the accumulating bacteria themselves while extracellular degradation is the utilization of an exogenous carbon source not necessarily by the accumulating microorganisms (Roohi *et al.,* 2017). During degradation extracellular enzymes from microorganism breakdown complex polymer yielding smaller molecules of short chains, for example, oligomers, dimers, and monomers, that are small enough to pass through the semipermeable outer bacterial membrane and then to be used as carbon and energy sources. The process is called depolymerization. This mechanistic approach can be determined by various methods. One of the commonly used methods is turbidity measurements of opaque suspensions of PHB granules. As hydrolysis proceeds, the diameter of PHB granules decreases, which results in decrease of the optical density of the PHB suspension. This method is sensitive, easy to perform, and can be applied for hydrolysis of both native PHB and denatured PHB (Korner *et al*., 2005).

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