



Biodegradation of plastics waste using fungi: A review

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Abstract

Plastics are synthetic polymers that are widely used in every field of life every day. Along with the increasing use of plastic, the amount of plastic waste produced and accumulated to environment will also increase. If the plastic waste is not handled properly, it will pollute the environment and threaten many living things including humans. Biodegradation is a promising method for dealing with plastic waste. This method includes many microbes including fungi such as *Aspergillus* spp. and *Penicillium* spp. as its biodegradation agents. Some plastics degrading fungi produce many specific enzymes that catalyze the degradation of plastic polymer into simpler and smaller fragments including oligomer, dimer, and monomer through several steps including biodeterioration, depolymerization, assimilation, and mineralization. The fragmented plastic particles are absorbed and used by plastic degrading fungi as their energy and carbon sources. Biodegradation is the appropriate method to overcome the plastics pollution because this method has no side effects as the conventional methods.

Key words – Biodegradation – Biodeterioration – Depolymeration – Fungi – Plastics Waste

Introduction

Plastics are relatively inexpensive, lightweight, resistant to water, and strong products can be used for many purposes. This polymer is widely used in almost all fields of life. The use of plastic products is likely to increase by 9% every year (Thompson et al. 2009). World plastic production reached 400 million tons annually (UNEP 2018). Unfortunately, plastic contains various toxic components hazardous to living things. The massive use of plastic products leads to plastics accumulation in the environment (Thompson et al. 2009), because these pollutants require a very long time to be completely degraded in nature, thus pollute the water, land, and air (Kathiresan 2003, Soud 2019).

In Western Europe, around 7.4% of municipal solid wastes are plastic wastes. As much as 65% of that amount is polyethylene/polypropylene, 15% polystyrene, 10% PVC, 5% PET, and the rest come from other plastic polymers (Premraj & Doble 2005). In 2010, China ranked first among the countries that were top producer of plastic wastes generating 27.7% of total global mismanaged plastic wastes or around 8.82 MMT/year (Millions of Metric Tons per Year). Indonesia was at second position (10.1%) in the list of the world's total mismanaged plastic wastes, followed by Philippines (third, 5.9%), Vietnam (fourth, 5.8%), Sri Lanka (fifth, 5%), Thailand (sixth, 3.2%), Egypt (seventh, 3%), Malaysia (eighth, 2.9%), Nigeria (ninth, 2.7%), Bangladesh (tenth, 2.5%), and

United States (twentieth, 0.9%) (Jambeck et al. 2015). North-East Asia was the highest single-use plastic producer in 2014, 26% in particular, followed by North America (21%), Middle East (17%), Europe (16%), Asia and the Pacific (12%), Central and South America (4%), and the former Uni Soviet countries (3%). Based on the industrial sector, packaging was the sector that consumed the most single-use plastic or about 36%. Plastic production in the building and construction sector was 16%, textiles 14%, consumer and institutional products 10%, transportation 7%, electronics/electrical 4%, machinery industry 1%, and about 12% used in other sectors (UNEP 2018).

Plastic wastes have become contaminants on land and in the aquatic environment. Thus, effective plastic waste processing methods are needed (Mandan & Arya 2017). The common methods used in processing plastic wastes are landfill, incineration, and recycling. Each of these methods has its weaknesses. Thus, they are not effective in solving plastics pollution. Landfill plastic wastes processing requires a long time without rotting. Incineration treatment will produce toxic gases into the environment (Al-Salem et al. 2009, Hopewell et al. 2009, Gan & Zhang 2019). The incineration process of PVC, PET, PS, and PE wastes produces carcinogenic substances as PAHs, nitro-PAHs, dioxins, and others (Al-Salem et al. 2009, Yang et al. 2018). Additionally, recycling plastic wastes is also relatively expensive (Al-Salem et al. 2009, Hopewell et al. 2009, Gan & Zhang 2019).

Some plastic wastes processing techniques that include energetic, chemical, and biological methods were developed three decades ago. The energetic processing technique uses radiation energies such as gamma rays, ion beams, electrons, and UV rays. The plastic wastes chemical processing technique involves certain chemicals such as acids and alkalis. While the biological plastic wastes processing technique utilizes various kinds of microbes, this technique is known as biodegradation (Premraj & Doble 2005). Of all the methods of handling plastic wastes, biodegradation is recognized as the most promising and environmentally safe method of processing plastic wastes (Gan & Zhang 2019). Biodegradation is the processing of plastic waste using microorganisms (Verma & Gupta 2019).

Types of Plastic

Plastics can be divided into thermoplastics and thermosets. Thermoplastic is a group of plastics that can be remelted and reprocessed back into a product as well as recyclable. Meanwhile, thermosets or thermosetting is a plastic group that cannot be remelted because the molecular bonds contained in that plastic polymers are tightly bound in crosslink (Chan & Ji 1999, Albano et al. 2009, Choi et al. 2009, Al-Salem et al. 2010, Bărbuță et al. 2010, Choi et al. 2005, Mohammadian & Haghgi 2013). The four most commonly used plastic polymers in daily life are high-density polyethylene (HDPE), low-density polyethylene (LDPE), polypropylene (PP), and polyethylene terephthalate (PET). They are the most common sources of plastic pollutants that are mainly used as plastic bags (Drzyzga & Prieto 2018). Polyethylene (PE) is the most widely used plastic. The use of that polymer reached 29.6% of the total daily plastic used, followed by polypropylene (PP) as much as 18.9%, polyvinyl chloride (PVC) 10.4%. Meanwhile, the use of polyurethane (PUR) reached 7.4%, polystyrene (PS) reached 7.1% and the use of PET reached 6.9% (Wu et al. 2017, Yang et al. 2018).

Polyethylene (PE) is a synthetic plastic that has a high molecular weight, complex three-dimensional structure, and hydrophobic nature (Hadad et al. 2005, Shah 2009). Polyethylene is composed of stable polymers containing bonds composed of ethylene monomers (Alshehrei 2017). The stability of the polymers makes this plastic resistant to degradation in nature (Nandi & Joshi 2013). PP is a synthetic plastic composed of a heat-and-chemical-resistant polymer. Because of this property, PP is resistant to degradation (Khoironi et al. 2019).

PET has two hydroxyls (OH) and dicarboxylic aromatic acids consisting of aromatic carbon rings and two carboxyls (CO₂H) (Venkatachalam et al. 2012, Farzi et al. 2019, Webb et al. 2013). This plastic is synthesized by involving two chemical reactions (Kint & Muñoz-Guerra 1999,

Awaja & Pavel 2005, Webb et al. 2013). The aromatic components contained in PET caused this synthetic polymer difficult to be degraded naturally in the environment (Webb et al. 2013).

The presence of ester bonds in PET polymers leads to their strong and recalcitrant characters causing them scarcely to be degraded in the environment (Lucas et al. 2008, Khoironi et al. 2019). However, many microbial communities can utilize PET as their carbon and energy source (Zhang et al. 2004, Webb et al. 2013). Extracellular polymeric components produced and excreted by some microbes are appropriate to overcome the recalcitrant properties of plastics including PET. Thus, they can be used as plastic polymers degrading agents. Extracellular components produced by microbes play an important role as bio surfactants that enhance the changing of hydrophilic and hydrophobic phases between the plastic surface and microbial cell surfaces. This change is important to help them to break down plastic polymers (Lucas et al. 2008, Khoironi et al. 2019).

Furthermore, biodegradable plastics and polymers are materials that are now widely used in various industries (Weng et al. 2013, Kim et al. 2017). Biodegradable polymers are polymers containing amides, esters, or ethers bonds. Biodegradable polymers can be distinguished into agro-polymers, and bio-polyesters (Tiwari et al. 2018). Some biodegradable plastics include polylactic acid (PLA) and polybutylene adipate-co-terephthalate (Weng et al. 2013, Kim et al. 2017). Although biopolymers are easier to be degraded than non-biodegradable synthetic polymers, biodegradable polymers also have strong carbon bonds that cannot be degraded in such a short time (Tiwari et al. 2018). The complete biodegradation process of a biodegradable polymers in nature takes months or even years (Kim et al. 2017).

The Negative Effects of Plastics Waste

The massive use of plastics generates the accumulation of plastic wastes in the environment (Ahsan et al. 2016). Plastic wastes cause many diseases for living beings, including humans. Plastic polymers accidentally ingested lead to immune system disorders, inhibit and disturb enzyme activities, and cause hormonal system disorders resulting in abnormalities of the endocrine system. The carcinogenic compounds of this synthetic polymer threaten many living creatures both on land and in aquatic environments (Pavani & Rajeswari 2014, Munir et al. 2018).

Plastic waste polluting the land can be imperfectly degraded by environmental factors. They cause the washing or decomposition of harmful substances of the plastic polymers into the environment. Hazardous substances such as heavy metals, plasticizers, stabilizers, and plastic dyes will be released into the environment. These pollutants pollute the terrestrial environment. Furthermore, they will be carried by the water stream then pollute the aquatic environment. In particular, around 80% of plastic pollution in the aquatic environment originated from the land (Sheavly 2005, Alabi et al. 2019).

Plastic wastes float from one place to another. They might bring many living things to new places, introduce some invasive species to a new aquatic environment that will compete with indigenous species (Derraik 2002, Hasnat & Rahman 2018). Fire is another adverse effect of plastic waste that is caused by the burning of flammable plastic waste. Not only causes many types of losses, but the smoke produced by the burning of the plastic wastes also emits toxic gases into the air like carbon monoxide (CO) and hydrogen cyanide (HCN) (Purwaningrum 2016).

Even though plastic might be degraded in nature after hundreds or thousands of years, the incomplete degradation of that recalcitrant polymer releases toxic fragments into the environment thus causing many problems. The fragmented toxic compounds might be accumulated in various living things resulting in some health disorders (Kang et al. 2019). Moreover, the accumulation of plastic wastes pollute the view which decreases the attractiveness of tourism objects in a country thus adversely affecting a country's economy, mainly a country that is highly depend on tourism as the main source of its GDP. It is because of the number of plastic wastes discomfort and reduce the tourists interest toward a tourism object (UNEP 2018). After all, the complete and environmentally safe plastic waste processing method is needed. Various studies have reported that the degradation of plastic waste using enzymes produced by microbes such as fungi is the appropriate method to overcome plastic pollution (Kang et al. 2019).

Plastics Waste Biodegradation

Plastic recycling activities have several weaknesses. Plastic wastes that piled up by the community become more difficult to separate based on its type. It will be worse if the plastic wastes mixed with various other wastes. The mixed plastic wastes will affect the plastic melting point and even get more complicated. Contaminated plastic waste might damage the plastic melting machine. Besides, adequate space is also needed in recycling (Sahwan et al. 2005).

The burning of plastic wastes as often done by the community, and incineration process produce pollutants that are harmful to the environment. The incineration process releases greenhouse gases such as furans and dioxins depleting the ozone layer. Dioxins are hazardous components interfering with the endocrine system in humans (Chaturvedi 2002, Pilz et al. 2010, Kumar et al. 2017). Therefore, biodegradation is the essential plastic wastes processing method to solve these problems as this process will completely degrade plastic polymers resulting in microbial biomass as its biological agents (Kumar et al. 2017).

Synthetic polymers are potential energy and carbon sources for microorganisms. For instance, complete oxidation of polyethylene polymers produces almost the same energy as glucose, a substrate for microbes. The complete oxidation of polyethylene produces usable energy ranging from -422 kJ to -425 kJ per mole of O₂. Meanwhile, the usable energy generated from glucose is -479 KJ per mole O₂. The complexity of synthetic polymers such as polyethylene and polypropylene, containing C-C skeletal bonds, makes them nonhydrolyzable. As they are nonhydrolyzable so they will be firstly broken down through redox reactions into simpler ones then assimilated by microbial cells. Meanwhile, hydrolyzable polymers such as polyethylene terephthalate and polyamide, containing amides and ester bonds, can be directly hydrated by enzymes produced by microbes (Gewert et al. 2015, Krueger et al. 2015, Oberbeckmann & Labrenz 2020).

Hydrolysis is the breaking down of the functional bonds of a polymer by recombining them with water (Tiwari et al. 2018). The hydrolysis of semi-crystalline polymers such as esters, amides, and anhydrides take place in two stages. The first stage is the diffusion of water into the amorphous with succeeding hydrolysis. The second step will begin when moisture penetrates and degrades the crystalline parts of the polymer. The process will reduce the crystallinity and molecular weight of the polymer which makes the polymer more easily dissolved in water thus increases the erosion rate of the polymer. Afterward, the plastic surface corrosion process will change the structural dimensions of the plastic polymer (Rabek 1975, Tiwari et al. 2018).

Biodegradation is a complete degradation process of a polymer through the activity of enzymes produced by microorganisms. The biodegradation process of plastic wastes is the breakdown of complex plastic polymers into simpler oligomers and monomers. These results will then be absorbed into the microbial cells (Gu 2003, Muhonja et al. 2018). In biodegradation, microbes will colonize the plastic surface to form biofilms. The colonization of plastic surfaces is affected by the hydrophobicity compatibility of plastic surfaces and microbes. Biofilms on plastic surfaces are useful for accelerating the degradation process of plastic polymers (Agrawal & Singh 2016). Furthermore, there are two main activities in the biodegradation process. The first activity is the process of converting molecules that have high molecular weight into organic monomers and organic acids that occur through biological and chemical hydrolysis. The second process is the decomposition of these materials into gas that is carried out by microorganisms (Bikiaris 2013, Kim et al. 2017).

Biodegradation has four stages consisting of biodeterioration, depolymerization, assimilation, and mineralization (Gu 2003, Muhonja et al. 2018). Biodeterioration is the cooperation activities between several microorganisms and abiotic factors that break down polymers into simpler ones. Depolymerization is a process in which microorganisms secrete catalytic compounds in the form of enzymes and free radicals to form biofilms helping them to break the polymer chain progressively become oligomers, dimers, and monomers (Marjayandari & Shovitri 2015). During the biodegradation process, the microbes expel various exoenzymes that break down complex polymers into simpler compounds that have shorter chains. These results will be used as carbon and

energy sources for microorganisms, the process is called depolymerization (Frazer 1994, Nandi & Joshi 2013).

Moreover, biodegradation also involves mineralization processes (Frazer 1994, Nandi & Joshi 2013). This is a stage of biodegradation in which produces CO₂, H₂O, or CH₄ (Premraj & Doble 2005). Mineralization occurs through a series of biological activities (Kim et al. 2017). Meanwhile, the degradation rate of plastic waste highly depends on the structure of the polymers forming the plastic, although the differences of the plastic structures are not too distinct. Other factors influencing the biodegradation of plastic waste are molecular weight, molecular shape, and crystallinity of plastic polymers (Premraj & Doble 2005).

Plastics Degrading Fungi

Many studies have reported the ability of fungi in plastic biodegradation (Table 1). Using fungi as bioremediation agents is an appropriate method to decrease the amount of plastic wastes polluting the environment. The rapid growth of fungi in many substrates is a beneficial factor in biodegradation. Because of their rapid growth, their mycelium will be spread out covering the entire substrate surfaces then penetrating them to start the biodegradation process. Fungi can be found in any kind of environment, including extreme environments such as low pH and arid (Kim & Rhee 2003, Nandi & Joshi 2013). Fungi isolated from soil exposed to plastics are important plastic wastes biodegradation agents (Nandi & Joshi 2013). There are many studies that reported some plastic degrading fungi including *Gliomastix* sp., *Chaetomium* sp., *Fusarium* sp., *Mortierella* sp., and *Paecilomyces* sp. (Nathania & Kuswytasari 2013, Hardiyanti et al. 2017). Indigenous fungi isolated from plastics contaminated landfill are potential agents of plastic biodegradation as *Trichoderma* sp., *Aspergillus flavus*, and *Aspergillus niger* (Hardiyanti et al. 2017).

Table 1 Some plastics degrading fungi

No.	Plastics Type	Fungi	Isolate Sources	References
1.	PE	<i>Aspergillus niger</i>	Garbage soil	Nandi & Joshi (2013)
		<i>Aspergillus flavus</i>	Garbage soil	Nandi & Joshi (2013)
		<i>Aspergillus sydowii</i>	Rhizosphere <i>Avicenia</i>	Sangale et al. (2019)
		PNPF15/TF	<i>marina</i>	
		<i>Aspergillus terreus</i>	Rhizosphere <i>Avicenia</i>	Sangale et al. (2019)
		MANGF1/WL	<i>marina</i>	
		<i>Chaetomium globosum</i>	Dumpsite soil	Sowmya et al. (2014)
		<i>Fusarium</i> sp. AF4	Sewage sludge	Shah et al. (2009)
		<i>Myceliophthora</i> sp.	Plastic garbage	Khalil et al. (2013)
2.	LDPE	<i>A.fumigatus</i>	Red sea	Alshehrei (2017)
		<i>Aspergillus flavus</i>	Red sea	Alshehrei (2017)
		<i>Aspergillus japonicas</i>	Polythene bags	Mandan & Arya (2017)
		<i>Aspergillus niger</i>	Red sea	Alshehrei (2017)
			Polythene bags	Mandan & Arya (2017)
			Culture	Ingavale et al. (2018)
		<i>Aspergillus nomius</i>	Landfill soil	Munir et al. (2018)
		<i>Aspergillus terreus</i>	Red sea	Alshehrei (2017)
		<i>Mucor</i> sp.	Polyethylene contaminated soil	Singh & Gupta (2014)
3.	HDPE	<i>Penicillium</i> sp.	Red sea	Alshehrei (2017)
		<i>Trichoderma viride</i>	Landfill soil	Munir et al. (2018)
		<i>Aspergillus fumigatus</i>	Plastic waste dumping site	Verma & Gupta (2019)
		<i>Aspergillus niger</i>	Culture	Ingavale et al. (2018)
4.	PET	<i>Bjerkandera adusta</i>	Wild fungi from Ohgap Mountains, South Korea	Kang et al. (2019)
		<i>Aspergillus niger</i>	Culture	Asmita et al. (2015)

Table 1 Continued.

No.	Plastics Type	Fungi	Isolate Sources	References
5.	PUR	<i>Monascus ruber</i>	Plastic contaminated soil	El-Morsy et al. (2017)
		<i>Monascus sanguineus</i>	Plastic contaminated soil	El-Morsy et al. (2017)
		<i>Monascus</i> sp.	Plastic contaminated soil	El-Morsy et al. (2017)
		<i>Pestalotiopsis</i> sp.	Isolated from <i>Nephentes ampullaria</i>	Lii et al. (2017)
6.	PHB	<i>Alternaria alternate</i>	Culture	Aburas (2016)
		<i>Aspergillus flavus</i>	Culture	Aburas (2016)
		<i>Aspergillus fumigatus</i>	Culture	Aburas (2016)
		<i>Aspergillus nidulans</i>	Culture	Aburas (2016)
		<i>Aspergillus niger</i>	Culture	Aburas (2016)
		<i>Aspergillus ochraceus</i>	Culture	Aburas (2016)
		<i>Aspergillus oryzae</i>	Culture	Aburas (2016)
		<i>Aspergillus parasiticus</i>	Culture	Aburas (2016)
		<i>Aspergillus terreus</i>	Culture	Aburas (2016)
		<i>Fusarium</i> sp.	Culture	Aburas (2016)
7.	PS-PUR	<i>Penicillium</i> sp.	Culture	Aburas (2016)
		<i>Trichoderma</i> sp.	Culture	Aburas (2016)
		<i>Trichoderma viride</i>	Soil	Râpă et al. (2014)
		<i>A. flavus</i> (FopI-2)	Soil, plastic debris, latex and plastic shield	Ibrahim et al. (2011)
		<i>A. fumigatus</i> (FopI-4)	Soil, plastic debris, latex and plastic shield	Ibrahim et al. (2011)
		<i>Aspergillus sonali</i> (FsH-3)	Soil, plastic debris, latex and plastic shield	Ibrahim et al. (2011)
		<i>A. terreus</i> (FsH-8)	Soil, plastic debris, latex and plastic shield	Ibrahim et al. (2011)
<i>Fusarium solani</i> (FsM-6)	Soil, plastic debris, latex and plastic shield	Ibrahim et al. (2011)		
<i>Spicaria</i> spp. (Fp-7)	Soil, plastic debris, latex and plastic shield	Ibrahim et al. (2011)		

There are many studies that reported polyethylene (PE) degrading fungi, *Myceliophthora* sp. is one of them. This fungus produces laccase catalyzing the degrading process of plastic polymers. It produces laccase optimally at pH 5.0 and temperature 30°C. This plastic degrading fungus can grow on a medium containing polyethylene. Electron microscopy analysis showed that the surface of the plastic exposed to the fungus was damaged, thus closely related to the activity of the laccase produced by the fungus (Khalil et al. 2013).

Two other polyethylene degrading isolates are *Aspergillus terreus* MANGF1/WL and *Aspergillus sydowii* PNP15/TF isolated from the *Avicennia marina* rhizosphere. *Aspergillus terreus* MANGF1/WL reduced 50% of the weight of plastic samples at pH 5.0 within 60 days. Whereas *Aspergillus sydowii* PNP15/TF reduced the weight of plastic samples about 94% at pH 3.5 within the same incubation period. These weight reductions were associated with depolymerase enzyme activity that broke the polyethylene bonds (Sangale et al. 2019). As well as those fungi, *A. fumigatus* reduced polyethylene weight about 24% and *A. oryzae* 36%. Fungi *Aspergillus* spp. such as *A. fumigatus*, *A. oryzae* and *A. flavus* produce laccase to degrade plastic polymers. While the highest laccase activity was found in *A. oryzae* (Muhonja et al. 2018).

Indumathi & Gayathri (2016), successfully obtained several plastic degrading fungi isolated from plastic-contaminated dumping sites consisting of *Aspergillus niger*, *A. oryzae*, *A. japonicus* and *Penicillium* sp. They found that *A. oryzae* dominated the soil samples. The fungus was then tested for its plastic degradation ability using plastic strips that are buried in soil pits. Based on the research, *A. oryzae* degraded those plastics about 20% within 15 days, 24% within 30 days, 26%

within 45%, and 30% within 60 days. SEM analysis revealed that the plastics were experiencing micro cracks (Indumathi & Gayathri 2016).

Chaetomium globosum was also reported as a plastic degrading fungus. The degradation rate of the fungus was higher after the plastic was given preliminary treatment by UV light and autoclaved. It reduced the plastic weight exposed to UV light by about 21% within 3 months. UV light is the oxidation initiator of polymers as well as accelerates the degradation rate. The initial treatment using UV light in plastic degradation was more effective. The degradation of autoclaved plastic using *Chaetomium globosum* was only 7.5%. Meanwhile, plastic samples that were only surface sterilized before exposed to this fungus were degraded by about 5.6% within the same incubation time (Sowmya et al. 2014). *Aspergillus niger* and *A. flavus* decreased the plastic weights which were previously exposed by UV light and 99.9% nitric acid around 19-24% within 2-4 months (Nandi & Joshi 2013). Moreover, *Fusarium* sp. AF4 was also reported that it was able to bind to the surface of PE. Based on the electron microscopy analysis, it was known that its hypha damaged the plastic surface. It thus facilitated the biodegradation process carried out by *Fusarium* sp. AF4 (Shah et al. 2009).

Alshehrei (2017), obtained LDPE plastic degrading fungi isolated from the Red Sea including *Aspergillus niger*, *A. flavus*, *A. terreus*, *A. fumigatus* and *Penicillium* sp. The percentage of weight loss of the plastic samples within 1 month exposed to *A. niger* was 19.5%, *A. flavus* 16.2%, *A. terreus* 21.8%, *A. fumigatus* 20.5%, and *Penicillium* sp. was 43.3% (Alshehrei 2017). *Aspergillus japonicas* degraded the plastic about 12% within the same incubation period. SEM analysis proved that the plastic surfaces were experienced cracking, corrosion, and folding compared to controls that seemed normal (Mandan & Arya 2017). Also, *Mucor* sp. isolated from soil exposed to polyethylene plastic degraded LDPE about 16% within 4 weeks incubation in synthetic media containing LDPE as the main carbon source (Singh & Gupta 2014). Still, there are many LDPE degrading fungi such as *Alternaria alternate*, *Emericella nidulans*, *Paecilomyces variotii*, *Penicillium duclauxii* and *P. vinaceum* (Ameen et al. 2015).

Aspergillus fumigatus also has a high HDPE plastic degradation ability. The fungus was able to reduce 59% of the plastic sample's weight, 60.4 mg to 33.6 mg, using soil media within 9 months. Whereas in the liquid medium, within 2 months it reduced HDPE weight about 29.1%. Based on SEM analysis, plastic surfaces tested with soil medium suffered more damages and perforations than liquid media compared to controls that were not damaged. FTIR analysis detected the presence of functional groups from the degradation process such as ketones, aldehydes, carboxylic acids, and others (Verma & Gupta 2019). *Bjerkandera adusta* was also reported to be able to degrade HDPE. The fungus produces oxidative extracellular enzymes potentially as plastic degrading enzymes. Raman analysis illustrated that the enzymes produced by the fungus degraded the amorphous structure of the HDPE (Kang et al. 2019).

Meanwhile, *A. niger* (Ingavale et al. 2018), *Penicillium oxalicum* NS4 (KU559906) and *P. chrysogenum* NS10 (KU559907) degraded both LDPE and HDPE (Ojha et al. 2017, Verma & Gupta 2019). The degradation of LDPE and HDPE by *A. niger* was characterized by the decreasing in plastic molecular weight, plastic thickness, and intensity of the functional plastic groups. The color of the plastic samples exposed to the fungus was faded as well as the surface was damaged. These were the signs of enzymatic activity carried out by *A. niger*. In particular, the weight reduction of LDPE by the submerged method was 3.97% and the composting method was 1.89%. Whereas the weight reduction of HDPE with the submerged method was 3.56% and 1.6% with the composting method within 60 days (Ingavale et al. 2018). Not only degraded polyethylene polymers but *A. niger* also degraded another polymer, PET. Within 1 month this fungus was reported to decrease 52.94% of PET weight in Rose Bengal Broth (Asmita et al. 2015).

Some fungi known as PHB plastic degradation agents are *Penicillium*, *Aspergillus*, *Fusarium*, *Alternaria*, *Trichoderma*, *A. fumigatus*, *A. oryzae* (Aburas 2016), and *Trichoderma viride* (Râpă et al. 2014). The percentage of PHB degradation using solid media containing PHB within 7 days by *Alternaria alternate* was 14%, *Aspergillus flavus* 23%, *Aspergillus fumigatus* 24%, *Aspergillus nidulans* 47%, *Aspergillus niger* 24%, *A. ochraceus* 24%, *Aspergillus parasiticus* 25%, *A. terreus*

29%, *Fusarium* sp. 34%, *Penicillium* sp. 34% and *Trichoderma* sp. 14%. The hydrolysis of the PHB polymers catalyzed by depolymerase enzymes produced by those fungi (Aburas 2016). Meanwhile, *Trichoderma viride* caused several changes in plastic samples after 10 days of incubation including change in the color of plastic, perforation, and damage of the plastic surface. The surface perforation is the initial stage carried out by fungi before their mycelium covering the entire surface of the plastic then breaking down the plastic polymer (Râpă et al. 2014).

Polyurethane plastic (PUR) can be degraded by several fungi including *Monascus sanguineus*, *Monascus* sp. (El-Morsy et al. 2017), and *Pestalotiopsis microspore* (Mandan & Arya 2017). *Monascus* spp. produce esterase, protease, and lipase that catalyze the degradation of polyurethane by breaking down the ester bonds of PUR (El-Morsy et al. 2017). Besides being able to produce various kinds of antibiotic compounds, endophytic fungi are also reported to be able to degrade plastics. Lii et al. (2017) reported that *Pestalotiopsis* isolated from *Nepenthes ampullaria* was able to degrade polyurethane (PUR). This endophytic fungus used PUR as its carbon source. The PUR degradation activity correlated with the activity of the esterase. Also, it was suspected that there was the involvement of the metallothionein-like protein in the degradation of the PUR (Lii et al. 2017). *Pestalotiopsis microspore* produces serine hydrolase enzymes and polyurethanase enzymes induced when the fungus is in a media containing PUR. It is also able to degrade plastics in anaerobic conditions which are very important for plastic waste degradation in extreme environments (Mandan & Arya 2017).

Polyester-polyurethane (PS-PUR) degrading fungi including *Spicaria* spp., *Aspergillus sonali*, *A. terreus*, *A. flavus*, *A. fumigatus* and *Fusarium solani* used this polymer as their carbon and nitrogen source. In liquid shaking culture using liquid basal media within 3 weeks of incubation, *Fusarium solani* degraded completely (100%) the plastic sample. Meanwhile, under the same condition, the plastic degradation by *Spicaria* spp. was 12.7%, *Aspergillus sonali* 71.8%, *A. terreus* 26.1%, *A. flavus* 40.5%, and *A. fumigatus* 43.5%. In solid basal medium for 3 weeks incubation, *Fusarium solani* decreased that plastic weight by about 72.5%, *Spicaria* spp. degraded it about 22.9%. While, the percentage of degradation by *A. solani*, *A. terreus*, *A. flavus*, and *A. fumigatus* were 63.6%, 58%, 98.4% and 39.5% (Ibrahim et al. 2011).

Besides being able to be used as biodegradation agents for plastic waste on the land, fungi can also be used as agents for the degradation of plastic waste in the aquatic environment. Paço et al. (2017) reported that fungi found in the sea, *Zalerion maritimum*, degraded PE polymer (Drzyzga & Prieto 2018).

Enzymes Involved in Plastics Biodegradation

During the biodegradation process, the fungal mycelium covers the entire plastic surfaces. They will synthesize and release various enzymes, such as lipase, esterase, urease, depolymerase, and hydrolase to break down the plastic polymers (Râpă et al. 2014). The ability of fungi to produce enzymes is influenced by the used substrate. The type of enzyme produced by fungi depends on the substrate. In particular, the differences between plastic polymers cause different types of enzymes produced by fungi. For instance, in the degradation of polyurethane, fungi will release some important enzymes to breakdown that plastic polymers such as proteases, esterases, lipases, laccases, and polyurethanases (Ibrahim et al. 2011, El-Morsy et al. 2017). These enzymes will break down polyurethane polymers by breaking the ester bonds (Bhardwaj et al. 2011, El-Morsy et al. 2017).

Aspergillus flavus is one of many fungi known to be able to produce laccase, amylase, lignin peroxidase, and manganese peroxidase. *Aspergillus niger* produces laccase, lignin peroxidase, and manganese peroxidase. *Fusarium graminearum* produces amylase, laccase, lignin peroxidase, and manganese peroxidase. These four types of enzymes are essential in the breaking down of carbon bonds in polyethylene polymers (Ganesh et al. 2017). Meanwhile, *Pestalotiopsis microspore* produces serine hydrolase enzyme which catalyzes the degradation of PUR polymers (Russell et al. 2011, Lii et al. 2017).

Not only useful in producing secondary metabolites but endophytic fungi also useful in plastic biodegradation. One of the plastic degrading endophytic fungi is *Pestalotiopsis microspora*. This fungus is a polyurethane plastic degrading agent. It produces serine hydrolase. This fungus also produces polyurethanase which is induced when the fungus is in a medium containing PUR. The fungus can degrade plastics in anaerobic condition, thus it is very important for processing plastic waste in extreme environments (Mandan & Arya 2017). Meanwhile, the degradation of PE by *Chaetomium globosum* catalyzed by laccase and manganese peroxidase. Manganese peroxidase activity is higher compared to laccase in the biodegradation process by this fungus (Sowmya et al. 2014).

Plastic polymers will be degraded through two steps. The first step is the release of enzymes into the substrate, the plastic polymers. The enzymes catalyze the hydrolysis reaction of the plastic polymers. Extracellular and intracellular depolymerase enzymes degrade plastic polymers. The smaller parts resulted from the early degradations will be absorbed by microbes which will then undergo intracellular biodegradation processes (Tokiwa & Calabria 2004, Bhardwaj et al. 2012).

Hydrolase is a class of enzymes that has broad substrates that catalyze hydrolysis reaction of many substrates including peptides, amides, and halides (Junge & Kirsch 1973, Fojan et al. 2000). The hydrolysis of a polymer is conducted by involving aspartate, histidine, and serine. Interaction between aspartate and histidine ring produces hydrogen bonds. Histidine deprotonating serine forms nucleophilic alkoxide (-O) which attacks ester bonds in plastic polymers. This reaction produces an alcoholic tip and an acyl-enzyme complex. The acyl-enzyme complex will be attacked by water forming a carboxyl-end and free enzyme (Lucas et al. 2008, Tiwari et al. 2018).

Two examples of hydrolase enzymes are esterase and lipase (Fojan et al. 2000). Esterase is an enzyme classified into hydrolase which catalyzes the breaking down and formation of ester bonds, involved in interesterification, intraesterification, and transesterification (Sayali et al. 2013). Ester bonds forming many polymers such as polyesters can be degraded by the esterase (Premraj & Doble 2005). This enzyme is often found in many microorganisms (Premraj & Doble 2005) including fungi such as *Monascus ruber* (El-Morsy et al. 2017).

Lipase is also categorized as a hydrolase enzyme which has broad substrates. Lipase catalyzes water-insoluble substrates like long-chain fatty acids (Verger 1997, Fojan et al. 2000). Lipase is also capable of catalyzing hydrolytic cleavage of the ester bonds found in polyester that has a low molecular weight (Premraj & Doble 2005). Enzymes such as lipases and PHA depolymerases break ester bonds found in aliphatic polyester including PLA. Before the hydrolysis reaction occurs toward the polymers, the hydrophobic side of the enzyme will bind to the solid substrate through hydrophobic interactions (Mochizuki & Hiramami 1997, Prema & Palempalli 2015). The optimum activity of PLA depolymerase isolated from *Bacillus licheniformis* was at pH 7.0, while the optimum temperature was around of 50°C-60°C. Heavy metals such as Mn^{2+} , Cu^{2+} , Na^+ , K^+ , Mg^{2+} , and Fe^{2+} are the inhibitors of this enzyme (Prema & Palempalli 2015).

Another important enzyme involved in plastic biodegradation is laccase. Laccase is an enzyme widely found in plants, fungi, other microorganisms (Sivakumar et al. 2010, Khalil et al. 2013) and insects (Dana et al. 2017). This enzyme catalyzes the phenol substrate and other similar molecules through the oxidation process. This enzyme is a common lignolytic and lignin-degrading enzyme (Sivakumar et al. 2010, Khalil et al. 2013).

Laccase is a polyphenol oxidase that oxidizes lignin by using molecular oxygen and producing water as its product. Laccase has broad substrates including phenols, ortho- and para-diphenols, amino phenols, methoxy phenols, polyphenols, polyamines, aryl diamines, and ascorbate. Laccase has various benefits, one of which is in the field of plastic bioremediation (Dana et al. 2017). This enzyme degrades xenobiotics, polycyclic aromatic hydrocarbons (PHAs), phenolic components, chlorinated phenolic pollutants, and diesel (Pannu & Kapoor 2014, Zarinkamar et al. 2014, Dana et al. 2017). This indicates great potential for environmental management caused by plastic wastes pollution (Gianfreda et al. 1999, Khalil et al. 2013).

Laccase catalyzes the breaking down of polyethylene to carboxylic acid through oxidation. The product will be transported to fl-oxidation with coenzyme-A which breaks two carboxylic acid

fragments into acetyl-S-CoA which will be further metabolized through the citric acid cycle. Water and carbon dioxide will be produced at the end of that reaction (Khalil et al. 2013). Multigene families associated with laccase production are mostly found in fungi. 17 genes that encode laccase production found in *Coprinopsis cinerea* (Lu et al. 2015, Dana et al. 2017). Fungal laccase is a blue enzyme that contains multicopper. It catalyzes the oxidation of one electron contained in the phenol components by reducing molecular oxygen into water (Reinhammar 1984, Nishida et al. 2001).

Other important enzymes are PHA depolymerases, PHB depolymerases, PLA depolymerases, PCL depolymerases, cutinases, ureases, proteinases (proteinase K in PLA degradation), and dehydratases (Pathak & Navneet 2017, Urbanek et al. 2018). PHA and PHB can be degraded by PHA and PHB depolymerases. PHB depolymerases degrade all (R) chains, cyclic-(R) oligomers, oligolides, and polymers composed of racemic hydroxybutanoate (Shimao 2001, Premraj & Doble 2005). PCL can be degraded by lipases, esterases, cutinases (Premraj & Doble 2005). PLA degraded by Proteinase K, pronase, and bromelain (Shimao 2001, Premraj & Doble 2005). The ability of proteases to degrade PLA is because they can recognize α -ester bonds found in PLA (Nakamura et al. 2001, Pranamuda et al. 2001, Jarerat et al. 2006, Panyachanakul et al. 2019). Meanwhile, polyurethane polymers can be degraded by proteases, esterases, lipases, laccases, and polyurethanases (Ibrahim et al. 2011, El-Morsy et al. 2017).

Conclusions and Future Perspective

Plastics biodegradation using fungi is considered a potential method in overcoming the problem of plastic wastes. It is safe and more environmentally friendly. Fungi produce various kinds of extracellular and intracellular enzymes that degrade plastic polymers. Fungi use plastic polymers as energy and carbon sources for their metabolism. The application of this method can be crucial to overcome the accumulation of plastic wastes.

However, the biodegradation of plastic waste has certain limitations. The plastics biodegradation process is affected by biotic factors, bioavailability, and characteristics of plastic polymers. Abiotic factors that affect the rate of plastic biodegradation are moisture, temperature, and pH. Biotic factors determining the rate of plastic biodegradation are enzymes and hydrophobicity. Meanwhile, the characteristics of plastic polymers that affect biodegradation are molecular weight, size and shape, additives, and bio surfactants (Ahmed et al. 2018). The type of fungus used also affects the biodegradation process of plastic waste. Each fungus is only effective in degrading certain types of plastic waste. This limitation is related to its ability to produce suitable enzymes to decompose plastic waste.

Additionally, the studies of plastics biodegradation still focus on a laboratory scale and macro plastic waste. Also, based on the reported researches, the biodegradation rate of plastic waste using microorganisms takes ages (Matjašič et al. 2020). Therefore, research and development regarding the ability of fungi to degrade plastic wastes need to be conducted in future. Understanding fungal metabolism in degrading plastic wastes is one of the main factors. Metabolic engineering and optimization of processes are also essential to increase the fungal ability to degrade plastic wastes.

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