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Degradation of Polyhydroxyalkanoate (PHA): a Review

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Polyhydroxyalkanoate (PHA) is an attractive material due to its mechanical properties and biodegradability. As a result of its ability to degrade naturally in the environment, utilization of PHA is a step closer towards a greener environment with the aim of reducing the dependency on the non-degradable synthetic plastic. PHA is degraded by microorganisms that could secrete extracellular PHA depolymerase. Besides that, other factors that could affect the degradation of PHA include the environmental condition and the properties of PHA such as composition, crystallinity, additives and surface area of the PHA. This review provides a summary of intracellular and extracellular degradation, factors affecting the degradation of PHA and the application of PHA degradation.

Keywords: PHA, degradation, PHA depolymerase.

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Деградация полигидроксиалканоатов (ПГА): обзор

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Полигидроксиалканоаты (ПГА) – привлекательный материал благодаря его механическим свойствам и биоразрушаемости. Из-за их способности разрушаться в окружающей среде естественным путем использование ПГА – ещё один шаг в направлении более зеленой окружающей среды, ставящий своей целью уменьшение зависимости от неразрушаемых синтетических пластиков. ПГА разрушаются микроорганизмами, способными секретировать внеклеточные ПГА-деполимеразы. Наряду с этим на деградацию ПГА могут влиять другие факторы, включая условия окружающей среды и такие свойства ПГА, как их состав, кристалличность, наличие добавок и площадь поверхности. Обзор обобщает закономерности внутриклеточной и внеклеточной деградации ПГА, факторы, влияющие на их деградацию, а также применение этих материалов, связанное с их деградацией.

Ключевые слова: ПГА, деградация, ПГА-деполимераза.

Introduction

The increase of environmental pollution caused by non-biodegradable petrochemical-based plastics have given rise to the development of polyhydroxyalkanoate (PHA) as a substitute of the synthetic plastics. Furthermore, the degradability of PHA offers the solution for the emerging environmental problem caused by the lack of degradability in conventional plastics which contributes to the high amounts of solid waste. This phenomenon has indirectly contributed to the continuation of studies done on PHA production. PHA is a type of polyester produced intracellularly by microorganisms as a form of carbon and energy storage when grown in environment with limited essential nutrients including nitrogen, magnesium, phosphorus or oxygen but surplus of carbon supply (Anderson and Dawes, 1990). PHAs are known to be renewable and environmentally friendly as well as have properties similar to numerous elastomers and thermoplastics (Holmes, 1988; Steinbüchel, 1991; Steinbüchel, 1992; Lee, 1996). PHAs can be categorized into two main groups depending on the number of carbon atoms in the monomeric units. Short-chain-length (SCL) consists of C3-C5 atoms while medium-chain-length (MCL) comprised of C6-C14 atoms. Rui Li and co-workers demonstrated that a limited number of bacteria are also able to synthesize PHA containing a combination of SCL-MCL (Li et al., 2007).

Biodegradation is defined as the fragmentation or breaking down of materials by bacteria, fungi or through other biological means whether aerobically or anaerobically. In short, biodegradable polymers are polymers that are degraded in biological environments through enzymatic and non-enzymatic hydrolysis and not through thermal oxidation, photolysis or

radiolysis (Ikada and Tsuji, 2000). The ability for PHA to degrade naturally in the environment has made it a promising and interesting material for many applications. PHA degradation is generally carried out by microorganisms that secrete intra- or extracellular PHA depolymerases, which vary in their molecular organization and substrate specificity (Jendrossek, 2007). Solid PHA could be hydrolyzed into water-soluble oligomers and monomers, by several microorganisms such as fungi and bacteria which are capable of secreting extracellular PHA-degrading enzymes. The resulting products were then served as a source of nutrient for the microorganisms (Sudesh and Abe, 2010).

PHA has been found to be able to degrade in environments with high microbial activity such as soil (Mergaert et al., 1993), lake water and marine water (Ohura et al., 1999) and even in sewage sludge (Lee and Choi, 1999). The occurrence of biodegradation is mainly due to the PHA degrading enzymes which is known as PHA depolymerase that are secreted by microorganisms to hydrolyze water-insoluble PHA into water-soluble forms so that it can be utilized by these microorganisms (Sudesh et al., 2000). PHA depolymerase is an enzyme made up of a catalytic domain and a substrate-binding domain. Both these domains are connected by a linker domain. The substrate binding domain of the enzyme binds to the crystalline PHA material. Subsequently, the catalytic domain starts to cleave the polymer chain (Numata et al., 2009).

Apart from the activity of depolymerase, the rate of biodegradation is also significantly influenced by several environmental factors such as temperature, microbial population, nutrient supply, pH, moisture level as well as the conditions of the PHA materials including the surface area of the PHA, its composition and crystallinity (Doi, 1990). In this review, we will revisit some of the aspects of PHA biodegradation mainly in terms of the intra- and extracellular depolymerases, as well as the factors that affect the degradation rate and the various studies on PHA degradation.

Intracellular and extracellular degradation of PHA

Intracellular degradation

Intracellular PHA is stored as insoluble inclusion bodies, approximately 200-500 nm in diameter and exist as membrane enclosed inclusion (Anderson and Dawes, 1990). In 1980's, Barnard and Sanders demonstrated that PHA inclusions inside the cells were neither in solid nor liquid state, but they were in the form of mobile amorphous elastomeric state whereby they do not behave as crystalline structures (Barnard and Sanders, 1988, 1989). The inclusions were thought to be coated by a layer of membranous phospholipids for the maintenance of native state of PHA (Fuller et al., 1992; Horowitz and Sanders, 1994; Foster, 2000). The transition of terminology from "PHA granules" to "PHA inclusion bodies" was covered by the same group of researchers in the early 1990's after they managed to illustrate the preparation of artificial amorphous, biomimetic granules of PHA (Horowitz and Sanders, 1994; Foster, 2000).

Many proteins are associated with PHA inclusion *in vivo*, enveloped by a phospholipid monolayer. It has been proposed that all enzymes necessary for the synthesis of PHA from acetyl-CoA associate with PHA inclusions in *C. necator* (Uchino et al., 2007). In *C. necator*, PHA synthase (PhaC) becomes insoluble by granule binding because the PHA chain remains covalently linked to the enzyme during the synthesis of the polymer (Liebergesell et al., 1991; Gerngross et al., 1993). In addition to PhaC, other granule-associated proteins include the intracellular PHA depolymerase (PhaZ), phasins (PhaP) and regulator protein of the phasin expression (PhaR) (Luengo et al., 2003). PhaZ is involved

in the degradation of PHA (Saegusa et al., 2001; Jendrossek and Handrick, 2002). Bringham and co-workers proposed that expression of PhaZ2 has an influence on PHB granules shaping and remodeling in *C. necator*. More granules per cell could be formed from deletion of *phaZ2* or *phaZ3* genes (Bringham et al., 2012).

Intracellular degradation or mobilization takes place when the bacterium is stressed under carbon limitation conditions. Accumulated PHA granules in the cells are hydrolyzed as carbon and energy sources (Madison and Huisman, 1999; Luengo et al., 2003). From there, PHA is broken down to 3-hydroxyalkanoic acid, a monomeric component by PHA depolymerase and oligomer hydrolase (Kobayashi et al., 2005). If the PHA is comprised of only one type of monomer, for example, 3-hydroxybutyrate, the resulting PHA is called poly(3-hydroxybutyrate) [P(3HB)] homopolymer. P(3HB) is the most common type of PHA synthesized by most bacteria naturally. The intracellular degradation of P(3HB) results in the liberation of 3-hydroxybutyric acid which is then oxidized by a dehydrogenase to acetoacetyl-CoA, which is finally converted into acetyl-CoA by β-ketothiolase (Eggers and Steinbüchel, 2013; Lemes et al., 2015). The breakdown products of PHA are naturally found in animals. Therefore, the biodegradation of PHA in living cells does not cause the formation of toxic compounds and therefore it is categorized as a biocompatible material.

Since both PHA synthase and PHA depolymerase are present in PHA accumulating microorganisms, studies were done to identify the rate of polymer hydrolysis to synthesis. According to Doi and co-workers, the rate of polymer hydrolysis in *C. necator* is about ten times slower than the rate of synthesis in a nitrogen-free medium (Doi et al., 1992). At early P(3HB) synthesis stage, depolymerase exhibited the highest activity which subsequently decreased and remained at lower level which dropped further when entering stationary phase. The specific depolymerase activity could be enhanced later by addition of nitrogen or depletion of carbon sources (Volova et al., 2013). Therefore, it is important to use optimum culture conditions in order to maximize PHA production with minimal *in vivo* hydrolysis. However, addition of Triton X-100 or phenylmethylsulphonyl fluoride (PMSF) could inhibit the depolymerase activity by interacting with the serine residues located at the enzyme active site (Foster et al., 1996). Similarly, the presence of bovine serum albumin (BSA) at different concentrations were found to inhibit the degradation of poly(3-hydroxyoactanoate) [P(3HO)] until completely ceasing activity at a concentration of 10 mg/mL (Foster et al., 1996).

The dynamic changes of PHA inclusion bodies along the production and utilization stages have been studied. Two hypotheses have been proposed to explain the morphology of the granules before and after intracellular degradation (Fig. 1). First hypothesis suggested that the granule sizes will decrease from the original sizes with the number of granules in one cell remaining unchanged. Meanwhile, the second hypothesis proposed that variation will happen in both the number and size (Low, 2003).

Transmission electron microscopy (Fig. 2) at early stage and 60 h after commencement of degradation stage showed reduction in poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)] granule sizes but increase in granule counts. This was observed in *Delftia acidovorans* cells (Low, 2003). An explanation for this observation is that these granules were being attacked by intracellular depolymerase enzymes at random directions causing the granules to break into numerous smaller granules. Smaller granules will have larger surface area for increased rate of degradation and utilization of the PHA. Nevertheless, it may also be possible

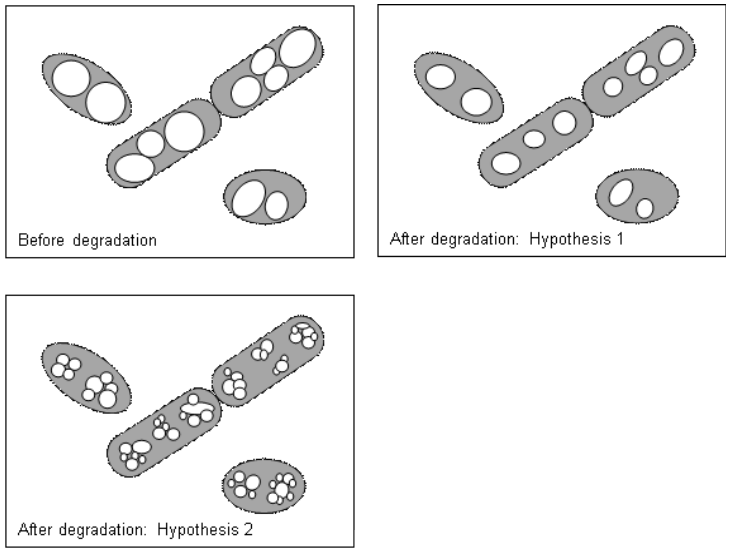

Fig. 1. Schematic diagrams showing the proposed two hypotheses on the morphologies of intracellular PHA inclusion bodies. Hypothesis 1 proposed that the granule sizes will reduce from the original sizes with the number of granules in every cell remaining unchanged. Hypothesis 2 proposed an increment of smaller granules besides the reduction in granule sizes (Low, 2003)

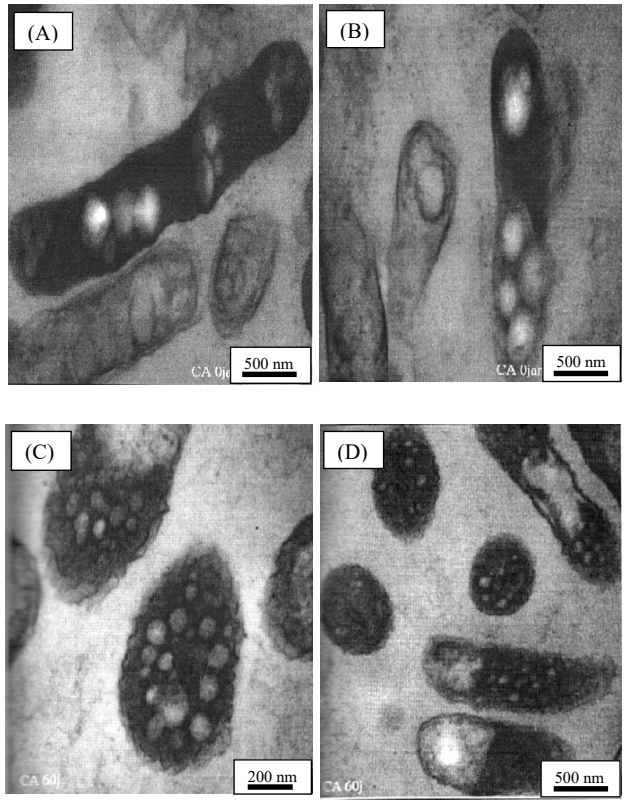

Fig. 2. Transmission electron microscopy at early stage and 60 h after commencement of degradation stage in *Delftia acidovorans*. (A) and (B) 0 hour with 37 wt % P(3HB-*co*-3HV) content; (C) and (D) after 60 hours of degradation with 4 wt % P(3HB-*co*-3HV) content (Low, 2003)

that the smaller granules found at the later stage of degradation are new PHA granules formed by some PHA synthases that remain active in the cells. A study done by Volova and co-workers revealed that heterogeneous bacterial cells could be seen with majority of the cells containing small number of diminished size granules, while others were devoid of granules, suggesting the degradation was performed at different rates (Volova et al., 2013).

Extracellular degradation

Extracellular depolymerases were found to possess the ability to hydrolyze partially crystallized P(3HB) (Handrick et al., 2001; Tseng et al., 2006). These depolymerases consist of signal peptide (22-58 amino acids) and three functional domains, catalytic domain (320-400 amino acids), linker domain (50-100 amino acids), and substrate binding domain (40-60 amino acids) from the N-terminus to C-terminus (Jaeger et al., 1995; Jendrossek and Handrick, 2002). The catalytic domain is further categorized into Type I and Type II depolymerase. The difference between both types of enzymes are the sequential order of active amino acids forming a catalytic triad and also their specific locations in which Type I starts from N-terminus to C-terminus whereas Type II is located at N-terminus. Besides PHA depolymerase, several lipases are capable of hydrolyzing poly(ω-hydroxyalkanoates) such as poly(6-hydroxyhexanoate) [P(6HHx)] and poly(4-hydroxybutyrate) [P(4HB)], specifically prone towards polymers with no side chains in the carbon backbone. It was also reported that both PHA depolymerase and lipase may share a similar mechanism of substrate hydrolysis (Jaeger et al., 1995).

There are few parameters such as monomeric composition and crystallinity of the PHA that can be included to evaluate the degradation rate of PHA (Sridewi et al., 2006). Zhenguo Li and co-workers suggested that enzymatic degradation of PHA is based on the length of the side chain of PHA, which implies that the longer side chains provide better degradability (Li et al., 2007). Furthermore, copolymers of PHA have been found to degrade better compared to homopolymers (Mergaert et al., 1993). This higher degradation capability is attributed to the surface morphology of copolymers which combines low crystallinity and porous surface (Wang et al., 2004; Sridewi et al., 2006).

Homopolymer is difficult to be disintegrated by microorganisms due to its high crystallinity whereas the presence of 3HHx monomers increases the amorphous proportion in the copolymer, therefore, the degradation rate increases as the crystallinity of polymers decreased. This phenomenon occurs because the depolymerase hydrolyzes the amorphous disordered region of PHA more efficiently than the crystalline part (Iwata et al., 1999). Besides that, addition of $CaCO_3$ could enhance degradation activity by stabilizing the pH in the culture medium as a consequences of acidification from the production of (*R*)-3-hydroxybutyric acid monomer (Li et al., 2016). SEM studies showed that the surface appearance of P(3HB-*co*-12 mol % 3HHx) was more porous compared to the surface of P(3HB) and P(3HB-*co*-8 mol % 3HHx) after undergoing degradation on the acidic soil surface for a period of four weeks (unpublished data).

Factors affecting the degradation of PHA

The degradation of PHA is determined by two main factors which are the environmental conditions and properties of the PHA materials (Fig. 3).

PHA can degrade naturally in the environment due to the microbial activity which has the PHA-degrading enzymes. Thus, the environmental conditions have an enormous

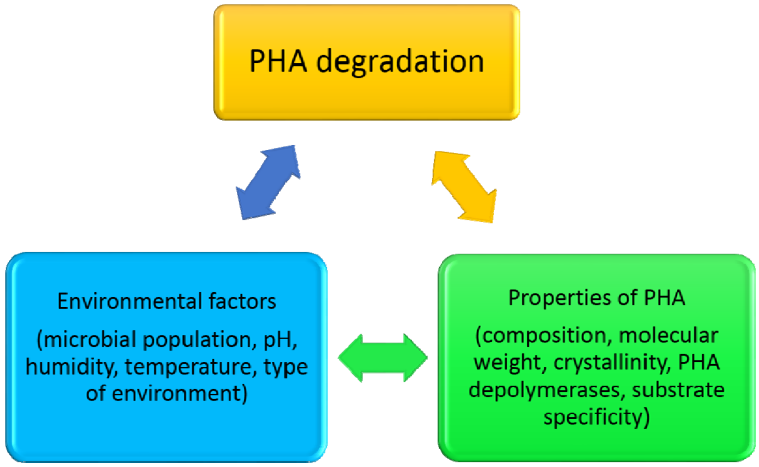

Fig. 3. Factors affecting the degradation of PHA

influence on the growth of the PHA-degrading microorganisms which will eventually change the production and secretion of PHA depolymerases as well as the degradation activity of the enzymes. Humidity (moisture level), temperature, presence or absence of oxygen and availability of nutrients are some of the key parameters that are crucial for the growth of microbial community in the environment. Apart from that, the composition and abundancy of microbial populations (bacteria, actinomycetes, fungi) will also determine the rate of PHA degradation.

In general, PHA will degrade faster in regions that have abundance of PHA-degrading microorganisms because there will be more contact between the microbes with the substrate to be degraded. Sang and coworkers showed that the microbial degradation rate of P(3HB-*co*-3HV) films in the soil relied on the microbial community and distribution. In addition, the degradation ability of the P(3HB-*co*-3HV)-degrading microorganisms also facilitated the colonizing on the surface of the incubated P(3HB-*co*-3HV) films (Sang et al., 2000). The attachment of the microbes facilitated the colonization of the PHA surface, followed by the release of the degrading enzymes (Holmes 1985, 1988; Brandl and Puchner, 1990). In addition, small-scale swelling and bursting were formed by the growth of fungi when they penetrate the polymer solids (Griffin, 1977). It has been reported that different PHA-degrading microbes were found in different environment (Shah et al., 2008) (Table 1). The kinetics of PHA degradation in natural environment in two water reservoirs having various ecological characteristics was studied by Voinova et al. (2008). It was shown that the biodegradation of PHA depends on the environment temperature and inorganic composition of water. Comparison of PHA degradation process under aerobic and anaerobic conditions revealed that the polymer degradation was slower under anaerobic conditions (Voinova et al., 2008).

However, PHA degrading microorganisms vary with respect to the type of PHA they can degrade. Most of the well-characterized microorganisms have the enzyme with substrate specificity for P(3HB) which means they are able to degrade P(3HB) most efficiently. On the other hand, there are other microorganisms that have enzymes with broader substrate specificity and capable of utilizing a wider range of PHA from scl-PHA to mcl-PHA (Schirmer et al.,

Table 1. PHA-degrading microbes from different environments

Environment	PHA-degrading microbe
Soil	*Acidovorax faecilis*, *Aspergillus fumigatus*, *Comamonas* sp., *Pseudomonas lemoignei*, *Variovorax paradoxus*
Activated sludge	*Alcaligenes faecalis*, *Pseudomonas*
Seawater	*Comamonas testosteroni*
Anaerobic sludge	*Ilyobacter delafieldii*
Lake water	*Pseudomonas stutzeri*

1995; Jendrossek, 1998). Some of the interesting discoveries of PHA degraders include the ability of *Xanthomonas*-like bacterium to degrade PHA with aromatic side chains (Doi et al., 1992) and the ability of *Comamonas* sp. strain to degrade scl- and mcl-PHA (Quinteros et al., 1999). In the latter study, it was discovered that clear zones on P(3HB), P(3HO) and poly(3-hydroxyphenylvalerate) [P3HPV] overlay plates were formed by Strain P37C, belonging to the genus *Comamonas*. They concluded that there was a direct correlation between PHA side chain length and the rate of hydrolysis of the PHAs.

In the work conducted by Kanesawa and coworkers, copolymers consisting of 3-hydroxyalkanoate units with various chain lengths (C4–C10) were produced by *C. necator* or *Pseudomonas oleovorans* by utilizing different carbon substrates. The enzymatic degradation processes of the PHA films were carried out in a phosphate buffer (pH 7·4) which contained the PHA depolymerase from *Alcaligenes faecalis* T1, at 37 °C. The rate of enzymatic degradation was found to be heavily dependent upon the composition of the PHA and decreased significantly with an increment of the side-chain length of the 3-hydroxyalkanoate monomeric units (Kanesawa et al., 1994).

Kusaka and coworkers investigated the degradation of high molecular weight P(3HB) synthesized by a recombinant *E. coli* through the depolymerase activity of *A. faecalis*. It was discovered that the degradation rate was related to the crystallinity of the material and the temperature of the medium (Kusaka et al., 1999). A similar phenomenon was observed in the study carried out by Volova and coworkers. In their study, it was discovered that the degradation rate of P(3HB-*co*-3HV) (containing 3 to 18 mol % 3HV) was 20-30 % higher than the homogenous P(3HB) under the similar conditions. The degradation of both of these PHAs in the soil and water was affected by the difference of crystallinity of the films, in which the crystallinity of the homopolymer and the copolymers was 65-80 % and 50-65 %, respectively. Thus, an increase in the degree of crystallinity will decrease the degradation rate. In parallel, it was also reported that the increase in temperature from 15-30 °C led to the linear increment of the degradation rate of the polymers (Volova et al., 1992). PHA degradability by microorganisms is inversely proportional to the increase in molecular weight. This is due to the ease of access of the monomers, dimers, and oligomers of a PHA repeating units to be mineralized. High molecular weights caused a sharp decrease in solubility and thus making them hostile for microbial attack. Furthermore, bacteria require the substrate to be assimilated through the cellular membrane which would be further degraded by the cellular enzymes (Doi, 1990; Gu et al., 2000).

In 1993, Mergaert and colleagues reported on the degradation of P(3HB) and P(3HB-*co*-10 mol % 3HV). They concluded that these PHAs were degraded in soils by the action of a wide range of microorganisms in which the degradation was further enhanced at higher temperatures. The hydrolysis of the PHAs (expressed in the form of decrease in molecular weight) was found to be similar at 40 °C in soils and sterile buffer. Interestingly, the weight loss was only observed in soils, and the rate of weight loss was shown to be dependent on the type of soil used. The copolymer was found to be degraded faster than the homopolymer, although the differences between the degradation rates varied widely between soils and temperatures (Mergaert et al., 1993). In order to get a better understanding of the effect of chemical structure on the biodegradability of PHA, the biodegradation behavior of P(3HB), P(3HB-*co*-40 mol % 3HV), P(3HB-*co*-20 mol % 3HV), P(3HB-*co*-3 mol % 3HV) and P(3HB-*co*-40 mol % 4HB) were studied under controlled composting conditions in accordance to ISO 14855-1. It was found that PHAs with different chemical structures can be biodegraded under the controlled composting conditions. The order of biodegradability was P(3HB-*co*-40 mol % 4HB) >P(3HB-*co*-40 mol % 3HV) >P(3HB-*co*-20 mol % 3HV) > P(3HB-*co*-3mol % 3HV)> P(3HB). The chemical structure of PHAs was found to be unchanged, but the thermal stability and the molecular weight were greatly decreased during the composting (Weng et al., 2011).

Besides that, different form of PHA will have different rate of degradation. A study using film and pellets was conducted by Volova and coworkers in the tropical marine environment. Their findings showed that the biodegradation patterns of PHAs were affected by the shape of the polymer and the preparation technique instead of by the chemical composition of the polymer. In comparison with the compacted pellets, the biodegradation rates of polymer films in seawater were found to be higher. The mass loss of P(3HB) and P(3HB-*co*-3HV) samples was relative to the reduction of molecular weight. There was a significant increase of the polydispersity index of both PHAs. Nevertheless, the degree of crystallinity of both PHAs was found to be unchanged (Volova et al., 2010). Another study on the two types of PHAs (film and pellets respectively) was conducted by Boyandin and coworkers. In that study, they discovered that the degradation of both types of PHA by soil microorganisms in Vietnamese soils was influenced by various factors such as the soil and climatic conditions of the study locations. The hot and humid climate of Vietnam was shown to facilitate the polymer biodegradation, and the daily mass loss of PHA specimens of different compositions amounted to 0.20 – 0.33 % (Hoa Lac) and 0.04 – 0.13 % (Dam Bai) for films; 0.12 – 0.18 % (Hoa Lac) and 0.02 – 0.08 % (Dam Bai) for pellets. The study revealed faster degradation of the homopolymer, P(3HB), as compared to P(3HB-*co*-3HV) (Boyandin et al., 2013). The findings were different from what other researchers have been reporting previously in which the copolymer was the one degraded faster as compared to the homopolymer.

PHA degradation and its application

The indicators showing that PHA degradation has taken place include the reduction of molecular mass and degree of crystallinity, change in the total mass of polymer and their strength properties. Many studies have been conducted in the area of PHA degradation. In the current era that is moving towards a green and sustainable lifestyle, biodegradable technology has been used widely in many sectors. Indeed, the focus is placed on making consumables that are biodegradable and environmentally friendly.

Besides that, PHAs were also used for biomedical applications such as biomaterials for cellular proliferation, suturing and controlled drug delivery. Ecological applications of biodegradable polymers include the making of fishing lines and nets, mulch films, delivery system for fertilizers and pesticides, manufacturing of toiletries, food packaging and daily necessities (Ikada and Tsuji, 2000). All these applications were made possible due to the fact that PHA is biodegradable.

One of the renowned examples of commercialized PHA was BIOPOL (Zeneca BioProducts), a copolymer of P(3HB-*co*-3HV) that was used as packaging material for shampoo bottles and other cosmetic products. In 1990, Dave and coworkers investigated the degradation of BIOPOL and its blends with other plastic materials such as polycaprolactone, polystyrene, poly-L-lactide and polystyrene-*co*-acrylonitrile. The study involved the use of PHA depolymerase from the culture supernatant of the fungus *Penicillium funiculosum*. As the content of BIOPOL component was increased, higher weight losses of the blends tested were reported (Dave et al., 1990). In 1992, similar results were also reported by Gassner and Owen who found out that only the BIOPOL component of blends with ethylene-vinylacetate was degraded by soil microorganisms (Gassner and Owen, 1992).

PHA has been tested for the application as slow release of fertilizer and pesticide. The potential of using P(3HB) as a fertilizer carrier was investigated by Volova and coworkers. The formulations of the nitrogen fertilizer urea loaded in a degradable matrix of the natural P(3HB) in the form of films, pellets and coated granules. It was observed that the nitrogen release into the soil occurred in parallel with the degradation of the polymer. The amount of nitrogen loaded into the carrier as well as the geometry of the carrier, have a direct influence on the nitrogen release. The result showed that nitrogen release can last for 30 days or longer and that release rate could be controlled by varying the fabrication technique employed. P(3HB) with urea formulations have a positive effect on the soil microbial community. The use of embedded urea showed beneficial impact on the growth of creeping bentgrass (*Agrostis stolonifera*) and lettuce (*Latuca sativa*) (Volova et al., 2016a).

The recent published work on the application of PHA by the same group of researchers was on the slow-release formulations of the herbicide metribuzin (MET) embedded in the polymer matrix of degradable P(3HB) in the form of microparticles, films, microgranules and pellets (Volova et al., 2016b). That study showed that the MET release could be controlled by using different methods of making the formulations and varying the MET loading. As the polymer started to degrade, the MET accumulation in soil occurred progressively. The results of this study showed that all P(3HB)/MET formulations exhibited evident herbicidal activity. Therefore, degradable P(3HB) can be regarded as a promising material for designing slow-release formulations of the herbicide metribuzin for soil applications.

In addition to environmental degradation and intracellular degradation of PHA, the biodegradation of various PHA-based biomaterials have also been investigated *in vivo*. It has also been demonstrated that some PHA can degrade when implanted in animal tissues. It was found that the degradation rate of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)] in animal is relatively high compared to other PHA and it can be controlled by varying the 4HB monomer composition (Saito et al., 1996; Ying et al., 2008; Bringham and Sinskey, 2012). Therefore, P(3HB-*co*-4HB) copolymer has gained interest in a wide range of medical applications (Yang et al., 2002; Martin and Williams, 2003; Chee et al., 2008;

Cahil et al., 2012). Besides being degraded by PHA depolymerases, P(3HB-*co*-4HB) can be also degraded by eukaryotic lipases and esterases (Mukai et al., 1994; Saito et al., 1996). This further aids the biodegradation of 4HB-based biomaterials in living systems.

It is important to understand this mechanism because when PHA material is used as scaffold in the application for tissue engineering, the rate of degradation have to be equivalent to the regenerative rate of the tissue. In the meantime, when it is used for as a drug carrier, the rate of degradation will determine the amount of drug released (Pouton and Akhtar, 1996). Many successful studies of PHA degradation in living organisms have been proven. Furthermore, formation of toxic compounds was not detected (Zinn et al., 2001). Incorporation of other monomers into P(3HB) will reduce the crystallinity, resulting in a decrease in the melting temperature, increase in the flexibility of polymer and degradation rate of the polymers.

Conclusion

This review has covered the major components of PHA degradation. It is evident that all the factors affecting PHA degradation are interrelated. The key player of PHA degradation is nonetheless the microorganisms that have the ability to secrete extracellular depolymerase. In addition, the degradation of PHA also play a significant role in determining the application of PHA in various areas. PHA is a promising material for a sustainable living. Understanding the mechanism of PHA degradation and the factors that affect its degradation will help the researcher in designing suitable material for the industrial needs.

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